



UNIVERSIDADE ESTADUAL DE CAMPINAS
FACULDADE DE ENGENHARIA DE ALIMENTOS

SABRINA ALVES LENQUISTE

CASCA DE JABUTICABA (*Myrciaria jaboticaba* (Vell.) Berg) LIOFILIZADA E CHÁ DA
CASCA DE JABUTICABA LIOFILIZADA COMO ESTRATÉGIAS NO COMBATE À
SÍNDROME METABÓLICA E ESTRESSE OXIDATIVO.

FREEZE-DRIED JABOTICABA (*Myrciaria jaboticaba* (Vell.) Berg) PEEL AND FREEZE-
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Tese apresentada a Faculdade de Engenharia de Alimentos da Universidade Estadual de Campinas como parte dos requisitos para obtenção do Título de Doutora em Alimentos e Nutrição - Área de Nutrição Básica Experimental Aplicada a Tecnologia de Alimentos.

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Orientador: Prof. Dr. Mário Roberto Maróstica Junior

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Albert Einstein

RESUMO GERAL

O presente estudo objetivou determinar os compostos bioativos e o potencial antioxidante *in vitro* e *in vivo* da casca de jabuticaba liofilizada (CJL) e do chá preparado a partir da casca de jabuticaba liofilizada (CJL-chá), bem como avaliar o potencial terapêutico desses produtos na obesidade e síndrome metabólica induzida por dieta. Compostos fenólicos totais, flavonoides e antocianinas totais foram quantificados por métodos colorimétricos. Ácido elágico, ácido gálico e cianidina-3-O-glicosídeo foram identificados e quantificados por HPLC. Os ensaios antioxidantes FRAP, ABTS+, DPPH e ORAC foram realizados com o extrato metanólico da CJL e o CJL-chá. Ratos, *Wistar*, adultos, foram randomizados em 6 grupos (n = 10): Controle magro (AIN93-M) que recebeu dieta AIN-93M por 12 semanas; Controle obeso que recebeu dieta hiperlipídica adicionada de frutose (HFF) por 12 semanas; Prevenção CJL (P. CJL) e Tratamento CJL (T. CJL) que receberam dieta HFF suplementada com 2% de CJL por 12 e 6 semanas, respectivamente; Prevenção chá de jabuticaba (P. CJL-chá) e Tratamento chá de jabuticaba (T. CJL-chá) que receberam dieta HFF e tiveram a água substituída pelo chá da CJL por 12 e 6 semanas, respectivamente. Os testes de tolerância à glicose (GTT) e tolerância à insulina (ITT) foram realizados. Glicose sérica e insulina plasmática foram mensuradas. Adiponectina e leptina séricas foram dosadas. O fígado e os tecidos adiposos epididimal (TAE), mesentérico (TAM) e retroperitoneal (TAR) foram coletados e pesados. Os lipídios nos fígados foram mensurados por Bligh & Dyer. Alíquotas do tecido hepático foram fixadas em formalina tamponada e encaminhadas para análise histológica. O conteúdo total de insulina nas ilhotas e a secreção de insulina após estímulo de glicose foram mensurados por radioimunoensaio (RIE). O potencial antioxidante plasmático e hepático foi mensurado por FRAP e TBARS. GSH total e as enzimas antioxidantes (GPx, GR, SOD e CAT) foram medidas no plasma e no fígado dos animais. Os resultados foram submetidos ao teste de variância ANOVA e teste de Tukey, com significância de 0,05. TBARS, GSH e CAT foram positivamente modulados pela suplementação com casca de jabuticaba, porém FRAP, SOD, GPx e GR, não responderam à suplementação. O ganho de peso, adiposidade visceral e alguns parâmetros relacionados à resistência à insulina foram positivamente alterados com 6 e 12 semanas de suplementação. A suplementação por 12 semanas elevou o HDL-colesterol e reverteu a esteatose hepática mostrando que a o consumo de CJL por mais longo período pode promover melhores resultados. Conclui-se que a CJL e CJL-chá possuem grande potencial funcional na prevenção e combate ao dano oxidativo, obesidade, resistência à insulina e esteatose hepática.

ABSTRACT

The aim of present study was to determine the bioactive compounds and antioxidant potential *in vitro* and *in vivo* of freeze-dried jaboticaba peel (FJP) and freeze-dried jaboticaba peel tea (FJP-T), as well as evaluate the therapeutic potential of these products in diet induced obesity and metabolic syndrome. Total phenolic compounds, flavonoids and anthocyanins were determined by colorimetric methods. Ellagic acid, gallic acid and cyanidin-3-O-glucoside were identified and quantified by HPLC. Antioxidant assays FRAP, ABTS+, DPPH and ORAC were done with FJP methanolic extract and FJP-T. *Wistar* rats, adults, were randomized in 6 groups (n = 10): normolipidic diet (AIN-93M) fed AIN93-M diet for 12 weeks; High-fat fructose diet (HFF) fed HFF diet for 12 weeks; Prevention FJP (P. FJP) and treatment FJP (T. FJP) fed HFF diet supplemented with 2% FJP for 12 and 6 weeks, respectively; Prevention FJP-T (P. FJP-T) and treatment FJP-T (T. FJP-T) fed HFF diet and had substituted water by FJP-T for 12 and 6 weeks, respectively. Glucose tolerance test (GTT) and insulin tolerance test (ITT) were done. Serum glucose and plasma insulin were measured. Serum adiponectin and leptin were dosed. Liver and adipose tissues epididymal (EAT), retroperitoneal (RAT) and mesenteric (MAT) were collected and weighted. Fat liver was measured by Bligh; Dyer (1959). Liver aliquots were fixed in buffered formalin and forwarded by histological analysis. Total pancreatic islets insulin content and glucose stimulated insulin secretion were measured by radioimmune assay (RIA). Liver and plasma antioxidant potential was measured by FRAP and TBARS assay. Total GSH and antioxidant enzymes (GPx, GR, SOD and CAT) were measured in plasma and liver. Results were submitted to ANOVA variance analysis and Tukey's test, with significance of 0.05. TBARS, GSH and CAT were positively modulated by jaboticaba peel supplementation, but FRAP, SOD, GPx and GR not respond to supplementation. Weight gain, visceral adiposity and some parameter related to insulin resistance were positively changed with 6 and 12 weeks of supplementation. Long term (12 weeks) supplementation increased HDL-cholesterol and reversed liver steatosis, showing that long term jaboticaba peel intake could promote better results. In conclusion, FJP and FJP-T have a great functional potential in prevent and treatment of oxidative damage, obesity insulin resistance and liver steatosis.

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1. INTRODUÇÃO E JUSTIFICATIVA

A obesidade aparece atualmente como uma epidemia mundial, especialmente nos países ocidentais e industrializados. Além dos aspectos psicossociais envolvidos neste processo patológico, a frequente associação da obesidade com complicações metabólicas e outras doenças como diabetes, hipertensão e doenças cardiovasculares preocupa pelo alto grau de morbidade e mortalidade (BRAY, 2004; OGLESBY et al., 2006).

A síndrome metabólica é descrita como um conjunto de anormalidades metabólicas e hemodinâmicas, sendo fortemente associada à obesidade. A resistência à insulina (RI) é vista atualmente como o elo entre a obesidade de distribuição central, intolerância à glicose, hipertensão arterial, dislipidemia, distúrbios da coagulação, hiperuricemia e microalbuminúria, os quais são fatores integrantes da síndrome metabólica. (SONNENBERG; KRAKOWER; KISSEBAH, 2004). As mudanças metabólicas decorrentes da obesidade e síndrome metabólica associam-se ainda a um estado de estresse oxidativo, por meio da produção excessiva de citocinas pró-inflamatórias (POULOS; HAUSMAN; HAUSMAN, 2010; WELLEN; HOTAMISLIGIL, 2005).

A adiposidade visceral e resistência à insulina desempenham um papel-chave inicial para o aparecimento e perpetuação de esteatose hepática, caracterizada pelo excessivo acúmulo de gordura no fígado. A doença hepática gordurosa não alcoólica (DHGNA) é uma condição clínico-histopatológica caracterizada pela esteatose, associada ou não a processo inflamatório (KRAWCZYK; BONFRATE; PORTINCASA, 2010).

Alguns caminhos têm sido apontados para que se entenda a relação entre os fatores dietéticos e o surgimento e progressão da síndrome metabólica e DHGNA. Estudos têm mostrado que dietas hipercalóricas / hiperlipídicas e ricas em frutose são capazes de induzir a obesidade, síndrome metabólica e estresse oxidativo (DELBOSC et al., 2005; KELLEY; ALLAN; AZHAR, 2004; RAINS; JAIN, 2011). Atualmente entende-se que o excesso de peso por si só, bem como a excessiva ingestão energética, não implicam diretamente no acúmulo de gordura no fígado. A composição de macronutrientes da dieta parece modular a DHGNA mesmo sem perda de peso (ZELBER-SAGI et al., 2007). Em modelos animais, as dietas com elevado teor de frutose induzem aspectos da síndrome metabólica, incluindo ganho de peso, resistência à insulina, hipertrigliceridemia, hipertensão e esteatose hepática (SANCHEZ-LOZADA et al., 2007).

Os compostos fenólicos representam uma importante classe de substâncias orgânicas presentes nos vegetais. O grupo fenol é uma característica química comum a todos os compostos fenólicos, porém, existem importantes diferenças estruturais e funcionais entre as substâncias pertencentes a esse grupo, sendo assim, há uma divisão em três classes de compostos fenólicos: flavonoides, ácidos fenólicos e taninos (GOBBO-NETO; LOPES, 2007; TAPAS; SAKARKAR; KAKDE, 2008). As antocianinas são compostos fenólicos da classe dos flavonoides. De origem grega, a palavra antocianina deriva de *anthos* (flor) e *kyanos* (azul). Essas substâncias são responsáveis pelas cores azul, violeta, vermelho, roxo, magenta e laranja de diversos vegetais. Pigmentos antocianínicos estão presentes em muitos alimentos da dieta humana, como uva, maçã, feijão preto, morango, amora, jabuticaba, cerejas, açaí e jambolão, bem como os sucos, geléias, licores e vinhos preparados a partir desses alimentos (BOBBIO et al, 2000; BRITO et al, 2007).

Nos últimos anos o interesse da comunidade científica pelos efeitos fisiológicos das antocianinas se intensificou, tendo em vista que diversos estudos demonstraram um grande potencial dessas substâncias atuarem como compostos bioativos benéficos à saúde (CROZIER et al., 2009; TAPAS et al., 2008). A propriedade mais comumente atribuída às antocianinas é a sua capacidade de redução do dano oxidativo, a qual está intimamente relacionada com inúmeros efeitos benéficos ao organismo, como proteção contra o aparecimento e a progressão de certos tipos de cânceres, proteção contra as doenças cardiovasculares e diabetes, atividade anti-inflamatória e de modulação da resposta imune, além da proteção contra distúrbios neuronais e doenças neurodegenerativas relacionadas ao envelhecimento (DORNAS et al, 2007). Além da atividade antioxidante, atribui-se as antocianinas a capacidade de redução da obesidade, da resistência à insulina (RI) e melhora do perfil lipídico (PRIOR et al., 2008).

A jabuticabeira (*Myrciaria spp.*) é uma árvore frutífera que pode ser encontrada na região que vai desde o Pará até o Rio Grande do Sul, tendo maior ocorrência e produtividade nos estados da região Sudeste. O fruto da jabuticabeira, a jabuticaba, possui forma globosa, tamanho pequeno, e polpa macia, esbranquiçada, mucilagínosa e de sabor sub-ácido e intensamente doce (DONADIO, 2000; MATTOS, 1983). Alguns efeitos medicinais são atribuídos a esta fruta há muitas décadas, incluindo ação antiasmática da polpa e ação contra diarreia e irritações cutâneas para a casca (LIMA et al., 2008). Estudos recentes detectaram alguns compostos fenólicos de interesse para a saúde em elevadas concentrações

na casca de jabuticaba. LEITE et al. (2011) identificaram altas concentrações de duas antocianinas na casca de jabuticaba: cianidina-3-O-glicosídeo e delphinidina-3-O-glicosídeo. Estudos subsequentes confirmaram a presença dessas antocianinas e identificaram outros compostos, dentre os quais se destacam o ácido gálico, ácido elágico e taninos (ABE; LAJOLO; GENOVESE, 2012; ALEZANDRO; DUBE; et al., 2013; WU et al., 2013).

A alta perecibilidade e sazonalidade tornam o consumo da jabuticaba esporádico, além disso, quando consumidas *in natura*, muitas vezes a casca é descartada. A elaboração de produtos como sucos, licores, vinagres, geleias, chás e compotas podem viabilizar o consumo da jabuticaba por períodos mais longos, bem como, quando preparadas utilizando a casca, extrair e preservar os compostos bioativos da fruta (REYNERTSON et al., 2006; SANTOS, VEGGI e MEIRELES, 2010; CLERICI e CARVALHO-SILVA, 2011). Além dos compostos fenólicos normalmente presentes em infusões de folhas e flores, chás de frutas são abundantes em pigmentos, como as antocianinas, derivados principalmente das cascas (PILJAC-ŽEGARAC et al., 2010). O chá da casca de jabuticaba é ainda um produto não explorado com potenciais efeitos positivos na saúde. Assim, o estudo dos compostos bioativos e dos efeitos biológicos da casca de jabuticaba e do chá da jabuticaba mostra-se como um caminho para elucidação dos potenciais terapêuticos dessa fruta, bem como valorização e estímulo do seu consumo pela população.

Nesse sentido, o objetivo do presente trabalho foi identificar os compostos bioativos presentes na casca de jabuticaba liofilizada e no chá da casca de jabuticaba, avaliar o potencial antioxidante *in vitro* desses produtos, bem como avaliar a capacidade antioxidante e o efeito em parâmetros de síndrome metabólica desses produtos em modelo animal de obesidade induzida por dieta.

2. REVISÃO BIBLIOGRÁFICA

2.1 Obesidade: complicações metabólicas relacionadas ao acúmulo de gordura tecidual

A obesidade aparece atualmente como uma epidemia mundial, especialmente nos países ocidentais e industrializados. Além dos aspectos psicossociais envolvidos neste processo patológico, a frequente associação da obesidade com outras doenças como diabetes, hipertensão e doenças cardiovasculares preocupa pelo alto grau de morbidade e mortalidade (BRAY, 2004; OGLESBY et al., 2006).

Define-se por obesidade o excessivo acúmulo de gordura nos adipócitos, geralmente associado à presença de estado inflamatório e a resistência periférica à insulina. O aumento do tecido adiposo é acompanhado de uma maior expressão de citocinas inflamatórias, como o TNF- α . Essas citocinas possuem a capacidade de inibir a sinalização celular da insulina, além de prejudicar a funcionalidade das células β -pancreáticas, responsáveis pela produção e liberação deste hormônio, levando a um quadro de resistência à insulina e, posteriormente, diabetes melitos tipo 2 (BRAY, 2006; WELLEN; HOTAMISLIGIL, 2005).

A hipertrofia do tecido adiposo, como ocorre na obesidade, particularmente na região abdominal, é associado à resistência à insulina (RI), hiperglicemia, dislipidemias, hipertensão e estados pró-trombóticos e inflamatórios. O estado inflamatório sub-clínico, comumente encontrado em indivíduos obesos associado ao aumento na secreção de adipocinas interferem negativamente na via de sinalização da insulina, ocasionando e instalando o quadro de RI (POULOS; HAUSMAN; HAUSMAN, 2010; WELLEN; HOTAMISLIGIL, 2005).

Anteriormente, pensava-se que o tecido adiposo tinha como única função, a manutenção de uma reserva energética, contudo as descobertas recentes têm rompido com esse conceito. Os adipócitos são responsáveis pela síntese e secreção de substâncias com atividades biológicas importantes, conhecidas como adipocinas, que incluem o TNF- α , interleucinas, leptina, adiponectina, resistina, entre outras, o que confere a esse tecido importante função endócrina. Tais substâncias atuam na regulação do metabolismo energético, funções neuroendócrinas, resposta inflamatória e sistema imune e, com exceção

da adiponectina, tem sua produção aumentada na presença da obesidade (GALIC; OAKHILL; STEINBERG, 2010; HAUNER, 2004; OGLESBY et al., 2006).

Entre as adipocinas de maior impacto sobre obesidade e síndrome metabólica estão a leptina e adiponectina. A leptina (do grego *Leptos* – magro) é uma proteína codificada pelo gene *Ob* descoberta em 1994 em estudo com camundongos obesos. Essa adipocina é produzida pelo tecido adiposo, proporcionalmente à gordura estocada, e é responsável pela regulação do apetite e aumento do gasto energético. Porém, já se sabe que indivíduos obesos apresentam resistência à ação da leptina e consequente prejuízo na regulação do apetite e gasto energético (LAGO et al., 2009). Contrária às demais adipocinas, a adiponectina possui sua expressão e secreção diminuídas em resposta ao excesso de tecido adiposo. Essa adipocina possui importantes efeitos na prevenção e tratamento da obesidade e síndrome metabólica, tais como anti-aterogênese, melhora da sensibilidade à insulina e redução do processo inflamatório. Além desses efeitos, são atribuídos à adiponectina aumento da oxidação de lipídios no músculo esquelético e diminuição da produção de glicose hepática (POULOS et al., 2010).

O excesso de tecido adiposo é acompanhado por importantes modificações de perfil lipídico que estão intimamente associadas ao desenvolvimento de doenças cardiovasculares, como aterosclerose. O aumento da adiposidade resulta em uma maior liberação de ácidos graxos livres (AGL), devido ao aumento na taxa de lipólise. Esses AGL estimulam a deposição de triglicerídeos em tecidos periféricos, sendo a causa mais provável para o desenvolvimento de RI em fígado e músculos. A resistência à insulina presente nesses tecidos desencadeia diversas alterações metabólicas, como hiperglicemia, hiperinsulinemia, elevação dos níveis de triglicerídeos e LDL colesterol, além de diminuição nos níveis de HDL colesterol (MESHKANI; ADELI, 2009). A influência do estresse oxidativo no processo aterosclerótico e na resistência à insulina tem sido estudada. Ainda não está claro se o estresse oxidativo apareceria como causa ou consequência das alterações metabólicas da obesidade, contudo, sabe-se que há uma produção exacerbada de radicais livres e aumento da resposta inflamatória nos indivíduos obesos. O papel dos AGL na ativação da enzima NADPH oxidase e o estímulo à produção de citocinas pró-inflamatórias são apontados como possíveis mecanismos da relação entre estresse oxidativo e obesidade (HUANG et al., 2015).

A Síndrome metabólica (SM) representa um grupo de fatores de risco cardiometabólico que inclui a obesidade abdominal combinada com três ou mais dos

seguintes fatores: hiperglicemia, hipertrigliceridemia, hiperinsulinemia, redução dos níveis de HDL colesterol e hipertensão. As modificações metabólicas oriundas do excesso de tecido adiposo são apontadas como fatores chave no surgimento e progressão da síndrome metabólica, a qual configura importante fator de risco cardiovascular e de diabetes mellitus tipo 2. A anormalidade central associada à síndrome metabólica parece ser a resistência dos tecidos periféricos à insulina, sendo esta a base etiopatogênica ou fisiopatológica para o excessivo acúmulo de gordura no tecido hepático (CAVALHEIRA; SAAD, 2006).

A doença hepática gordurosa não alcoólica (DHGNA) é vista como uma manifestação precoce da obesidade e síndrome metabólica, sendo a causa mais comum de doença hepática crônica em todo o mundo. A DHGNA é uma condição clínico-histopatológica caracterizada pelo acúmulo de gordura nos hepatócitos, associada ou não a processo inflamatório. A adiposidade visceral e resistência à insulina desempenham um papel-chave inicial para o aparecimento e perpetuação de esteatose hepática (KRAWCZYK; BONFRATE; PORTINCASA, 2010). A esteatose hepática é caracterizada como a forma menos progressiva da DHGNA, a qual pode evoluir para a esteato-hepatite não alcoólica (NASH) com maior potencial de evoluir para cirrose e carcinoma hepatocelular (NOBILI; CARTER-KENT; FELDSTEIN, 2011). A DHGNA é multifatorial, de amplo espectro e tem potencial evolutivo. Além disso, seu desenvolvimento está intimamente associado às modificações metabólicas decorrentes da obesidade, tais como estresse oxidativo, produção de endotoxinas no intestino, aumento na secreção de citocinas inflamatórias e resistência à insulina (CAVE et al., 2007).

2.1.1 Fatores dietéticos no surgimento e progressão de obesidade e suas complicações

Alguns caminhos têm sido apontados para que se entenda a relação entre os fatores dietéticos e o surgimento e progressão da síndrome metabólica e DGHNA. Estudos têm mostrado que dietas hipercalóricas / hiperlipídicas e ricas em frutose são capazes de induzir a obesidade, síndrome metabólica e estresse oxidativo (DELBOSC et al., 2005; KELLEY; ALLAN; AZHAR, 2004; RAINS; JAIN, 2011). A produção excessiva de espécies reativas de oxigênio é apontada como um dos mecanismos chaves no desenvolvimento da obesidade e outros distúrbios metabólicos, como a resistência à insulina (RI) e diabetes e DGHNA (CAVE et al., 2007). Segundo FEILLET-COUDRAY et al. (2009) em animais obesos há uma super produção de espécies reativas de oxigênio, e esse dano oxidativo

excessivo é um importante iniciador da patogênese do diabetes e outras complicações. De acordo com ANDO; FUJITA (2009) a indução da obesidade por meio de dieta hiperlipídica em ratos aumenta o estresse oxidativo, e o mecanismo ao qual se atribui tal efeito é a produção de adipocinas, como o Fator de Necrose Tumoral Alfa (TNF- α) e interleucina-6 (IL-6), pelos adipócitos. A elevada produção de Espécies Reativas de Oxigênio e Nitrogênio (ERONs) pode também induzir um quadro de resistência à insulina e o consumo de drogas antioxidantes demonstram melhorar a RI.

Atualmente entende-se que o excesso de peso por si só, bem como a excessiva ingestão energética, não implicam diretamente no acúmulo de gordura no fígado. A composição de macronutrientes da dieta parece modular a DHGNA mesmo sem perda de peso (ZELBER-SAGI et al., 2007). O efeito dos lipídios dietéticos na DGHNA é ainda incerto, estudos apontam o excesso de colesterol e de gordura saturada como um importante fator predisponente, enquanto outros mostram que os ácidos graxos de cadeia longa e o aumento da relação ômega 6 / ômega 3 teriam maior impacto no acúmulo hepático de gordura (CORTEZ-PINTO et al., 2006). Contudo, sabe-se que o consumo elevado de gordura saturada está relacionado ao aumento dos fatores inflamatórios e pior evolução do quadro hepático, geralmente com esteato-hepatite e níveis maiores de fibrose e que a ingestão excessiva de colesterol acarreta aumento da síntese de oxycolesterol, ativando vias de sinalização que estimulam a síntese *de novo* de ácidos graxos (YASUTAKE et al., 2012).

Expressiva ênfase tem sido dada a possível relação entre a maior ingestão de frutose e síndrome metabólica e DHGNA. O aumento substancial da ingestão de frutose, um adoçante muito utilizado pela indústria de alimentos, principalmente em refrigerantes e bebidas açucaradas, tem sido apontado como responsável pela maior prevalência de síndrome metabólica e DHGNA em adultos e crianças em estudos populacionais (VARTANIAN; SCHWARTZ; BROWNELL, 2007). Ainda não há um consenso sobre a quantidade de frutose poderia ser consumida de forma segura. Em modelos animais, as dietas com elevado teor de frutose induzem aspectos da síndrome metabólica, incluindo ganho de peso, resistência à insulina, hipertrigliceridemia e hipertensão (SANCHEZ-LOZADA et al., 2007). Em estudo com humanos acima do peso, foi avaliado o efeito metabólico do consumo diário de refrigerante de cola, leite ou bebida sem açúcar durante 6 meses. Observou-se que os indivíduos que consumiram a bebida açucarada de cola apresentaram esteatose hepática,

hipercolesterolemia, hipertrigliceridemia e acúmulo de gordura corporal (MAERSK et al, 2012).

Diante do impacto prejudicial que as dietas excessivas em calorias, lipídios e frutose possuem no surgimento e progressão da obesidade e síndrome metabólica, bem como da relação desses distúrbios com o processo de estresse oxidativo, faz-se necessário encontrar alternativas nutricionais capazes de auxiliar no combate aos prejuízos causados por tais dietas, de modo a evitar o surgimento e progressão da obesidade e doenças crônicas não transmissíveis (DCNT).

2.2 Compostos fenólicos: características e potencial terapêutico

Diversos processos fisiológicos são responsáveis pela formação de espécies reativas de oxigênio e nitrogênio, tais como a respiração celular e as reações de oxidação. Essas espécies reativas, ou radicais livres, são imprescindíveis a processos celulares, como a fagocitose, porém, quando produzidas em excesso, podem ocasionar danos ao organismo, contribuindo para o desenvolvimento de processos inflamatórios, aterosclerose e outras alterações metabólicas (SIKORA et al., 2008). Para combater os possíveis danos causados pela excessiva produção de radicais livres, os organismos possuem mecanismos antioxidantes enzimáticos, como a glutatona e as enzimas glutatona peroxidase e redutase, superóxido dismutase (SOD) e catalase (CAT), além de contar com a ação antioxidante de alguns componentes dietéticos, como o ácido ascórbico, o tocoferol, os carotenoides e os compostos fenólicos, os quais representam a principal classe de antioxidantes dietéticos (MCLEAN et al., 2005). O potencial antioxidante dos compostos fenólicos associado à alta disponibilidade de alimentos fontes de tais compostos estimulou pesquisas a cerca dos efeitos benéficos de dietas ricas em compostos fenólicos (CROZIER; JAGANATH; CLIFFORD, 2009)

Resultantes do metabolismo da via dos fenilpropanóides dos tecidos vegetais, os compostos fenólicos são encontrados em folhas, frutos e flores na forma de glicosídeos ou agliconas. Fatores ambientais, como radiação solar, nutrição, pluviosidade, estação do ano, e ainda a presença de poluentes, podem influenciar o metabolismo e a produção desses compostos e, consequentemente, sua concentração final no vegetal (GOBBO-NETO; LOPES, 2007). O grupo fenol é uma característica química comum a todos os compostos fenólicos, porém, existem importantes diferenças estruturais e funcionais entre as substâncias pertencentes a esse grupo, sendo assim, há uma divisão em três classes de compostos

fenólicos: flavonoides, ácidos fenólicos e taninos (CROZIER; JAGANATH; CLIFFORD, 2009; TAPAS; SAKARKAR; KAKDE, 2008).

As antocianinas são classificadas quimicamente como flavonóides devido a sua estrutura carbônica característica ($C_3-C_6-C_3$). De origem grega, a palavra antocianina deriva de *anthos* (flor) e *kyanos* (azul). Essas substâncias são responsáveis pelas cores azul, violeta, vermelho, roxo, magenta e laranja de diversos vegetais. Pigmentos antocianínicos estão presentes em muitos alimentos da dieta humana, como uva, maçã, feijão preto, morango, amora, jabuticaba, cerejas, açaí e jambolão, bem como os sucos, geléias, licores e vinhos preparados a partir desses alimentos (BOBBIO et al, 2000; BRITO et al, 2007).

Alimentos e bebidas ricos em flavonóides têm sido associados à redução de risco de doenças relacionadas ao envelhecimento, devido a sua potente ação antioxidante, sendo importantes na remoção de ERONs. Além disso, eles são capazes de formar quelatos com íons metálicos, diminuindo a atividade pró-oxidante de metais como ferro e cobre (HALLIWELL; RAFTER; JENNER, 2005). Compostos polifenólicos, bem como as antocianinas, possuem efetiva capacidade sequestradora de radicais livres. Essa propriedade é influenciada pelo número e posição dos grupos OH, assim como pelas posições de glicosilação. Os compostos polifenólicos se diferenciam de outros antioxidantes, como ácido ascórbico e α -tocoferol, que agem em meio aquoso e na camada fosfolipídica, respectivamente, por sua capacidade de agir em ambas as fases. Os polifenóis são capazes de captar diversos radicais como a alcóxila ($RO\bullet$), alquilperóxila ($ROO\bullet$), superóxido ($O_2 \bullet^-$), radical hidroxila ($HO\bullet$), óxido nítrico ($NO\bullet$) (CERQUEIRA; MEDEIROS; AUGUSTO, 2007).

Nos últimos anos o interesse da comunidade científica pelos efeitos fisiológicos das antocianinas se intensificou, tendo em vista que diversos estudos demonstraram um grande potencial dessas substâncias como compostos bioativos benéficos à saúde (CROZIER et al., 2009; TAPAS et al., 2008). A propriedade mais comumente atribuída às antocianinas é a sua capacidade de redução do dano oxidativo, a qual está intimamente relacionada com inúmeros efeitos benéficos ao organismo, como proteção contra o aparecimento e a progressão de certos tipos de cânceres, proteção contra as doenças cardiovasculares e diabetes, atividade anti-inflamatória e de modulação da resposta imune, além da proteção contra distúrbios neuronais e doenças neurodegenerativas relacionadas ao envelhecimento (DORNAS et al, 2007). Além da atividade antioxidante, atribui-se as antocianinas a

capacidade de redução da obesidade, da resistência à insulina (RI) e melhora do perfil lipídico (PRIOR et al., 2008).

A ingestão regular e por longo prazo de compostos fenólicos contribui para a defesa do organismo e está relacionada com a prevenção de doenças crônicas não transmissíveis. Estudos epidemiológicos sugerem que uma dieta rica em vegetais tem efeito benéfico contra vários tipos de câncer e sugerem que tal efeito estaria relacionado com o potencial antioxidante e anti-inflamatório destes compostos (HOLLMAN; FESKENS; KATAN, 1999).

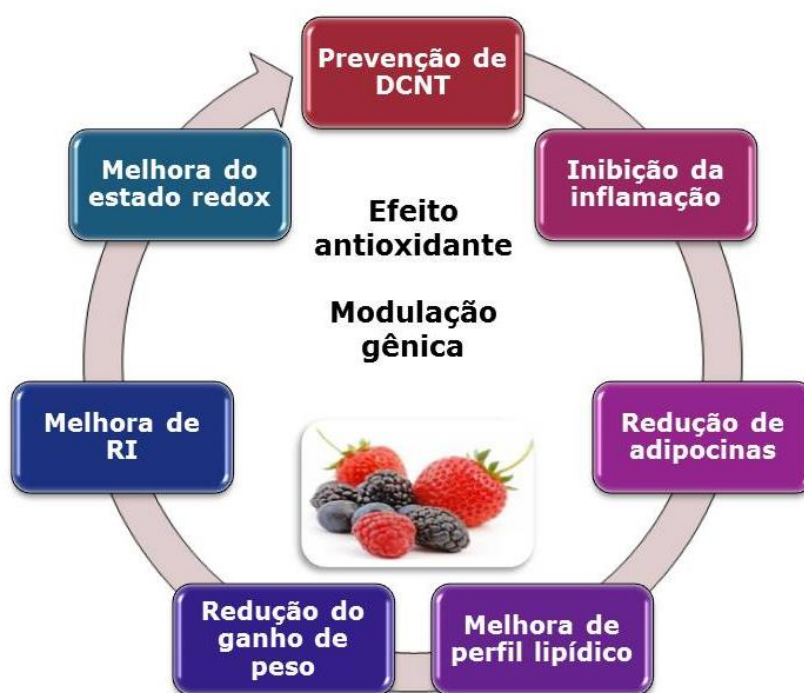


Figura 1. Efeitos das antocianinas em parâmetros relacionados à obesidade e prevenção de doenças crônicas não transmissíveis (DCNT).

A atividade antioxidante de antocianinas de vinho tinto envelhecido foi descrita por TEDESCO et al. (2001) em um modelo *in vitro* de eritrócitos humanos cuja atividade da enzima catalase havia sido inibida por ácido sódio. Nesse modelo experimental, as antocianinas foram capazes de elevar a atividade da enzima catalase, bem como reduzir a formação de metahemoglobina. HAN et al. (2007) avaliaram os efeitos do consumo de *red potato flakes* (RPF) em ratos machos da linhagem F344/DuCrj, que receberam dieta AIN-93G modificada, enriquecida com 25% de RPF por 4 semanas. Os animais tratados mostraram diminuição nos níveis de TBARS séricos e maior expressão hepática da enzima SOD. Dessa

forma, sugere-se que as antocianinas possuem efeito positivo sobre a inibição da peroxidação lipídica sérica e no sistema antioxidante enzimático, podendo aumentar o potencial antioxidante em diferentes modelos experimentais.

Efeitos benéficos no desenvolvimento da obesidade e síndrome metabólica em modelos animais foram encontrados com o consumo de antocianinas. Diminuição no ganho de peso, diminuição nos níveis séricos de insulina, normalização da intolerância à glicose e diminuição dos lipídios hepáticos são alguns dos principais efeitos apontados. Melhoras nos níveis plasmáticos de colesterol e na deposição hepática de gordura também são apontados como efeitos das antocianinas. Além disso, regulação do processo inflamatório parece ser um dos mecanismos de controle da obesidade por meio das antocianinas. Portanto, a dieta rica em antocianinas parece reduzir o risco de obesidade e diabetes tipo 2 (JAYAPRAKASAM et al., 2006; PRIOR et al., 2010; TAKIKAWA et al., 2010; TSUDA et al., 2003).

O efeito das antocianinas de cereja foi testado em ratos com obesidade e síndrome metabólica induzida por dieta. Durante 90 dias, os ratos foram alimentados com uma dieta hiperlipídica suplementada com 1% de cereja liofilizada. A ingestão de cereja foi associada com redução da hiperlipidemia, do percentual de massa gorda, gordura abdominal e citocinas inflamatórias (IL-6 e TNF- α), além de outros marcadores inflamatórios. Assim, as antocianinas da cereja foram capazes de auxiliar na reversão da obesidade e síndrome metabólica, reduzindo o risco para o desenvolvimento de diabetes do tipo 2 e doenças cardiovasculares (SEYMOUR et al., 2009).

SUWANNAPHET et al. (2010) mostraram em estudo com ratos alimentados com dieta rica em frutose suplementada com 1% de sementes de uva, que tais sementes, ricas em compostos fenólicos, podem exercer papel preventivo no estresse oxidativo e resistência à insulina, por meio da ativação da enzima superóxido dismutase no fígado dos animais, além de melhora de hiperglicemia e hiperinsulinemia. Resultados similares foram encontrados por ABDUL KADIR; RAHMAT; JAAFAR (2015) em ratos com obesidade induzida por dieta hiperlipídica e suplementados com um extrato de tamarillo, um fruto com polpa e casca ricas em compostos fenólicos e antocianinas. O tratamento com o extrato de tamarillo levou a uma diminuição significativa no colesterol total e aumento significativo nos níveis de HDL-C, além de uma tendência de redução positiva dos níveis de glicemia, triglicérides e LDL-C. Houve ainda melhora aumento da atividade de superóxido dismutase (SOD) e glutational

peroxidase (GPx), bem como da capacidade antioxidante total. O processo inflamatório mediado por TNF- α e IL-6 foi positivamente modulado.

As evidências de que os compostos fenólicos, em especial as antocianinas, podem exercer efeitos benéficos para a prevenção e tratamento do estresse oxidativo, obesidade e síndrome metabólica são muitas. Assim, o estudo de alimentos fonte desses compostos é de suma importância a fim de se entender os mecanismos, bem como estabelecer dose e, futuramente, utilizar esses alimentos como estratégia dietética na prevenção e tratamento de doenças metabólicas.

2.3 Jabuticaba, a berrie brasileira

Nativa do Brasil, a jabuticabeira (*Myrciaria spp.*) é uma árvore frutífera que pode ser encontrada na faixa que vai desde o Pará até o Rio Grande do Sul, tendo maior ocorrência e produtividade nos estados da região Sudeste. Seu cultivo é basicamente doméstico, em pequenas propriedades rurais, como chácaras, sítios e fazendas (SATO; CUNHA, 2007). Dentre as espécies atualmente conhecidas, destaca-se a *Myrciaria cauliflora* (DC) Berg (jabuticaba Paulista ou Açu) e a *Myrciaria jabuticaba* (Vell) Berg (jabuticaba Sabará), com a última ocupando a maior área cultivada no Brasil. O fruto da jabuticabeira possui forma globosa, tamanho pequeno, e polpa macia, esbranquiçada, mucilagínosa e de sabor sub-ácido e intensamente doce (DONADIO, 2000; MATTOS, 1983). Apesar dos estudos que buscam evidenciar o potencial para saúde da casca da jabuticaba serem recentes, efeitos medicinais são atribuídos a esta fruta há muitas décadas. Tais efeitos incluem ação antiasmática da polpa, além de ação contra diarreia e irritações cutâneas para sua casca (LIMA et al., 2008).

Do ponto de vista nutricional, a jabuticaba é um fruto bastante interessante. Sua polpa apresenta quantidades consideráveis de vitamina C e minerais, com destaque para ferro, cálcio, fósforo e potássio (OLIVEIRA et al, 2003). Quanto à casca, embora esta seja comumente desprezada, possui elevados teores de minerais e fibras solúveis e insolúveis. Além disso, a casca de jabuticaba apresenta altos níveis de compostos fenólicos totais, cerca de 2,70 g/100g na variedade Paulista e 1,89 g/100 g na Sabará, de acordo com Lima et al (2008). As antocianinas presentes nesta fração da fruta também aparecem em quantidades consideráveis. Segundo Terci (2004) o teor de antocianinas da casca, obtido por três diferentes métodos espectrofotométricos, variou entre 310 e 315 mg de antocianinas por 100 g de fruta. Favaro (2008) também determinou o teor de antocianinas da casca de jabuticaba,

utilizando métodos espectrofotométricos e CLAE, encontrando valores de 29,1 a 40,0 mg de antocianinas por 100 g de fruta.

Estudos recentes detectaram alguns compostos fenólicos de interesse para a saúde em elevadas concentrações na casca de jabuticaba. LEITE et al. (2011) identificaram altas concentrações de duas antocianinas na casca de jabuticaba: cianidina-3-O-glicosídeo e delphinidina-3-O-glicosídeo. Estudos subsequentes confirmaram a presença dessas antocianinas e identificaram outros compostos, dentre os quais se destacam o ácido gálico, ácido elágico e taninos (ABE; LAJOLO; GENOVESE, 2012; ALEZANDRO; DUBE; et al., 2013; WU et al., 2013).

A casca de jabuticaba pode ser interessante no controle de parâmetros de saúde em modelos animais de estresse oxidativo e obesidade. LENQUISTE et al. (2012) demonstraram que a casca de jabuticaba liofilizada (CJL) adicionada a dieta hiperlipídica oferecida a ratos *Sprague-Dawley* nas concentrações de 1, 2 e 4% reduziu a hiperinsulinemia e o índice de HOMA-IR, atuando positivamente sobre a resistência à insulina. Além disso, os animais alimentados com 2% da CJL mostraram maiores níveis de HDL-colesterol, possivelmente devido a redução de LDL-colesterol. Desse modo, é possível que a CJL atue positivamente sobre a RI e o perfil lipídico em animais experimentais.

ALEZANDRO; GRANATO; GENOVESE (2013) avaliaram o efeito do consumo de um pó da casca de jabuticaba disperso em água em ratos com diabetes induzida por streptozotocina em alguns aspectos de saúde e observaram significativas melhoras nos níveis séricos de colesterol e triglicerídeos, além de expressivo aumento na atividade antioxidante no plasma (ensaio FRAP) e de enzimas antioxidante (SOD, CAT e GPx) em alguns tecidos, como cérebro e rins. Além disso, demonstraram uma significativa atividade inibidora *in vitro* de enzimas α -amilase e α -glucosidase nesses animais, associando essa inibição enzimática a possíveis efeitos anti-obesogênicos da casca de jabuticaba.

Em estudo com ratos com obesidade induzido por dieta hiperlipídica, suplementada com 1, 2 e 4% de casca de jabuticaba liofilizada, BATISTA et al (2013) observaram aumento da excreção de lipídeos e triglicerídeos fecais, além de diminuição na peroxidação lipídica nos fígados dos ratos alimentados com 2% de casca de jabuticaba liofilizada. Em estudo subsequente, utilizando o mesmo modelo experimental, esses autores observaram melhora da capacidade antioxidante e redução da peroxidação lipídica no fígado e

cérebro, além de redução nos níveis de ácidos graxos saturados circulantes nos animais alimentados com casca de jabuticaba liofilizada (BATISTA et al., 2014).

Camundongos alimentados com dieta hiperlipídica (35% de banha suína) quatro semanas e posteriormente adicionada de 1, 2 ou 4% de casca de jabuticaba liofilizada por mais seis semanas mostraram expressiva melhora da resistência à insulina no teste intraperitoneal de tolerância à insulina (ITT) além de modulação positiva de proteínas da via de sinalização de insulina no tecido adiposo. Adicionalmente, esses animais apresentaram melhora no processo inflamatório hepático, evidenciada pela redução de interleucinas inflamatórias, evidenciando o efeito benéfico da casca de jabuticaba liofilizada na obesidade, resistência à insulina e inflamação (DRAGANO et al., 2013).

Recentemente, ARAUJO et al. (2014) mostraram o efeito hipolipemiante de uma farinha da casca de jabuticaba adicionada, nas concentrações de 7, 10 e 15%, a uma dieta moderadamente hiperlipídica (7% de banha de porco) oferecida a ratos *Wistar*. O consumo da casca de jabuticaba reduziu os níveis séricos de colesterol e triglicerídeos totais dos animais, independente da concentração oferecida. Na concentração de 15%, a casca de jabuticaba foi capaz ainda de aumentar o HDL-colesterol e diminuir a glicose sérica. Os autores atribuem tais efeitos à presença de compostos fenólicos e fibras na farinha da casca de jabuticaba.

Embora bastante popular em todo o país, a jabuticaba tem seu comércio limitado devido à alta perecibilidade, provocada principalmente pelo elevado teor de água e açúcares na polpa. Essa limitação comercial, além de reduzir a quantidade produzida, compromete a qualidade e valor comercial do fruto (DONADIO, 1993; BARROS, FINGER e MAGALHAES, 1996). A alta perecibilidade e sazonalidade tornam o consumo da jabuticaba esporádico, além disso, quando consumidas *in natura*, muitas vezes a casca é descartada. A elaboração de produtos como sucos, licores, vinagres, geléias, chás e compotas podem viabilizar o consumo da jabuticaba por períodos mais longos, bem como, quando preparadas utilizando a casca, extrair e preservar os compostos bioativos da fruta (REYNERTSON et al., 2006; SANTOS, VEGGI e MEIRELES, 2010; CLERICI e CARVALHO-SILVA, 2011). As antocianinas apresentam elevada solubilidade em água e, estudos tem mostrado que o aquecimento pode aumentar a extração desses compostos, por meio da quebra de ligações de hidrogênio. Portanto, o preparo de uma infusão pode otimizar a extração das antocianinas da casca de jabuticaba (AZSMIR et al, 2013).

O chá, obtido através da infusão aquosa de uma porção do vegetal, é a segunda bebida mais consumida no mundo (WANG, PROVAN e HELLIWELL, 2000; PILJAC-ŽEGARAC et al., 2010). Além dos compostos fenólicos normalmente presentes em infusões de folhas e flores, chás de frutas são abundantes em pigmentos, como as antocianinas, derivados principalmente das cascas (PILJAC-ŽEGARAC et al., 2010). O chá da casca de jabuticaba é ainda um produto não explorado com potenciais efeitos positivos na saúde. Assim, o estudo dos compostos bioativos e dos efeitos biológicos da casca de jabuticaba e do chá da jabuticaba mostra-se como um caminho para elucidação dos potenciais terapêuticos dessa fruta, bem como valorização e estímulo do seu consumo pela população.

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CAPÍTULO 1

Jaboticaba peel and jaboticaba peel aqueous extract shows *in vitro* and *in vivo* antioxidant properties in obesity model.

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ABSTRACT

This study evaluated the chemical composition of freeze-dried jaboticaba peel (FJP) and jaboticaba peel aqueous extract (JE) and their antioxidant capacity *in vitro* and *in vivo* in obesity model. Phenolic compounds, total anthocyanins, flavonoids and antioxidant capacity (DPPH, FRAP, ABTS and ORAC assays) in FJP methanolic extract and JE were measured. Specific phenolics (ellagic and gallic acids) and anthocyanin (cyanidin-3-*O*-glucoside) were measured by LC-DAD-ESI/MS. In the biological assay thirty-six *Wistar* rats were divided in six groups: AIN-93M normal control diet; HFF (obese control) feed a high-fat and fructose diet; Prevention FJP (P. FJP) and Treatment FJP (T. FJP) feed HFF diet with 2% of FJP powder, for 12 and 6 weeks respectively; Prevention JE (P. JE) and Treatment JE (T. JE) were feed with HFF diet and the water was substituted by JE, for 12 and 6 weeks, respectively. FRAP, TBARS, GSH and antioxidant enzymes (GPx, GR, CAT and SOD) were determined in the plasma and liver. The limit of significance was set at $P < 0.05$. The FJP methanolic extract showed higher levels of total phenolics and anthocyanins, ellagic acid and DPPH, FRAP and ORAC assay, but JE showed higher levels of cyanidin-3-*O*-glucoside, gallic acid and ABTS assay. Antioxidant potential of the FJP and JE were confirmed by important markers in animals, such as TBARS and GSH levels and CAT activity, but not by FRAP assay, and SOD, GR and GPx enzymes. Thus, FJP and JE showed an important antioxidant effect *in vitro* and *in vivo*.

Keywords: *Myrciaria Jaboticaba* (Vell.) Berg., obesity, oxidative stress, antioxidant enzymes, lipid peroxidation

1. INTRODUCTION

Excessive production of reactive oxygen species (ROS) is identified as one of the key mechanisms in the development of obesity and other metabolic disorders such as insulin resistance (IR) and diabetes (Feillet-Coudray et al, 2009; Ando & Fujita, 2009). The excess of reactive species can damage cell lipids, proteins and DNA by oxidative action, which might result in loss of function and even cellular death (Habib & Ibrahim, 2011), which has linked the oxidative stress to some diseases (Durackova, 2010).

Studies have shown that hypercaloric / high-fat diets can induce oxidative stress and metabolic disorders in obesity induced animal model by excessive production of reactive species and decrease in the antioxidant protection (Tsuchiya et al, 2013; Aschbacher et al, 2014). The combat against oxidative damage is endogenously mediated by enzymatic antioxidant system, compound for the enzymes superoxide dismutase (SOD), glutathione reductase (GR), glutathione peroxidase (GPx) and catalase (CAT), and non-enzymatic systems: thiol reduced (GSH), vitamins, minerals and polyphenols (Rezaie, Parker & Abdollahi, 2007).

Nutritional alternatives to counteract the oxidative damage are necessary, in order to avoid the appearance and progression of obesity and chronic diseases. The role of phytochemicals in the oxidative damage combat it has been studied (Han, Shen & Lou, 2007; Habib & Ibrahim, 2011). Phenolic compounds are the principal class of dietary phytochemical, naturally present in plants foods, especially fruits, and have important antioxidant potential/capacity (Prior et al, 2010).

The jaboticaba (*Myrciaria Jaboticaba* (Vell.) Berg), a Brazilian typical fruit ,has high nutritional value. Mainly, jaboticaba peel has a significant content of minerals, soluble and insoluble fiber, and phenolic compounds (Lima et al, 2008; Alezandro et al, 2013). Leite et al (2011) identified cyanidin-3-*O*-glucoside and delphinidin-3-*O*-glucoside as predominant anthocyanins in freeze-dried jaboticaba peel. In addition to anthocyanins, jaboticaba peel has high concentrations of tannins, gallic acid and ellagic acid that have important antioxidant effect (Wu et al, 2012; Wu, Long & Kennelly, 2013; Abe, Lajolo & Genovese, 2012).

Due to high bioactive compounds concentration, the *in vitro* and *in vivo* antioxidant potential of jaboticaba peel has been reported by several authors (Leite et al, 2012; Alezandro et al, 2013; Batista et al, 2014). In these studies, the jaboticaba peel showed elevated values of DPPH, FRAP, ABTS and ORAC assays, as well increases GSH and antioxidant enzymes in animal model. Thus, the objective of present study was to identify and quantify the bioactive compounds in the Freeze-dried Jaboticaba Peel (FJP) and in the FJP aqueous extract (JE) and to evaluate their antioxidant capacity by *in vitro* assays. Furthermore, we evaluated the antioxidant potential of the FJP and JE supplementation in animals with oxidative stress induced by high fat and high fructose diet.

2. METHODS

2.1. Preparation of Freeze-dried jaboticaba peel (FJP)

Jaboticabas (*Myrciaria Jaboticaba* (Vell.) Berg) were acquired in Campinas Central Supply (CEASA), Brazil. Fruits were selected and cleaned. The peels were manually separated and were frozen, freeze-dried and milled to get a homogeneous powder. The powder was stored at – 80 °C in dark flasks, prior to analysis and preparation for the *in vivo* assays.

2.2. Proximate centesimal composition of FJP

Analyses of humidity, total protein and ash were performed according to methods described by Association of Official Analytical Chemists (AOAC, 2002). Total lipids were determined by Bligh & Dyer (1959). Soluble and insoluble fiber were determinate according to ASP et al. (1983). Carbohydrates content was obtained by difference using the equation: $100 - (\text{moisture} + \text{protein} + \text{lipid} + \text{ash} + \text{dietary fiber})$ and energy value was determined in isoperibol automatic calorimeter (PARR 1261) with oxygen pump (PARR 1108).

2.3. FJP extracts preparation

FJP MeOH extract was prepared mixing 0.5 g of FJP powder with 12.5 mL of MeOH:H₂O (70:30) (v/v). After vortex-mixing, sample was sonicated for 10 minutes. Extracts were filtered on paper filter and re-extracted twice. Filtrates were mixed and filtered again on 0.45 µm filter. FJP aqueous extract (JE) were obtained by weighing 2.0 g FJP powder and 100 mL boiling water addition. The extract was maintained in infusion for 30 minutes and manually homogenized each 15 minutes. Afterward extract was filtered under vacuum pressure and stored at 4 °C until analysis.

For phenolic LC-DAD-ESI/MS analysis FJP powder (1.0 g) was mixed with 15 mL of MeOH:H₂O:Acetic acid (85:15:0.5) (v/v), vortex-mixed for 30 seconds and sonicated for 5 minutes. Samples were vortex-mixing for 30 seconds, two times, until complete 10 minutes. Samples were centrifuged at 3500 rpm for 10 min at 25 °C. The extraction process was repeated with 10 mL of the solution. Thereafter, extracts were combined and diluted to 25 mL with the same solvent. All samples were filtered through 0.45 µm filter. HPLC analyzes used the same aqueous extract as described above. Extracts were prepared in triplicate for all analyzes,

2.4. Total phenolic determination

Total phenolic content was determined using the Folin-Ciocalteu method as described by Singleton, Orthofer, Lamuela-Raventós et al (1999). Samples and a gallic acid standard curve were read at 725 nm. Results were expressed as mg gallic acid equivalent g^{-1} or mL^{-1} . Total flavonoid concentration was quantified using the colorimetric method described by Herald, Gadgil e Tilley (2012). Samples and catechin standard curve were read at 510 nm. Results were expressed as mg catechin equivalent g^{-1} or mL^{-1} . Total anthocyanin concentration was performed by the pH differential method described by Fuleki and Francis (1968), with some modifications. The samples were read at 300 and 700 nm. Results were expressed as mg 100g or mL^{-1} . Assays were performed in triplicate for all samples.

2.5. Phenolics compounds analysis by LC-DAD-ESI/MS

Cyanidin-3-*O*-glucoside identification and quantification was performed according to general procedure for screening of phenolics in plant materials with modifications (Lin & Harnly, 2007). LC-DAD-ESI/MS instrument consisted of a Varian 250 HPLC (Varian, CA) coupled with a diode array detector (DAD) and a 500-MS IT mass spectrometer (Varian, CA). A Symetry C18 (Varian Inc., Lake Forest, CA) column (3 μm , 250 x 2 mm) was used at a flow rate of 0.4 mL min^{-1} . The column oven temperature was set at 30 °C. Mobile phase consisted of a combination of A (0.1% formic acid in water) and B (0.1% formic acid in acetonitrile). Gradient was varied linearly from 10% to 26% B (v/v) in 40 min, to 65% B at 70 min, and finally to 100% B at 71 min and held at 100% B to 75 min. The DAD was set at 270 and 512 nm for real-time read-out and UV/VIS spectra, from 190 to 650 nm, were continuously collected. Mass spectra were simultaneously acquired using electrospray ionization in the positive and negative ionization modes (PI and NI) at a fragmentation voltage of 80 V for the mass range of 100–1000 amu. A drying gas pressure of 35 psi,

nebulizer gas pressure of 40 psi, a drying gas temperature of 370 °C, capillary voltages of 3500 V for PI and 3500 V for NI, and spray shield voltages of 600V were used. LC system was coupled to the MSD with a splitting of 50%. Results were expressed as $\mu\text{g g}^{-1}$ or mL^{-1} of cyanidin equivalent. Determinations were performed in triplicate for all samples.

Ellagic acid and gallic acid were quantified in a HPLC (HPLC Agilent 1100 Series, Englewood, CO, USA) with manual injection. 20 μL sample loop and ternary pump, coupled to a diode array detector (DAD Agilent G13158). The oven (Agilent 1100) was operated at 25 ± 2 °C. Data was obtained and processed using the software ChemStation (Hewlett Packard, Germany). A reverse phase chromatographic column (C18 Eclips XDB (5 $\mu\text{m} \times 250 \text{ mm} \times 4.6 \text{ mm}$), Agilent, Englewood, CO, USA) was used. Mobile phase was 1% orthophosphoric acid in water (v/v) (A) and acetonitrile (B). Elution gradient started at 95:05 (A:B) at 0.7 mL min^{-1} . This condition was maintained for 5 min and then concentration of A was decreased (75:25, A:B), and at 25 min it reached 60:40 (A:B) followed by a linear increase of solvent A to 95% until 35 min. Detection was done at 210, 254, 280, 300 and 340 nm, which allowed simultaneous quantification and identification of the phenolic compounds separated by the HPLC. Content of identified compounds were calculated from the analytical curves. The results were expressed as $\mu\text{g g}^{-1}$ or mL^{-1} . Determinations were performed in triplicate for all samples.

2.6. Antioxidant assay *in vitro*

Antioxidant capacity in FJP MeOH and FJP aqueous extract was determined by DPPH, ABTS⁺, FRAP and ORAC assays. The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay was done according to Brand-Williams, Cuvelier & Berset (1995), with some modifications. The decreasing in absorbance of samples and Trolox standard curve was measured after 30 minutes of reaction and read at 515 nm. Results were expressed as $\mu\text{M Trolox g}^{-1}$ or mL^{-1} .

The ABTS⁺ assay was based on the method developed by Miller et al (1993), with modifications according to Rufino et al (2010). The working solution was prepared by reacting 7 mM ABTS (2,2 azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt) stock solution with 140 mM potassium persulfate after 16 h incubation in the dark at room temperature. The monitored decrease of absorbance over time (30 min with 1 min interval) was plotted and the differences between samples and control were calculated. The samples and Trolox standard curve were read at 734 nm. Results were expressed as $\mu\text{M Trolox g}^{-1}$ or mL^{-1} .

The ferric reducing antioxidant power (FRAP) of samples was determined according to Rufino et al (2010). The FRAP reagent was prepared in the dark with 300 mmol L^{-1} acetate buffer (pH 3.6), 10 mmol L^{-1} TPTZ (2,4,6-tris(2-pyridyl)-S-triazine) in a 40 mmol L^{-1} HCl solution and 20 mmol L^{-1} FeCl_3 . The samples and Trolox standard curve were read at 595 nm. Results were expressed as $\mu\text{M Trolox g}^{-1}$ or mL^{-1} .

ORAC assay (oxygen radical absorbance capacity test) was carried out adding 20 μL of samples extract or standard solutions, 120 μL of fluorescein diluted in phosphate buffer (pH 7.4), and 60 μL of AAPH (2,2'-azobis (2-methylpropionamidine) dihydrochloride) to black microplates, in the dark (Dávalos, Gómez-Cordovés, & Bartolomé, 2004). ORAC values were expressed in $\mu\text{M Trolox g}^{-1}$ or mL^{-1} . Appropriate calculations were used for check the linearity between the net area under the curve and the concentration for the samples.

The absorbances or fluorescences of these assays were read in a microplate reader SynergyHT, Biotek (Winooski, USA); with Gen5™2.0 data analysis software spectrophotometer.

2.7. Antioxidant assay *in vivo*

2.7.1. Animals and diets

Thirty-six male *Wistar* rats, recently weaned, were obtained from the Multidisciplinary Center for Biological Research at Unicamp (CEMIB). The experiment was approved by the Ethics Committee on Animal Experiments (CEUA / UNICAMP), protocol number 3272-1, and followed all the ethical requirements of the Brazilian College of Animal Experimentation (COBEA). Animals were maintained in growth period for 4 weeks, feed by commercial pelleted diet. Afterward, animals were randomly distributed into six groups ($n = 6$) and remained at individual cages with food and water under the system of free access, controlled temperature and humidity, with a range of 22 ± 1 °C and 60-70% respectively, and light / dark cycle of 12 hours, throughout the experimental period.

Two control diets were given during the experiment: a normal control diet, prepared in accordance with the American Institute of Nutrition (Reeves, Nielsen & Fahey, 1993), AIN-93M, with protein concentration of 12%, lipid concentration of 4% and 70% approximately of total carbohydrates and a high-fat fructose control diet, AIN-93M-modified with 12% protein and 35% of fat, 4% vegetable oil (soybean) and 31% of animal origin (lard) and 20% of fructose (HFF) (Shapiro et al, 2011). The HFF-FJP experimental diet was formulated from high-fat fructose diet adding 2% of FJP powder. Diets composition is showed in the table 1. The prevention groups (P. FJP and P. JE) consumed the supplemented diet or JE for 12 weeks and the treatment groups (T. FJP and T. JE) consumed HFF diet for 6 weeks to induce obesity and started the supplementation as from the sixth week. Groups P. JE and T. JE had the substitution of water by FJP aqueous extract (JE). The experimental design is presented in the figure 1.

Table 1. Composition of modified AIN-93M diets fed to rats.

INGREDIENTS	AIN-93M	HFF	HFF – FJP
	(g Kg ⁻¹)	(g Kg ⁻¹)	(g Kg ⁻¹)
Casein (78% prot.)	143.89	143.89	143.89
Corn starch	461.69	133.18	133.18
Maltodextrin	155.00	44.67	44.67
Sucrose	100.00	28.82	28.82
Soybean oil	40.00	40.00	40.00
Cellulose	50.00	50.00	50.00
Fructose	-	200.00	200.00
Mineral mix	35.00	35.00	35.00
Vitamin mix	10.00	10.00	10.00
L-cystine	1.80	1.80	1.80
Choline bitartarate	2.50	2.50	2.50
Tert-Butyl Hydroquinone	0.008	0.008	0.008
Lard	-	310.00	310.00
FJP Powder	-	-	20.00
Energy value (Kcal g ⁻¹)	4.17	5.45	5.51

In the HFF diet, 31% of lard was added and, consequently, starch, sucrose and maltodextrin contents were reduced. HFF – FJP diet was added of 2% of freeze-dried jaboticaba peel powder. Value expressed in Kcal g⁻¹ diet, obtained by calorimetry.

Diet intake was monitored every 2 days and weight gain once a week. The FJP aqueous extract (JE) was prepared daily and its consumption was monitored every day: JE was measured (25 mL) and placed in animal's bottles, in the next day the JE leftover was measured and the two volumes were recorded. The water consumption of HFF group was monitored to be used as control for the P. JE and T. JE groups.

AIN-93M	Normal control diet for 12 weeks	
HFF	High-fat fructose control diet for 12 weeks	
P. FJP	HFF diet + 2% FJP powder for 12 weeks	
T. FJP	HFF diet for 6 weeks	HFF diet + 2% FJP powder for 06 weeks
P. JE	HFF diet + FJP aqueous extract for 12 weeks	
T. JE	HFF diet for 6 weeks	HFF diet + FJP aqueous extract for 06 weeks

Fig.1. Experimental design. The animals were maintained on a commercial pelleted diet for 4 wk for growth. After 4 wk, they were divided in six groups (n = 6): AIN-93M (normal control) standard diet; HFF (obese control) received a high-fat and fructose diet containing 4% (w/w) soybean oil, 31% (w/w) lard and 20% fructose (w/w); P. FJP and T. FJP received the HFF diet with 2% of FJP powder (w/w), for 12 and 6 weeks respectively; P. JE and T. JE received the HFF and had the water substituted by FJP aqueous extract, for 12 and 6 weeks respectively. All diets were based on the AIN-93M diet.

At 16 experimental weeks, animals were euthanized by decapitation preceded by 12 h fasting. Blood was collected in tubes with anticoagulant EDTA to obtain plasma. After exsanguination, liver was removed, cleaned with saline solution, weighed, frozen in liquid nitrogen and stored in a freezer at – 80 °C. Frozen liver was divided in aliquots of the 100 mg approximately and the remaining tissue was freeze-dried. The freeze-dried liver was stored in freezer at – 20 °C for further analysis.

2.7.2. Lipid peroxidation by thiobarbituric acid reactive substances (TBARS) assay

TBARS determinations were done in liver and plasma according to Ohkawa, Ohishi & Yagi (1979), with adaptations. For liver 10 mg of the freeze-dried tissue were mixed with 1 mL of acetate buffer pH 3.6 and sonicated for 30 minutes in ice bath. The plasma (100µL) was directly pipetted in the tubes by reaction. The 8.1% sodium dodecyl sulfate (SDS) and working reagent (2-thiobarbituric acid — TBA, 5% acetic acid and 20% sodium hydroxide)

were added in the samples. After heating at 95 °C for 60 min, samples remained in ice bath for 10 min and centrifuged at 14.000 rpm for 10 min. Supernatant was read at 532 nm, using a clear 96-well microplate. Standard curve was obtained using malondialdehyde standard (MDA). Results were expressed in nmol MDA mg tissue⁻¹ or nmol MDA mL⁻¹ plasma.

2.7.3. Antioxidant potential in liver and plasma by FRAP assay

FRAP assay was used for determination of the antioxidant capacity in the tissues. Plasma was treated with ethanol, ultrapure water and 0.75 mol L⁻¹ metaphosphoric acid (Leite et al, 2011). Liver homogenate in phosphate buffer (PB) was centrifuged and supernatant was used in FRAP assays (Rufino et al, 2010) as described above.

2.7.4. Enzymatic and non-enzymatic endogenous antioxidant system

2.7.4.1. Thiol group content (GSH)

GSH levels were determined in the PB homogenates of liver and plasma using Ellman's reagent (DTNB) (Ellman, 1959), with modifications. GSH solution (2.5– 500 nmol GSH mL⁻¹) was used as standard and absorbance was read at 412 nm. Reduced thiol contents were expressed in nmol GSH mg protein⁻¹.

2.7.4.2. Glutathione peroxidase activity (GPx)

GPx activity was quantified in plasma and PB homogenate of liver. The oxidation of 10 mmol reduced glutathione by glutathione peroxidase coupled to the oxidation of 4 mmol NADPH by 1 U enzymatic activity of GR in the presence of 0.25 mmol H₂O₂ is measured in this assay. The rate of NADPH oxidation was monitored by the decrease in absorbance at 365 nm (Flohe & Gunzler, 1984). Results were expressed in nmol NADPH consumed min⁻¹ mg protein⁻¹.

2.7.4.3. Glutathione reductase activity (GR)

GR activity was measured in plasma and in liver PB homogenates, following the decrease in absorbance at 340 nm induced by 1 mmol oxidized glutathione in the presence of 0.1 mmol NADPH in phosphate buffer (Carlberg & Mannervik, 1985). Results were expressed in nmol NADPH consumed $\text{min}^{-1} \text{mg protein}^{-1}$.

2.7.4.4. Superoxide dismutase activity

SOD activity was analyzed in liver and plasma. Samples (100 μL) were added in 96-well microplate and 150 μL of the working solution (0.1 mmol hypoxanthine, 0.07 U xanthine oxidase, and 0.6 mmol NTB in PB in 1:1:1 proportions) and the kinetic reaction was monitored at 560 nm (Winterbourn, Hawkins, Brian, & Carrell, 1975). Area under the curve (AUC) was calculated and the SOD activity was expressed as U mg protein^{-1} .

2.7.4.5. Catalase activity

Catalase assay is based on reaction of the enzyme with methanol in optimum concentrations of H_2O_2 . The produced formaldehyde is measured colorimetrically with Purpald (chromophore) (Johansson & Borg, 1988; Wheeler, et al, 1990). Liver homogenate and plasma were pipetted (20 μL) in 96-well microplate with 100 μL assay buffer, 30 μL methanol and 20 μL H_2O_2 . Reaction occurred for 20 min in shaker of the dark. Thirty μL of potassium hydroxide stop the reaction. Purpald was added (30 μL) and the plate was read at 540 nm. The formaldehyde standard curve was done and used for calculate catalase activity. The results were expressed as $\text{nmol min}^{-1} \text{mL}^{-1}$ or g protein^{-1} .

For FRAP assay, enzymatic and non-enzymatic endogenous antioxidant system analyze in liver, the protein concentration of tissue homogenates was done by Bradford method (Bradford, 1976).

2.8. Statistical analysis

Phenolics compounds and antioxidant capacity between the FJP MeOH extract and FJP aqueous extract were analyzed by Student's *t-test* and the limit of significance was set at $p < 0.05$. For biological assays, difference in averages between AIN-93M and HFF groups was analyzed by Student's *t-test* with limit of significance of 0.05. Difference between HFF and supplemented groups was analyzed by Analysis of Variance (ANOVA; $\alpha = 5\%$) with post hoc Tukey's range test with significant differences between means ($p < 0.05$). Data analyses were carried out with GraphPad Prism 5.0 (GraphPad Software, Inc. La Jolla, CA, USA) software.

3. RESULTS AND DISCUSSION

3.1. Chemical composition

The jaboticaba peel is composed mainly of carbohydrates, including soluble and insoluble fiber, and water (Table 2). Possibly, the high content of carbohydrate corresponds the large amount of simple and complex sugars which are the major constituents of fruit peel (Damodaran et al, 2010). The content of insoluble and soluble fibers, protein and lipids has higher in the present study than in similar studies (Leite et al, 2011; Lenquiste et al, 2012; Alezandro et al, 2013). These results in the approximate composition can be justified by harvest time and ripeness of used jaboticaba across studies.

Table 2. Proximate composition of freeze-dried jaboticaba peel.

NUTRIENTS	g 100 g ⁻¹
Protein	7.31 ± 0.17
Lipids	2.71 ± 0.17
Moisture	13.88 ± 0.08
Total solids	86.12 ± 0.08
Ashes	3.84 ± 0.02
Soluble fibers	24.95 ± 0.15
Insoluble fibers	7.88 ± 0.22
Carbohydrates	33.96
Energy value (Kcal g ⁻¹)	2.55 ± 0.06

Data are presented as means ± standard deviation. The values are expressed as percentage. The carbohydrates were calculated by difference.

3.2. Antioxidant assay *in vitro*

3.2.1. Phenolic compounds and antioxidant capacity in FJP extracts

Jaboticaba peel has a great potential as functional food and as an additive in processed foods (Dessimoni-Pinto et al, 2011). On the other hand, jaboticaba peel can be consumed as tea such as formulated in the present study. This proposal becomes practicable once tea is the second most popular beverage in the world. Furthermore, plants infusion contains several flavonoids, substances that are among the polyphenolic compounds, which can have positive biological effects (El-Beshbishy, 2005).

Phenolics compounds and antioxidant capacity in freeze-dried jaboticaba peel (FJP) MeOH extract and FJP aqueous extract are showed in the table 3. Total phenolic compounds, anthocyanins and flavonoids were higher in FJP MeOH extract than in FJP aqueous extract. Total phenolics and anthocyanins in the FJP MeOH extract were similar to those obtained by Alezandro et al (2013), but lower than those obtained by Batista et al (2014).

Table 3. Phenolics compounds and antioxidant capacity in freeze-dried jaboticaba peel (FJP) MeOH extract (g), FJP aqueous extract (g) and FJP aqueous extract (mL).

	FJP MeOH extract (g)	FJP aqueous extract (g)	FJP aqueous extract (mL)
<i>Phenolic Compounds</i>			
Total Phenolics	48.61 ^a ± 1.18	36.12 ^b ± 3.05	0.72 ± 0.06
Anthocyanins	630.46 ^a ± 21.76	404.56 ^b ± 35.85	8.09 ± 0.72
Flavonoids	22.31 ^a ± 1.58	18.45 ^b ± 1.21	0.40 ± 0.02
<i>Specific Phenolics</i>			
Gallic acid	36.92 ^b ± 2.28	177.76 ^a ± 2.26	3.55 ± 0.05
Ellagic acid	3045.44 ^a ± 90.97	1581.61 ^b ± 135.50	31.63 ± 2.71
Cyanidin-3- <i>O</i> -glucoside	32945.24 ^b ± 347.80	34242.88 ^a ± 594.70	685.20 ± 11.90
<i>Antioxidant Capacity</i>			
DPPH	346.77 ^a ± 30.23	320.85 ^b ± 29.90	6.42 ± 0.60
FRAP	449.68 ^a ± 18.72	410.65 ^a ± 10.60	8.21 ± 0.21
ABTS	194.95 ^b ± 4.66	223.10 ^a ± 2.28	4.46 ± 0.05
ORAC	317.98 ^a ± 9.03	213.16 ^b ± 15.06	4.26 ± 0.31

Data are presented as means ± standard deviation. Total phenolics are expressed as mg gallic acid equivalent g or mL⁻¹. Total flavonoids are expressed as mg catechin equivalent g or mL⁻¹. Total anthocyanins are expressed as mg 100 g or mL⁻¹. Specific flavonoids are expressed as µg g or mL⁻¹. Cyanidin-3-*O*-glucoside is expressed as µg g or mL⁻¹ of cyanidin equivalent. Antioxidant activities are expressed as µM Trolox g or mL⁻¹. Statistical differences between FJP MeOH 70% and FJP aqueous extract are represented by different letters according Student's *t*-test (*p* < 0.05). The FJP aqueous extract (mL) column was not statistically compared to other columns.

The gallic acid and cyanidin-3-*O*-glucoside content were higher in FJP aqueous extract. However, ellagic acid was higher in the FJP MeOH extract. Gallic acid values showed in FJP MeOH extract was 18% lower than found by Wu et al (2012), which evaluated the phenolic compounds by HPLC in the jaboticaba peel and jaboticaba juice. Nevertheless, in the present study results are in agreement with those demonstrate by Batista et al (2014). Ellagic acid content in FJP MeOH extract was similar to those reported by Abe, Lajolo & Genovese

(2013) – 22.50 g Kg⁻¹ – and higher than the 348.08 mg 100 g⁻¹ obtained by Batista et al (2014). In Alezandro et al (2013) and Wu et al (2012) studies ellagic acid levels were lower than those obtained in this study. Anthocyanin cyanidin-3-*O*-glucoside in FJP MeOH was higher than those showed by Leite et al (2011) and Wu et al (2012), 1964.00 mg 100 g⁻¹ and 29.80 mg 10 g⁻¹, respectively.

Anthocyanins are more soluble in water and methanol. However, it is known that heating may enhance the extraction of certain compounds, by breaking hydrogen bonds. Therefore, the concentration of anthocyanins in aqueous extract can be improved by increasing solubility of these compounds through boiling water infusion (Azsmir et al, 2013). Daneshfar, Ghaziaskar & Homayoun (2008) evaluated the solubility of gallic acid in different solvents such as methanol, ethanol and water and its relationship with temperature. These authors observed that heating increases gallic acid solubility of in water. This may explain the higher concentration of gallic acid in the aqueous extract since it was heated to 95 °C.

Antioxidant capacity of jaboticaba peel extracts was measured by DPPH, FRAP, ABTS and ORAC assays (Table 3). In DPPH and ORAC assays, methanol extract was more efficient than the aqueous extract. The results of FRAP assay were statistically similar for both extracts and the ABTS assay showed higher values in the aqueous extract.

Leite-Legatti et al (2012) and Abe, Lajolo & Genovese (2012) evaluated the antioxidant capacity by DPPH assay in jaboticaba peel and showed lower values, 45.38 µg mL⁻¹ and 62.60 mmol TE kg⁻¹ fresh weight, respectively, than those presented in this study. Otherwise, Alezandro et al (2013) showed higher DPPH value (600.00 TE 100g⁻¹). FRAP assay carried out by Alezandro et al (2013) and ABST by Leite-Legatti et al (2012) showed 68% and 51% lower values than those showed in this study. However, ORAC values obtained

by Leite-Legatti et al (2012) and Batista et al (2014) were higher when compared to the present results – 25514.24 $\mu\text{M TE g}^{-1}$ and 519.11 $\mu\text{M TE g}^{-1}$, respectively.

The bioactive compounds in plants, as well as their antioxidant capacity and biological effects, could be an arduous work due to the large content and composition variation that depends of geography, photoperiod and climate. These differences have been reported as influence factors on secondary metabolites biosynthesis such as many flavonoids. Thus, it can affect antioxidant potential range (Jaakola & Hohtola, 2010; Kumazawa, Hamasaka, & Nakayama, 2004)

3.3. Antioxidant capacity *in vivo*

3.3.1. Weight gain and intake parameters

Weight gain, food intake, energy intake and FJP aqueous extract (JE) intake was showed in the table 4. Daily and cumulative weight gains were higher in the HFF group compared to AIN-93M. Supplemented groups had lower weight gain than the HFF group. Thus, the HFF diet was able to induce obesity in animals when compared to the AIN-93M group. Furthermore, FJP and JE supplemented groups were able to counteract this process. Food intake was higher in the AIN-93M groups than in the HFF group, however energy intake did not differ among groups. There was no difference in JE consumption in P. JE and T. JE groups when compared to water consumption of HFF group

Table 4. Weight gain, food and tea intake

	AIN-93M	HFF	P. FJP	T. FJP	P. JE	T. JE
Daily weight gain (g rat day ⁻¹)	2.65 ^B ± 0.16	3.51 ^{Aa} ± 0.40	3.05 ^b ± 0.51	3.09 ^b ± 0.16	2.77 ^b ± 0.27	2.65 ^b ± 0.37
Cumulative weight gain (g rat ⁻¹)	226.30 ^B ± 15.94	307.90 ^{Aa} ± 25.81	250.41 ^b ± 40.38	259.42 ^b ± 13.68	233.30 ^b ± 23.07	235.40 ^b ± 31.83
Food intake (g rat day ⁻¹)	23.81 ^A ± 1.28	17.49 ^{Ba} ± 1.22	17.54 ^a ± 1.12	17.63 ^a ± 1.49	16.60 ^a ± 1.35	16.14 ^a ± 1.10
Energy intake (Kcal rat day ⁻¹)	91.94 ^B ± 4.31	95.71 ^{Aa} ± 4.02	97.20 ^a ± 8.18	96.10 ^a ± 5.56	91.15 ^a ± 7.39	89.98 ^a ± 4.71
Water or JE intake (mL rat day ⁻¹)	-----	18.18 ^a ± 2.90	-----	-----	17.98 ^a ± 2.68	15.97 ^a ± 1.38

Data presented as means ± standard deviation. Statistical differences between AIN-93M and HFF groups are represented by different uppercase letters and statistical differences between HFF and supplemented groups are represented by different lowercase letters. HFF group had the water consumption measured to order to serve as a control for P. JE and T. JE groups. AIN-93M (normal control) standard diet; HFF (obese control) received a high-fat and fructose diet containing 4% (w/w) soybean oil, 31% (w/w) lard and 20% fructose (w/w); P. FJP and T. FJP received the HFF diet with 2% of FJP powder (w/w), for 12 and 6 weeks respectively; P. JE and T. JE received the HFF and had the water substituted by FJP aqueous extract, for 12 and 6 weeks respectively.

Studies have been evaluated the effects of purified anthocyanins and fruits rich in these compounds supplementation in high-fat diet on the development of obesity. Jayaprakasam et al (2006) and Prior et al (2008) showed that the supplementation of different sources of cyanidin-3-*O*-glucoside (C3G) extracted in a high-fat diets reduced mice weight gain. However, the consumption of freeze-dried foods as a source of anthocyanins added to high-fat diet has not shown effects on weight gain neither in body fat in animal models (Batista et al, 2014; Dragano et al, 2013; Lenquist et al, 2012; DeFuria et al, 2009; Prior et al, 2010).

The effects of phenolic compounds on obesity have been studied and its effect on weight gain and body mass are still divergent. Nevertheless, acceptable mechanisms for antiobesogenic potential of these compounds have been proposed. Alezandro, Granato & Genovese (2013) demonstrated significant inhibitory activity of α -amylase and α -glucosidase enzymes in induced diabetic rats fed with jaboticaba peel. Sergent et al (2012) showed that phenolic compounds of green tea inhibited pancreatic lipase activity which delayed or decreased *in vivo* fatty acid absorption by enterocytes. Thus, the enzymatic inhibition can be a mechanism in the obesity regulation. In recent study, Dragano et al (2013) reported that despite the consumption of FJP added to the high-fat did not effected on mice weight gain, insulin resistance and modulated proteins associated with obesity, inflammation and diabetes were improved.

3.3.2. Antioxidant potential and lipid peroxidation

Bioactive compounds have been studied due to their effects on preventing damage caused by ROS and free radicals which are widely produced during metabolic processes. In order to reduce oxidative stress, the organisms have developed mechanisms. These mechanisms include the non-enzymatic and enzymatic antioxidant defenses produced in indogenously and others provided by diet (exogenous) (Han, Shen & Lou, 2007).

There were no difference in plasma and liver FRAP assay (Figure 2A, 2B). Leite et al (2011) evaluated plasma antioxidant capacity by ABTS assay after FJP diet supplementation. They reported that the addition of 1 and 2% de FJP powder in a normolipidic diet increased the antioxidant potential. However at 4% FJP powder diet it showed an inverse effect. Additionally, Batista et al (2014) evaluated the antioxidant potential in several organs of rats wich consumed HF diet supplemented with FJP at 1, 2 and 4%. These authors observed that in plasma samples TEAC assay showed higher values in animals fed with 2 and 4% of FJP. Nevertheless, ORAC and FRAP assay did not differ among groups. In liver samples, they

showed that ORAC assay was higher in the FJP supplemented groups compared to HF group, but TEAC and FRAP assay did not differ among groups. FRAP assay was used by Alezandro, Granato & Genovese (2013) to evaluated oxidative stress in streptozotocin-mediated diabetic rats. In this study, plasma antioxidant capacity of diabetic rats was increased (2 to 2.5 times) after both jaboticaba doses (1.0 and 2.0 g dry weight kg^{-1} body weight) supplementation for 40 days.

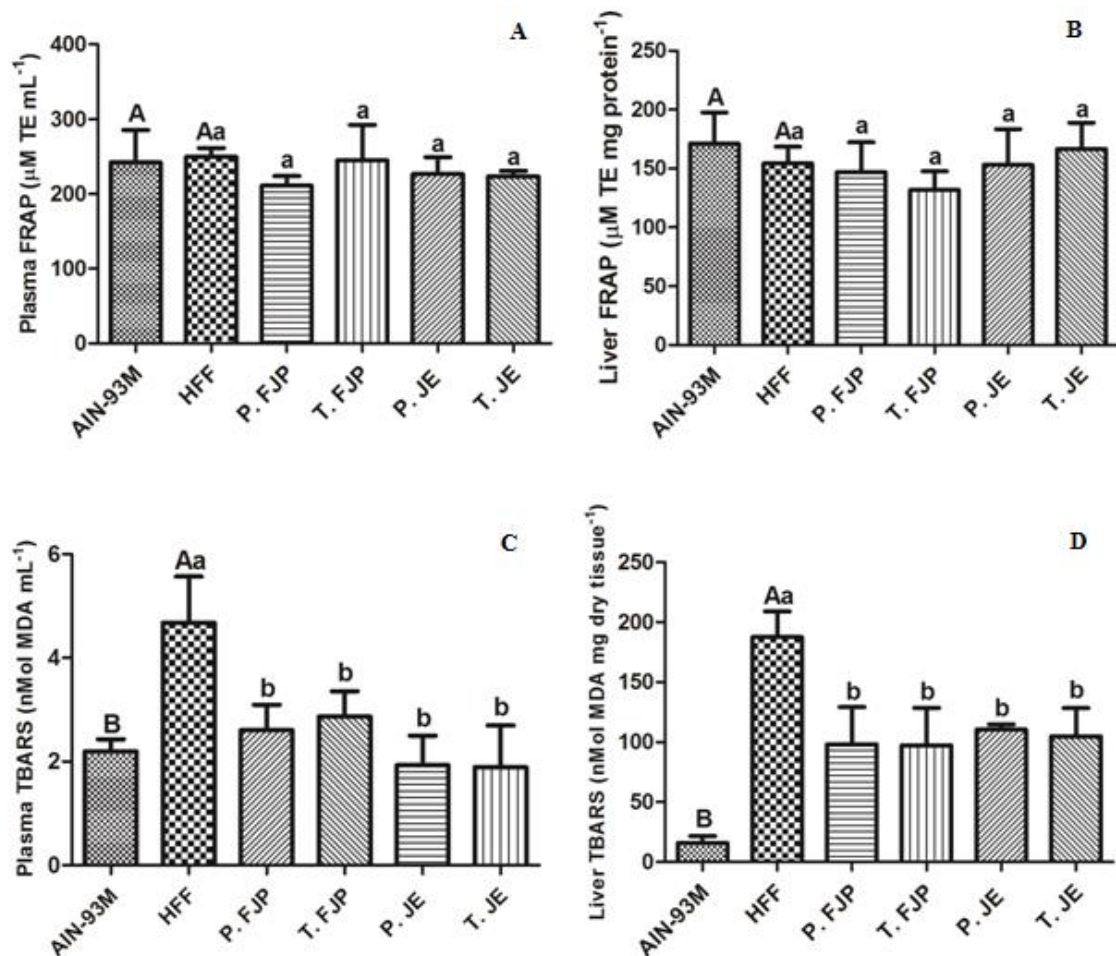


Fig. 2. FRAP and TBARS levels in plasma and liver of experimental animals. A – plasma FRAP; B – liver FRAP; C – plasma TBARS; D – liver TBARS. Data presented as means $\pm$ standard deviation. Statistical differences between AIN-93M and HFF groups are represented by different uppercase letters and statistical differences between HFF and supplemented groups are represented by different lowercase letters.

Lipid peroxidation in plasma and liver was measured by TBARS assay. HFF group showed higher MDA level in plasma and liver than AIN-93M, showing increased lipid

peroxidation. This damaging process was reversed by FJP and JE supplementation (Figure 2C, 2D). Wu et al (2015) evaluated serum MDA levels in mice fed with normal or three types of flavonoids extracts in supplemented diets. They observed that the extracts groups had lower MDA level than the control groups. Several studies has also shown positive effects of foods rich in phenolic compounds on lipid peroxidation (Alezandro, Granato & Genovese, 2013; Batista et al, 2014; Silva et al, 2013). Thus, MDA levels are an important marker of lipid peroxidation and can be modulated by phenolic compounds intake.

3.3.3 .Thiol group content and antioxidant enzymes

The increased oxidative stress is related to an overproduction of free radicals or deficiency in the antioxidant defense system (Habib & Ibrahim, 2011). Plasma GPx and GR did not differ among groups (Figure 3A, 3B). However, plasma GSH level was significantly lower in HFF group compared to the AIN-93M group. FJP and JE supplemented groups showed an increase in this biomarker compared to HFF group (Figure 3C). In liver, GPx level was higher in AIN-93M group compared to HFF group and there was no difference among HFF and supplemented groups (Figure 4A). Liver GR level was lower in AIN-93M group compared to HFF group. Supplements FJP and JE did not reduce the GR level compared to HFF group (Figure 4B). Liver GSH level was lower in HFF group compared to AIN-93M. FJP and JE groups showed an increase in this biomarker compared to HFF group (Figure 4C). Thus, only plasma and liver GSH levels were positively modulated by FJP and JE intake.

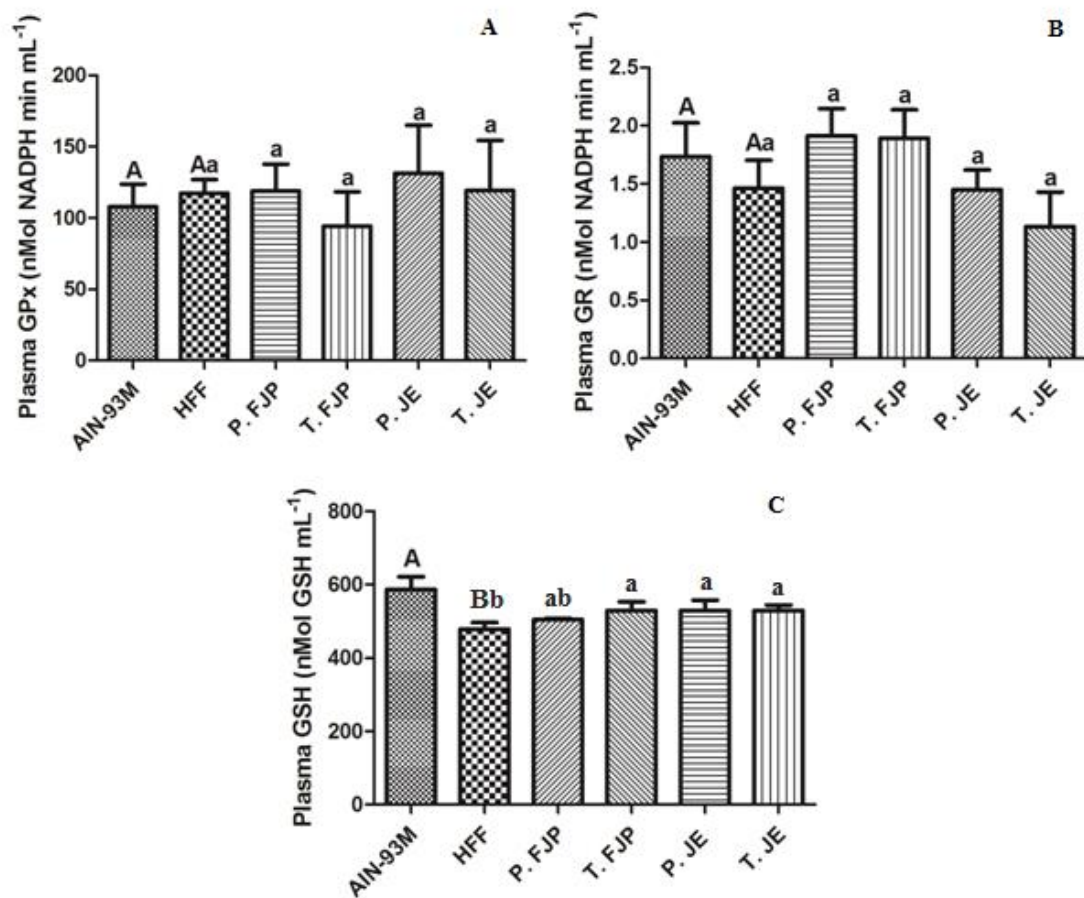


Fig. 3. Glutathione Peroxidase (GPx) and Glutathione Reductase (GR) enzymes and Reduced Glutathione (GSH) in plasma of experimental animals. A – GPx enzyme; B – GR enzyme; C – GSH. Data presented as means $\pm$ standard deviation. Statistical differences between AIN-93M and HFF groups are represented by different uppercase letters and statistical differences between HFF and supplemented groups are represented by different lowercase letters.

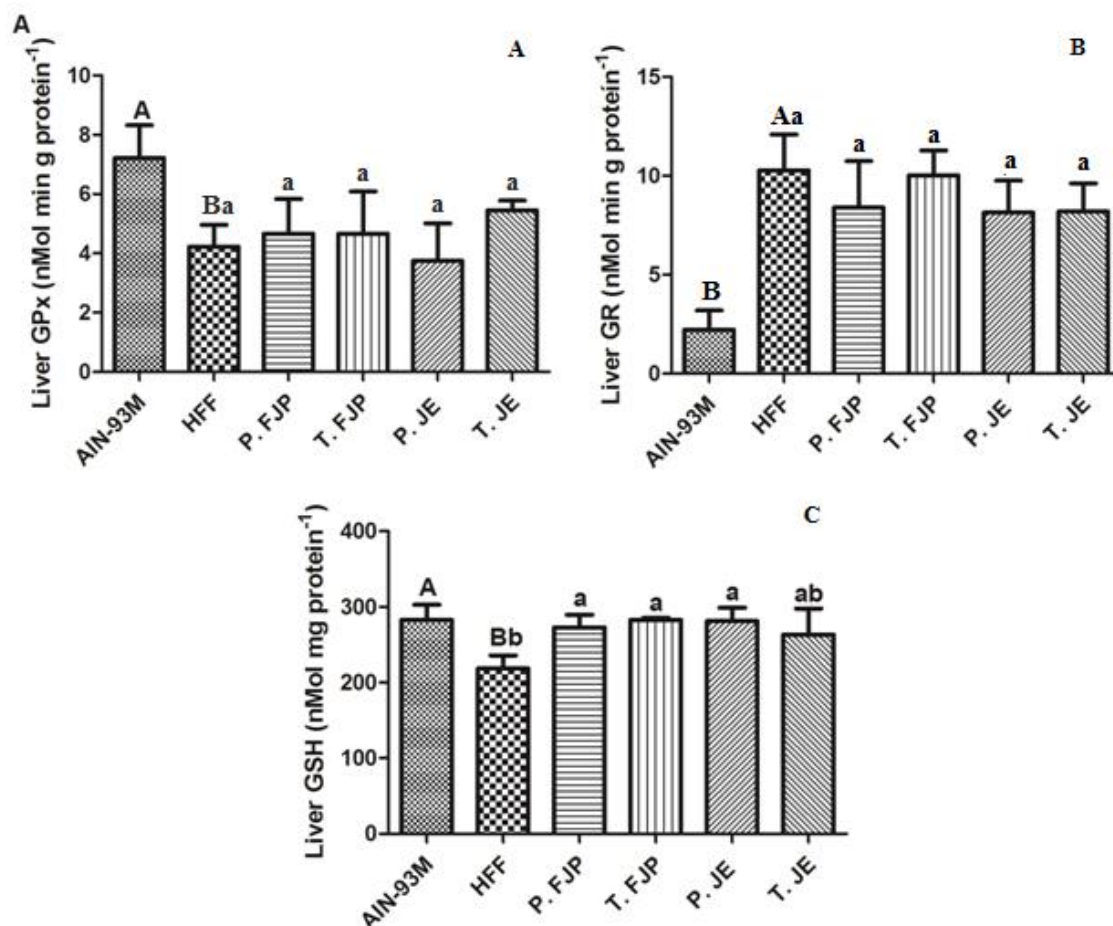


Fig. 4. Glutathione Peroxidase (GPx) and Glutathione Reductase (GR) enzymes and Reduced Glutathione (GSH) in liver of experimental animals. A – GPx enzyme; B – GR enzyme; C – GSH. Data presented as means $\pm$ standard deviation. Statistical differences between AIN-93M and HFF groups are represented by different uppercase letters and statistical differences between HFF and supplemented groups are represented by different lowercase letters.

Plasma and liver SOD activity was significantly lower in HFF group compared to AIN-93M group. There was no significant difference in plasma and liver SOD activities among HFF and supplemented groups (Figure 5A, 5B). There was no plasma significant difference in CAT activity among groups (Figure 5C). Liver CAT level was lower in HFF group than in AIN-93M group. The P. FJP and P. JE groups showed higher CAT activity than HFF group, whereas T. FJP and T. JE groups showed an increase in CAT activity. However, these values were not statistically different compared to HFF group (Figure 5 D). Thus, FJP and JE supplementation for 12 weeks increased CAT levels in animal's liver.

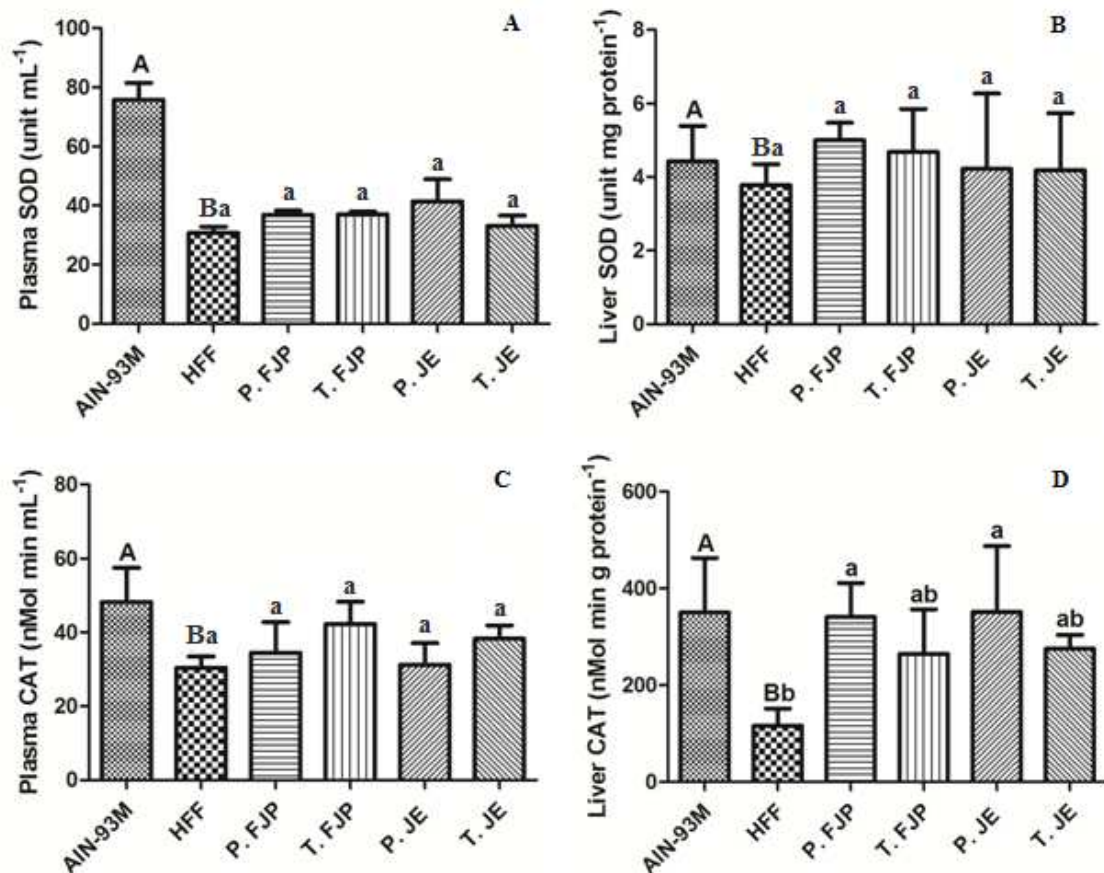


Fig. 5. Superoxide dismutase (SOD) and Catalase (CAT) activities in plasma and liver of experimental animals. A – plasma SOD; B – liver SOD; C – plasma CAT; D – liver CAT. Data presented as means $\pm$ standard deviation. Statistical differences between AIN-93M and HFF groups are represented by different uppercase letters and statistical differences between HFF and supplemented groups are represented by different lowercase letters.

Glutathione is the main source of reducing power. It is maintained in reduced form of GR, which acts with NADPH. Therefore, increasing or maintaining of total GSH level added up an improvement of GR level could be an important indicator of antioxidant system enhancement (Skrzydłowska et al., 2002). Wu et al (2015) showed an increasing in GPx and SOD activity in mice plasma supplemented with extract rich in flavonoids. Antioxidant enzymes were measured in plasma and liver of diet-induced obese rats after FJP supplementation. It was shown that the FJP promoted an increase in plasma SOD and CAT, as well as liver SOD, CAT and GPx levels (Batista et al, 2014). Similar results were obtained

by Alezandro, Granato and Genovese (2013). They showed an increase of antioxidant enzymes in plasma, kidneys, brain and liver in diabetic rats supplemented with FJP extract by gavage.

In vivo studies have been evaluated the supplementation effects of foods rich in phenolics compounds on antioxidant enzymes activity (Han et al, 2007; Tedesco et al, 2001; Suwannaphet et al, 2010). Scientific evidence has shown that these compounds, especially anthocyanins, can have a powerfull antioxidant effect, *in vitro* and *in vivo* studies (Alezandro, Granato & Genovese, 2013; Habib & Ibrahim, 2011; Batista et al, 2014). However, the results are still inconsistent and studies focused in possible mechanisms of action are needed.

4. CONCLUSION

Jaboticaba peel is a good source of bioactive compounds. FJP could have an excellent application as a food additive. Furthermore, FJP aqueous extract can be consumed as a tea which could provide an alternative bioactive compound source for human consumption. FJP and FJP aqueous extract had a higher antioxidant potential *in vitro*. The *in vivo* antioxidant potential of FJP and JE was confirmed by remarkable biomarkers, such as TBARS and GSH levels and CAT activity, but did not by FRAP assay, and SOD, GR and GPx enzymes. Thus, further studies are necessary in order to confirm the *in vivo* effects of the FJP and JE.

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CAPÍTULO 2

Jaboticaba peel powder and jaboticaba peel aqueous extract reduces obesity, insulin resistance and hepatic fat accumulation in rats.

(Artigo em fase de submissão para a revista Nutrition)

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This work does not present conflicts of interest.

ABSTRACT

Jaboticaba (*Myrciaria Jaboticaba* (Vell.) Berg) is a typical Brazilian fruit. Its peel has a great potential as functional food and as an additive in processed foods. This study investigated the effects of freeze-dried jaboticaba peel (FJP) and jaboticaba tea (JE) on obesity parameters and associated complications in diet-induced obese rats. Therefore, sixty *Wistar* rats were divided in six groups: control (AIN-93M), high-fat and high-fructose diet (HFF), HFF supplemented FJP and JE prevention groups (12-weeks) and treatment groups (6-weeks). Lipid and hormonal profile, glucose and insulin tolerance and insulin secretion by pancreas islet isolation were determined. Fat liver accumulation was evaluated by lipid tissue content measurement and histological analyses. Results showed that HFF diet induced weight gain, dyslipidemia, glucose intolerance, insulin resistance and liver fat accumulation. FJP and jaboticaba tea reduces weight gain and adiposity. FJP and jaboticaba tea supplementation for 12 weeks increases HDL-cholesterol, but not alter total cholesterol and serum leptin and adiponectin. Insulin resistance was positively modulated by FJP and jaboticaba tea supplementation, in 6 and 12 weeks. In addition, FJP and jaboticaba tea supplementation for 12 weeks reversed liver steatosis. Our results therefore suggest that FJP and jaboticaba tea could serve as a functional food and may be a dietary strategy to prevent or control obesity and related diseases. Thus, FJP and jaboticaba tea supplementation by 12 weeks showed positive effects in important parameters of obesity and insulin metabolism, as well as fat accumulation, reverting liver steatosis in diet-induced obese rats.

Keywords: *Myrciaria Jaboticaba* (Vell.) Berg., obesity, adiposity, insulin resistance, liver steatosis.

1. INTRODUCTION

Obesity appears as a worldwide epidemic, especially in Western and industrialized countries. Obesity is often associated with diabetes, hypertension and cardiovascular disease, which show a high degree with morbidity and mortality (Bastien, Poirier et al. 2014). Adipose tissue hypertrophy, especially in abdominal area, is related insulin resistance, hyperglycemia, dyslipidemia and inflammatory process. Sub-clinical inflammatory condition demonstrated in obesity leads to increase of adipokines secretion that interfere negatively in insulin signaling pathway, contributing to occurrence and progression of resistance to insulin (Wellen and Hotamisligil 2005). Other important obesity manifestation is nonalcoholic fatty liver disease (NAFLD), clinico-histopathological condition characterized by fat accumulation in hepatocytes. Visceral adiposity and insulin resistance play an initial key role for the onset and perpetuation of liver steatosis (Krawczyk, Bonfrate et al. 2010). Consumption of a high-fat and high fructose diet (HFF) have been related with the increase in obesity, glucose intolerance, insulin resistance, inflammation and nonalcoholic fatty liver disease in animals (Shapiro, Tumer et al. 2011, Poudyal, Panchal et al. 2012, Vos and Lavine 2013).

Development of functional foods containing beneficial phytochemicals and offering health and wellness benefits beyond basic nutrition may provide additional advantages in combating the obesity (Cheng, Pogrebnyak et al. 2014). Polyphenols are the most studied phytochemical class with beneficial metabolic effects. Antioxidant capacity of polyphenols is well described, but other healthy properties have been attributed to these compounds, as anti-proliferative and anti-inflammatory effects, insulin resistance and diabetes prevention, and prevention of chronic diseases non communicable (Crozier, Jaganath et al. 2009).

Jaboticaba (*Myrciaria Jaboticaba* (Vell.) Berg), a Brazilian typical fruit, has high nutritional value. Jaboticaba peel has been studied as great phenolic compounds source

(Alezandro, Dube et al. 2013, Lenquiste, Marineli et al. 2015). Anthocyanins are the major phenolic compounds class found jaboticaba peel, especially cyanidin-3-*O*-glucoside and delphinidin-3-*O*-glucoside (Leite et al, 2011). Other phenolics compounds in high concentrations in jaboticaba peel are tannins, gallic acid and ellagic acid (Abe, Lajolo et al. 2012, Leite-Legatti, Batista et al. 2012, Wu, Wu et al. 2013, Lenquiste, Marineli et al. 2015).

Thus, jaboticaba peel has a great potential as functional food and as an additive in processed foods. On the other hand, jaboticaba peel can be consumed as tea such as formulated in the present study. This proposal becomes practicable once tea is the second most popular beverage in the world. Furthermore, plants and fruits infusion contains several flavonoids, which have positive biological effects demonstrated (El-Beshbishy 2005). Therefore, the present study aimed to evaluate the beneficial effects of freeze-dried jaboticaba peel (FJP) and jaboticaba tea (JE) ingestion on obesity parameters and associated complications in high fat and fructose diet-induced obese *Wistar* rats.

2. METHODS

2.1 Jaboticaba peel powder and jaboticaba peel aqueous extract

Jaboticabas (*Myrciaria Jaboticaba* (Vell.) Berg) were acquired in Campinas Central Supply (CEASA), Brazil. Fruits were selected and cleaned. The peels were manually separated and were frozen, freeze-dried and milled to get a homogeneous powder (FJP). The powder was stored at – 80 °C in dark flasks, prior to addition in the experimental diets. FJP aqueous extract (JE) were obtained by weighing 2.0 g FJP powder and 100 mL boiling water addition (2% concentration). The extract was maintained in infusion for 30 minutes and manually homogenized each 15 minutes. This extract can be compared to a tea due to its form of preparation.

The chemical characterization and bioactive compounds presented by freeze-dried jaboticaba peel (FJP) and jaboticaba peel aqueous extract (JE) were previously described (Lenquiste, Batista et al. 2012).

2.2 Animals and diets

Thirty-six male *Wistar* rats, recently weaned, were obtained from the Multidisciplinary Center for Biological Research at Unicamp (CEMIB). For four weeks the animals were maintained in growth period, fed commercial pelleted diet and, subsequently divided randomly into six groups ($n = 6$): AIN-93M fed a normal control diet, prepared in accordance with the American Institute of Nutrition (Reeves, Nielsen et al. 1993); HFF, fed a high-fat fructose control diet, modified from AIN-93M, with 35% of fat, 4% vegetable oil (soybean) and 31% of animal origin (lard) and 20% of fructose (HFF) (Marineli, Moura et al. 2015); P. FJP and T. FJP fed high-fat fructose diet (HFF) supplemented with 2% of FJP powder by 12 and 6 weeks, respectively; P. JE and T. JE fed high-fat fructose diet (HFF) and had the substitution of water by FJP aqueous extract (JE) by 12 and 6 weeks, respectively. FJP and JE supplementation in prevention groups started concomitantly with HFF diet. The experimental period was determined by pilot study and previous studies of research group (Marineli, Moura et al. 2015; Dragano, Marques et al. 2013). Diets composition is showed in table 1 and the experimental design is presented in figure 1.

Throughout the experimental period, animals are maintained in individual cages with food and water under the system of free access, controlled temperature and humidity, with a range of 22 ± 1 °C and 60-70% respectively, and light / dark cycle of 12 hours. Diet intake was monitored every 2 days and weight gain once a week. The experiment was approved by the Ethics Committee on Animal Experiments (CEUA / UNICAMP), protocol number 3272-

1, and followed all the ethical requirements of the Brazilian College of Animal Experimentation (COBEA).

Table 1. Composition of modified AIN-93M diets fed to rats.

INGREDIENTS	AIN-93M	HFF	HFF – FJP
	(g Kg ⁻¹)	(g Kg ⁻¹)	(g Kg ⁻¹)
Casein (78% prot.)	143.89	143.89	143.89
Corn starch	461.69	133.18	133.18
Maltodextrin	155.00	44.67	44.67
Sucrose	100.00	28.82	28.82
Soybean oil	40.00	40.00	40.00
Cellulose	50.00	50.00	50.00
Fructose	-	200.00	200.00
Mineral mix	35.00	35.00	35.00
Vitamin mix	10.00	10.00	10.00
L-cystine	1.80	1.80	1.80
Choline bitartrate	2.50	2.50	2.50
Tert-Butyl Hydroquinone	0.008	0.008	0.008
Lard	-	310.00	310.00
FJP Powder	-	-	20.00
Energy value (Kcal g ⁻¹)	4.17	5.45	5.51

In the HFF diet, 31% of lard was added and, consequently, starch, sucrose and maltodextrin contents were reduced. HFF – FJP diet was added of 2% of freeze-dried jaboticaba peel powder. Value expressed in Kcal g⁻¹ diet, obtained by calorimetry.

FJP aqueous extract (JE) was prepared daily and its consumption was monitored every day: JE was measured (25 mL) and placed in animal's bottles, in the next day the JE leftover was measured and the two volumes were recorded. The water consumption of HFF group was monitored to be used as control for the P. JE and T. JE groups.

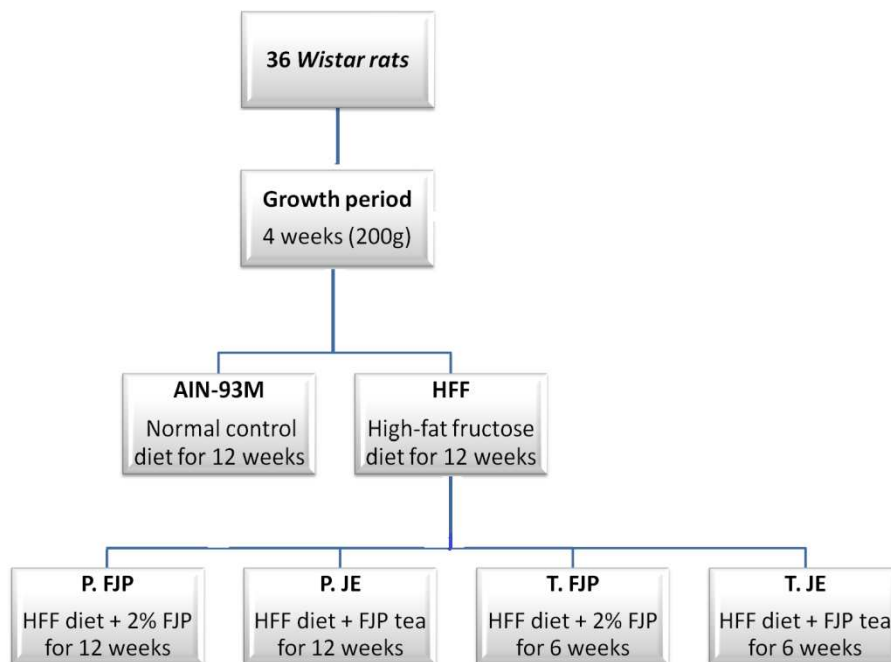


Figure 1. Experimental design. The animals were maintained on a commercial pelleted diet for 4 wk for growth. After 4 wk, they were divided in six groups ($n = 6$): AIN-93M (normal control) standard diet; HFF (obese control) received a high-fat and fructose diet containing 4% (w/w) soybean oil, 31% (w/w) lard and 20% fructose (w/w); P. FJP and T. FJP received the HFF diet with 2% of FJP powder (w/w), for 12 and 6 weeks respectively; P. JE and T. JE received the HFF and had the water substituted by FJP aqueous extract, for 12 and 6 weeks respectively. All diets were based on the AIN-93M diet.

2.3 Glucose Tolerance Test (GTT) and Insulin Tolerance Test (ITT)

Intraperitoneal glucose tolerance test (iGTT) and insulin tolerance test (ITT) were performed on food-deprived (12 h) non anaesthetized rats after 10 and 11 weeks of treatment, respectively. Blood glucose levels were measured with an Optium mini (Abbott Diabetes Care) handheld glucometer using appropriate test strips. For the iGTT, a solution of 50% D-glucose ($2.0 \text{ g kg body weight}^{-1}$) was administered into the peritoneal cavity. Blood samples were collected from the tail vein at 30, 60, 90 and 120 min for the determination of glucose concentrations. The area under curve (AUC) of glucose was calculated. For the ITT, glucose

blood levels were sampled at 5, 10, 15, 20, 25 and 30 min following intraperitoneal injection of human insulin ($0.75\text{U kg body weight}^{-1}$; Novolin R, Novo Nordisk® Farmacêutica do Brasil LTDA). The constant rate for glucose disappearance during the ITT ($k_{\text{ITT}} \text{ \% min}^{-1}$) was calculated using the formula $(0.693/t_{1/2})$. The glucose $t_{1/2}$ was calculated from the slope of the least-square analysis of the plasma glucose concentrations during the linear decay phase (Bonora, Manicardi et al. 1987).

2.4 Blood and tissue collection for biochemical analysis

At 16 experimental weeks, animals were euthanized by decapitation preceded by 12 h fasting. Blood was collected in tubes without and with anticoagulant EDTA to obtain plasma and serum, respectively. After exsanguination, pancreatic tissue was immediately extracted and taken to static insulin secretion analysis. Liver was removed, cleaned with saline solution and weighed. An aliquot of liver was fixed in a formaldehyde 10% solution for histological analysis. Remaining liver was frozen in liquid nitrogen and stored in a freezer at $-80\text{ }^{\circ}\text{C}$ for further analysis. Adipose tissues were removed, cleaned with saline solution, weighed and discarded.

2.5 Blood parameters

The serum total cholesterol, serum HDL cholesterol and serum glucose were assayed enzymatically using commercial kits (Winner lab®). The enzyme-linked immunosorbent assay (ELISA) method was used to determine serum levels of leptin and adiponectin using commercial kits from Millipore®. Plasma insulin levels were determined by radioimmunoassay (RIA) as described by Scott, Atwater et al. (1981).

2.6 Static insulin secretion analysis

Pancreatic islets were isolated by collagenase digestion of the pancreas as previously described (Ribeiro, Vanzela et al. 2010). For islet insulin content, groups of four islets were collected and transferred to 1.5 ml tubes. One millilitre of deionized water was added to these samples, followed by sonication of the pancreatic cells (three times, 10 s pulses), and the islet insulin content was measured by radioimmunoassay. For static incubations, four islets from each group were first incubated for 30 min at 37°C in Krebs–bicarbonate buffer of the following composition (mM): 115 NaCl, 5 KCl, 2.56 CaCl₂, 1 MgCl₂, 10 NaHCO₃ and 15 Hepes, supplemented with 5.6 mM glucose and 3g.L⁻¹ bovine serum albumin, and equilibrated with a mixture of 95% O₂–5% CO₂ to give a pH of 7.4. The medium was then replaced with fresh buffer and the islets were incubated for 1 h with two glucose concentrations: physiological concentration (2.8 mMol L⁻¹ glucose) and supraphysiological concentration (16.7 mMol L⁻¹ glucose). At the end of the incubation period, the insulin content of the medium was measured by radioimmunoassay (Scott, Atwater et al. 1981).

2.7 Liver evaluation

2.7.1 Liver fat percentage

Hepatic fat percentage was measured in the freeze-dried liver by extraction and quantification of lipids by Bligh and Dyer (1959) method.

2.7.2 Liver morphology

Histological analysis of liver morphology was done with fixed tissue in formaldehyde solution. Initially, proceeded to wash in alcohol 70 °GL for dehydration in ascending alcohol series, diaphanization in serial xylols, tissue was embedded in paraffin, followed by

microtomy 5 μm and staining according to standard hematoxylin-eosin protocols. Histological sections were analyzed and photomicrographed with light microscope (Nikon eclipse E-400). Four measures were performed by liver blade in light microscope coupled to an ocular micrometer and 40x objective. At the light microscope were observed their structures and microscopic changes and was performed a descriptive analysis of the morphological characteristics observed.

2.8 Statistical analysis

For all analyzes were considered six animals for group ($n = 6$). The data were presented as means $\pm$ standard deviation. Difference in averages between AIN-93M and HFF groups was analyzed by Student's *t-test* with limit of significance of 0.05. Difference between HFF and supplemented groups was analyzed by Analysis of Variance (ANOVA; $\alpha = 5\%$) with post hoc Tukey's range test with significant differences between means ($p < 0.05$). Data analyses were carried out with GraphPad Prism 5.0 (GraphPad Software, Inc. La Jolla, CA, USA) software.

3 RESULTS

Weight gains, food intake and bioactive compounds intake

Weight gain, food intake, energy intake, FJP aqueous extract (JE) intake and bioactive compounds intake are shown in table 2. Cumulative weight gain was higher in the HFF group compared to AIN-93M. Thus, the HFF diet was able to induce obesity in animals in relation to the AIN-93M group. Supplemented groups had lower weight gain than the HFF group. Furthermore, FJP and JE supplemented groups were able to counteract this process. Food intake was higher in the AIN-93M groups than in the HFF group, however energy

intake did not differ among groups. There was no difference in JE consumption in P. JE and T. JE groups compared to water consumption of HFF group.

The bioactive compounds intake of supplemented groups was calculated according to the previous analysis in the FJP and JE (Lenquist et al, 2015), considering the medium intake of FJP and JE of each group. Total phenolic and ellagic acid intake were higher in P. FJP and P. JE compared to T. FJP and T. JE. Total flavonoids was similar in P. FJP, P. JE and T.FJP, but was lower in T. JE group. Cyanindin-3-Glucoside (C3G) intake was similar in all supplemented groups. Differences in bioactive compounds intake can be attributed to supplementation period, considering that treatment groups received the supplementation for lower period (6 weeks) and this period was marked by fasting for glucose and insulin tolerance test.

Cholesterol levels, adipokynes levels and fat accumulation

Table 3 shown total and HDL cholesterol levels, leptin and adiponectin levels and lipid accumulation in liver and adipose tissues. Serum total cholesterol levels were similar among all groups, showing that supplementation FJP and JE was not able to change this parameter. HDL cholesterol levels were lower in HFF group compared to AIN-93M group, while P. FJP and P. JE groups showed significant increase in serum levels of this lipoprotein. Treatment groups (T. FJP and T. JE) showed increase in HDL cholesterol levels, but this increase was not statistically significant. Thus, HDL cholesterol levels were positively modulated by FJP and JE supplementation for 12 weeks.

Serum leptin and adiponectin levels (Table 3) were lower in HFF group when compared to AIN-93M group. FJP and JE groups showed leptin and adiponectin levels similar to HFF group, showing that these markers were unmodulated by FJP and JE consumption.

Liver weight (g fresh tissue 100g⁻¹ body weight) and liver fat (%) were higher in HFF group when compared to AIN-93M group. Supplemented groups showed lowers liver weight and liver fat that HFF group. Thus, FJP and JE supplementation by 6 and 12 weeks was able to reduce this lipid accumulation in liver tissue.

Table 2. Weight gain, diet and JE consumption and bioactive compounds consumption by experimental animals

	AIN-93M	HFF	P. FJP	P. JE	T. FJP	T. JE
Cumulative weight gain (g ⁻¹)	217.00 ^B ± 7.97	312.18 ^{Aa} ± 24.51	250.41 ^b ± 40.38	220.71 ^b ± 15.67	253.62 ^b ± 9.36	216.15 ^b ± 16.69
Energy intake (Kcal day ⁻¹)	93.52 ± 2.88 ^A	96.60 ^{Aa} ± 1.55	90.82 ^a ± 7.17	89.64 ^a ± 7.01	94.61 ^a ± 3.34	89.99 ^a ± 5.55
Food intake (g day ⁻¹)	24.17 ^A ± 0.82	17.13 ^{B a} ± 1.07	16.91 ^a ± 1.46	17.34 ^a ± 1.12	17.68 ^a ± 0.74	16.61 ^a ± 0.99
Total Phenolics (mg day ⁻¹)	NC	NC	17.04 ^a ± 1.65	17.33 ^a ± 1.02	12.96 ^b ± 1.11	11.48 ^b ± 0.98
Total Flavonoids (mg day ⁻¹)	NC	NC	7.82 ^a ± 0.76	7.95 ^a ± 0.46	7.31 ^a ± 0.33	6.49 ^b ± 0.46
Ellagic acid (mg day ⁻¹)	NC	NC	1.07 ^a ± 0.11	1.08 ^a ± 0.06	0.58 ^b ± 0.05	0.51 ^b ± 0.04
C3G (mg day ⁻¹)	NC	NC	11.55 ^a ± 1.11	11.75 ^a ± 0.69	12.33 ^a ± 1.05	11.12 ^a ± 0.79

Data presented as means ± standard deviation. Statistical differences between AIN-93M and HFF groups are represented by different uppercase letters and statistical differences between HFF and supplemented groups are represented by different lowercase letters. HFF group had the water consumption measured to order to serve as a control for P. JE and T. JE groups. AIN-93M (normal control) standard diet; HFF (obese control) received a high-fat and fructose diet containing 4% (w/w) soybean oil, 31% (w/w) lard and 20% fructose (w/w); P. FJP and T. FJP received the

HFF diet with 2% of FJP powder (w/w), for 12 and 6 weeks respectively; P. JE and T. JE received the HFF and had the water substituted by FJP aqueous extract, for 12 and 6 weeks respectively. The total bioactive compounds in the FJP and JE were determined in a previous study (Lenquiste et al, 2015). NC = no consumed. C3G = cyanidin-3-*O*-glucoside. The bioactive compounds consumption was calculated to multiplying the daily consumption of diet or extract (g or mL) by concentration of each compound in the FJP or JE.

Table 3. Lipid profile, serum adipocytokines and fat accumulation in experimental animals

	AIN-93M	HFF	P. FJP	P. JE	T. FJP	T. JE
Serum Total cholesterol (mg dL ⁻¹)	48.31 ^A ± 7.03	39.67 ^{Aa} ± 2.14	44.42 ^a ± 5.13	41.78 ^a ± 8.53	41.27 ^a ± 4.77	42.56 ^a ± 9.87
Serum HDL cholesterol (mg dL ⁻¹)	40.20 ^A ± 3.27	25.03 ^{Bb} ± 1.99	32.57 ^a ± 4.50	34.06 ^a ± 5.22	27.50 ^{ab} ± 1.04	29.36 ^{ab} ± 1.26
Serum leptin (pg dL ⁻¹)	35.06 ^A ± 3.24	23.85 ^{Bb} ± 3.85	30.54 ^b ± 2.72	19.93 ^b ± 7.25	17.18 ^{bc} ± 4.10	16.50 ^{bc} ± 6.99
Serum adiponectin (pg dL ⁻¹)	22.41 ^A ± 3.22	16.64 ^{Bb} ± 1.50	20.97 ^b ± 4.25	19.93 ^b ± 5.44	19.85 ^b ± 2.62	18.50 ^b ± 3.18
Liver (g 100g ⁻¹)	2.60 ^B ± 0.16	3.19 ^{Aa} ± 0.08	2.84 ^b ± 0.08	2.76 ^b ± 0.17	2.77 ^b ± 0.25	2.85 ^b ± 0.15
Liver Fat (%)	19.20 ^B ± 3.35	54.18 ^{Aa} ± 3.45	44.12 ^b ± 3.51	42.05 ^b ± 6.12	44.13 ^b ± 2.81	43.15 ^b ± 5.21
EAT (g 100g ⁻¹)	2.62 ^B ± 0.41	4.14 ^{Aa} ± 0.55	3.23 ^b ± 0.56	3.14 ^b ± 0.13	3.29 ^b ± 0.29	2.92 ^b ± 0.10
MAT (g 100g ⁻¹)	1.44 ^B ± 0.27	2.92 ^{Aa} ± 0.21	2.28 ^b ± 0.41	2.27 ^b ± 0.18	2.31 ^b ± 0.25	2.01 ^b ± 0.36
RAT (g 100g ⁻¹)	3.45 ^B ± 0.32	5.61 ^{Aa} ± 0.62	4.59 ^b ± 0.82	4.27 ^b ± 0.43	4.44 ^b ± 0.60	3.95 ^b ± 0.80

Data presented as means ± standard deviation. Statistical differences between AIN-93M and HFF groups are represented by different uppercase letters and statistical differences between HFF and supplemented groups are represented by different lowercase letters. EAT = Epididymal Adipose Tissue. MAT = Mesenteric Adipose Tissue. RAT = Retroperitoneal Adipose Tissue.

Experimental animals fed with HFF diet showed increase of 58.00% in EAT weight, 102.80% in MAT weight and 62.61% in RAT weight compared to AIN-93M group. FJP and JE consumption for 6 and 12 weeks reduced to fat accumulation in these adipose tissues. Thus, jaboticaba peel and jaboticaba tea consumption could have a positive impact on the obesity prevention and treatment, especially in abdominal fat accumulation.

Glucose and insulin metabolism

In our study, FJP and JE supplementation improved significantly the glucose and insulin metabolism. Figure 2 shown serum glucose (Fig. 2A) and plasma insulin (Fig. 2B), both markers were higher in HFF group when compared to AIN-93M, indicating that HFF diet induced hyperglycemia and insulin resistance. Groups supplemented with FJP and JE shown reverse this situation, except T.JE group.

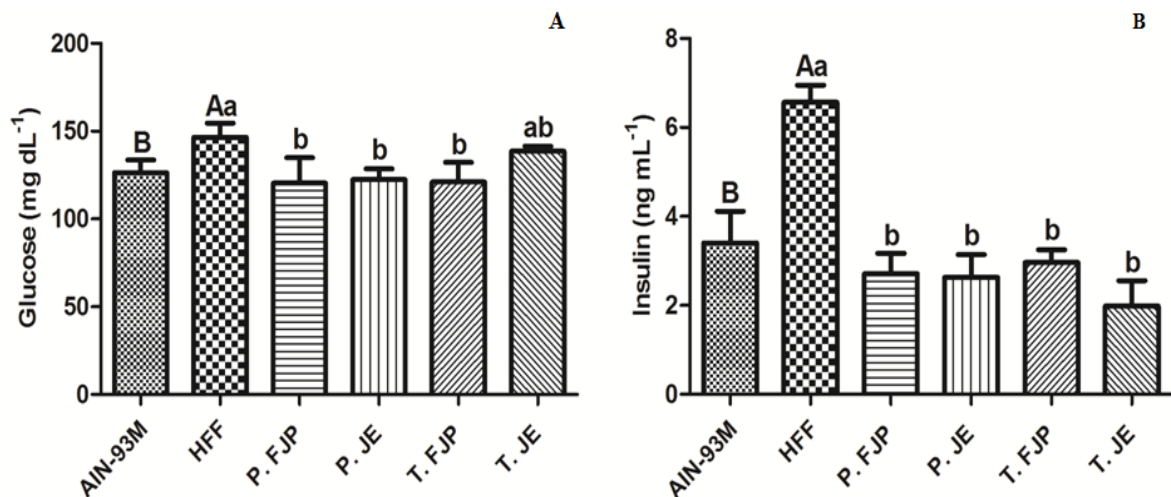


Figure 2. Serum glucose and plasma insulin of experimental animals. A – Serum glucose; B – Plasma insulin. Data presented as means $\pm$ standard deviation. Statistical differences between AIN-93M and HFF groups are represented by different uppercase letters and statistical differences between HFF and supplemented groups are represented by different lowercase letters.

Glucose and Insulin Tolerance Tests (GTT and ITT) are shown in Figure 3. HFF diet intake induced glucose and insulin intolerance compared to AIN-93M group. Glucose tolerance was not significantly higher in FJP and JE supplemented groups, but the absolute values of AUC were lower compared to HFF group (Fig. 3A), despite no statistic difference. However, T. FJP and T. JE were more insulin sensitive, as revealed by the lower blood glucose values observed during the ITT (Fig. 3D) and increased K_{ITT} (Fig. 3C). As regard the P. FJP and P. JE, shown improvement in insulin sensibility, but not showed statistic difference of the HFF group.

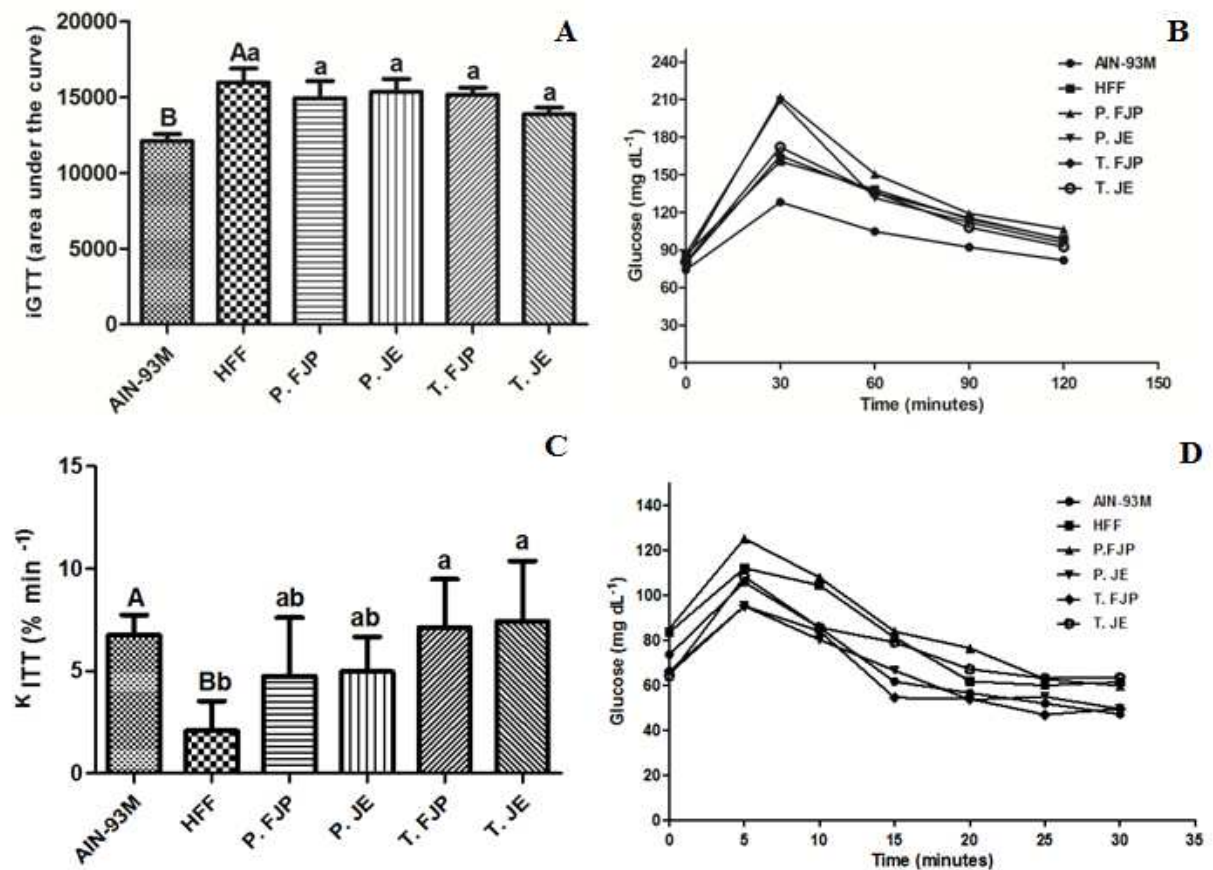


Figure 3. Intraperitoneal Glucose Tolerance Test (iGTT) and intraperitoneal Insulin Tolerance Test (ITT) of experimental animals. A – Area Under Curve (AUC) in iGTT test; B – Mean of blood glucose levels at 0, 30, 60, 90 and 120 min after intraperitoneal infusion of glucose solution; C – Mean values of K_{ITT} (curve slope in % min⁻¹); D – Mean blood glucose

at 0, 5, 10, 15, 20, 25 and 30 min after intraperitoneal infusion of insulin. Data presented as means $\pm$ standard deviation. Statistical differences between AIN-93M and HFF groups are represented by different uppercase letters and statistical differences between HFF and supplemented groups are represented by different lowercase letters.

Evaluation of pancreatic islet insulin secretion is shown in Figure 4. Total insulin content (Fig. 4A) was measured without glucose stimulus. HFF group showed higher islet insulin content compared to AIN93-M group. FJP and JE supplemented groups showed lower islet insulin content compared to HFF group. Insulin content secreted by islets after glucose stimulus is shown in figure 4B. In sub-stimulatory glucose levels (2.8 mMol/L) the insulin secretion by HFF group was similar to AIN93-M group. P. FJP and T. JE groups showed lower insulin secretion in relation to HFF group. T. FJP and P. JE groups showed insulin secretion similar to HFF group and others supplemented groups. In supra-stimulatory (16.7 mMol/L) glucose conditions, HFF group showed higher insulin secretion in comparison to AIN93-M group. FJP and JE supplemented groups showed lower insulin secretion that HFF group, indicating that the jaboticaba peel consumption could reverse the hyper insulin secretion by pancreatic islets in response to hyperglycemia.

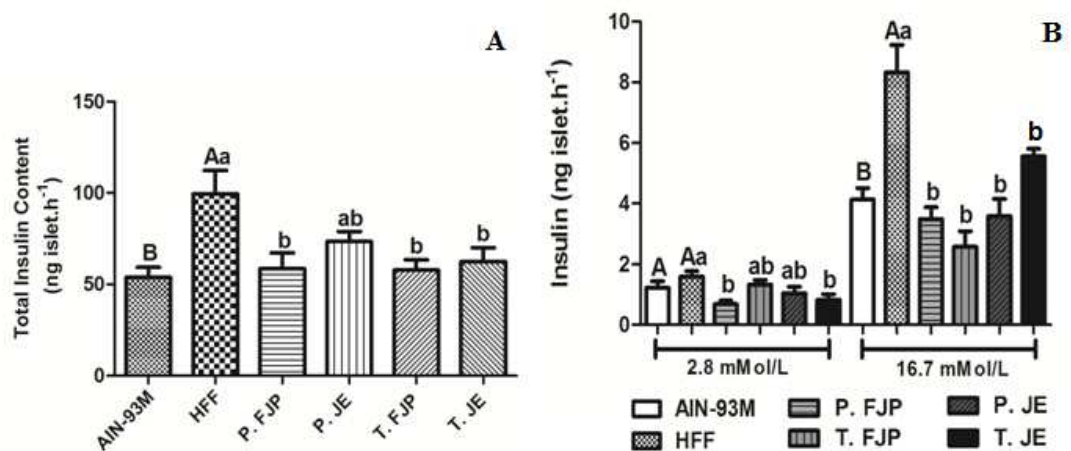


Figure 4. Estatic Insulin Secretion on pancreatic islets of experimental animals. A – Total insulin content on islets without stimulus; B – Insulin content secreted by islets after glucose

stimulus. Data presented as means $\pm$ standard deviation. Statistical differences between AIN-93M and HFF groups are represented by different uppercase letters and statistical differences between HFF and supplemented groups are represented by different lowercase letters.

Liver histology

Qualitative histological analysis of liver morphology showed liver tissue with typical structure in AIN93-M group (Fig. 5A). Hepatocytes showed hexagonal structure and central nucleus arranged in form of strands. In addition, this cells provision determined the occupied spaces by the sinusoid, which showed a regular shape. In HFF group (Fig. 5B) a higher fat accumulation was observed. This group presented a cell swelling with the nucleus of hepatocytes moved to periphery. As a result of swelling, lower sinusoid shape was found, which made it difficult to see. Extensive areas of inflammatory infiltrates were observed, as shown in detail. Long term FJP and jaboticaba tea (JE) intake by 12 weeks (Fig. 5C and 5E) led to tissue structure similar to that found into the AIN93-M group. Although, there is a small fat accumulation in some regions, the tissue showed hepatocytes with regular morphology, visible sinusoids and occasional inflammatory infiltrate foci. However, short term FJP and jaboticaba tea (JE) intake by 6 weeks (Fig. 5D e 5F) was not able to attenuate the harmful effects of high fat diet consumption on liver morphology, presenting liver tissue characteristics similar to HFF group. Thus, the long term of FJP and jaboticaba tea could have a positive effect in liver fat accumulation and inflammatory process.

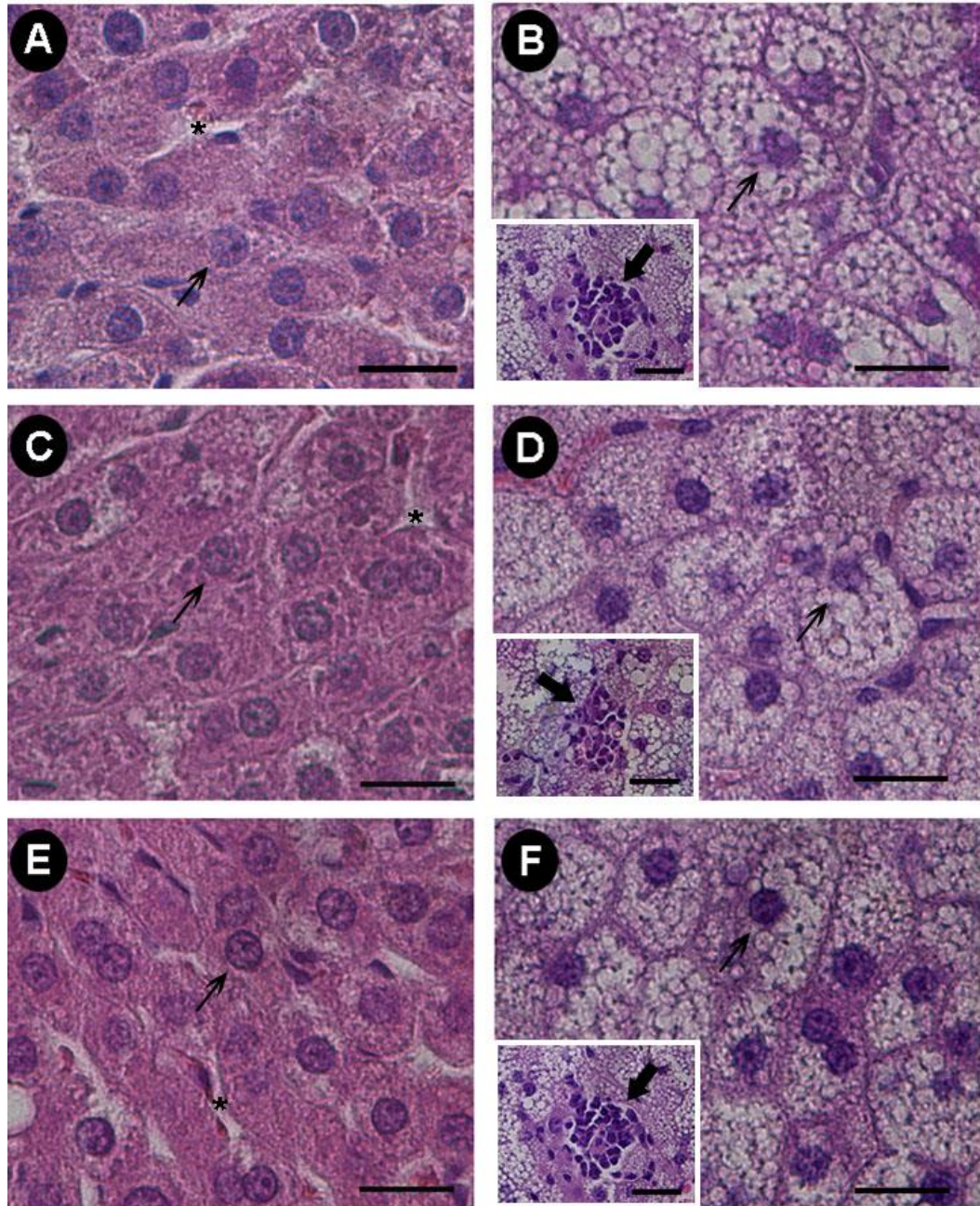


Figure 5. Histological analysis of liver. A – AIN-93M group; B – HFF group; C – P. FJP group; D – T. FJP group; E – P. JE group; F – T. JE group. Thin arrow indicates hepatocytes. Thick arrow points inflammatory infiltrate. Star indicates sinusoidal capillary. Scale bar = 20μm. Histological sections were stained with hematoxylin and eosin (HE).

4 DISCUSSION

Recent studies demonstrated that high-fat and high-carbohydrate diets are able to induce obesity, dyslipidemia, hepatic fat accumulation, inflammation and impaired glucose and insulin tolerance, similarly to many signs of human metabolic syndrome associated with obesity development (Shapiro, Tumer et al. 2011, Poudyal, Panchal et al. 2012). Fructose intake is associated to effects in carbohydrate and lipid metabolism, inducing metabolic abnormalities in humans and animals (Tappy and Le 2010, Vos and Lavine 2013).

Jaboticaba (*Myrciaria* spp.) is a typical Brazilian fruit whose peel is a great source of fibers, soluble and insoluble, and phenolic compounds especially anthocyanins and ellagic acid (Leite, Malta et al. 2011, Abe, Lajolo et al. 2012, Alezandro, Dube et al. 2013, Lenquiste, Marineli et al. 2015). Besides phenolic compounds naturally present in leaves and flowers infusions, fruit teas are abundant in pigments, such as anthocyanins, derived mainly from the peel (Piljac-Zegarac, Valek et al. 2010). Despite nutritional proprieties, jaboticaba peel is not consumed conventionally. Therefore, the preparation of tea with jaboticaba peel could be an alternative for consumption.

The effects of phenolic compounds on obesity have been studied and its effect on weight gain and adipose mass are still controversial. Our data demonstrated that jaboticaba peel (FJP) and jaboticaba tea (JE) by 12 or 6 weeks were able to reduce weight gain and adipose tissue weight. These findings are in accordance with studies that evaluated the effects of purified anthocyanins supplementation in high-fat diet on the development of obesity. Jayaprakasam, Olson et al. (2006) and Prior, Wu et al. (2008) showed that the supplementation of different sources of cyanidin-3-*O*-glucoside (C3G) extracted in a high-fat diets reduced mice weight gain. In previous study, we showed that FJP and JE supplementation reduced the weight gain in *Wistar* rats (Lenquiste, Marineli et al. 2015).

Esposito, Damsud et al. (2015) showed that the daily consumption of low- or high-fat diet supplemented with 1% black currant powdered extract (32% anthocyanins) for 8 weeks reduced body weight gain in obese mice. However other studies showed that the consumption of freeze-dried foods as a source of anthocyanins added to high-fat diet had not effects on weight gain neither in body fat in animal models (DeFuria, Bennett et al. 2009, Prior, Wilkes et al. 2010, Lenquiste, Batista et al. 2012, Dragano, Marques et al. 2013, Batista, Lenquiste et al. 2014).

Nevertheless, acceptable mechanisms for antiobesogenic potential of these compounds have been proposed. Inhibitory activity of α -amylase and α -glucosidase enzymes in induced diabetic rats fed with jaboticaba peel was demonstrated by Alezandro, Granato et al. (2013). Similar results were presented with two ellagitannins isolated from jaboticaba indicating that this edible fruit may also have α -glucosidase inhibitory activity (Wu, Wu et al. 2013). Sergent, Vanderstraeten et al. (2012) showed that phenolic compounds of green tea inhibited pancreatic lipase activity which delayed or decreased *in vivo* fatty acid absorption by enterocytes. Thus, the enzymatic inhibition can be a mechanism in the obesity regulation by phenolic compounds.

Obesity is associated with several metabolic complications, which have significant impact on health. Lipid profile is an important metabolic variables altered in obesity. Elevated levels of total cholesterol, LDL-cholesterol, triglycerides and low levels of HDL-cholesterol are associated with higher incidence of cardiovascular diseases, as atherosclerosis, and diabetes (Bastard, Maachi et al. 2006, Sasaki, Nishimura et al. 2007).

Our results showed an increase of 37 and 42% in HDL-cholesterol in animals that received FJP diet or jaboticaba tea (JE) for 12 weeks, respectively, in relation to the HF group. Groups supplemented for 6 weeks presented an increase in HDL cholesterol, but did

not differ from HFF group. As there was no reduction in circulating levels of total cholesterol, it is assumed that this increase in HDL-cholesterol is a consequence of decreased levels of LDL-cholesterol. Thus, it is suggested that FJP and jaboticaba tea (JE) long term consumption could have a protective effect against cardiovascular disease by means of elevation levels of HDL-cholesterol and possible decrease of LDL-cholesterol. Previous studies with FJP-fed obese animals showed no significant differences in the serum concentration of total cholesterol (Lenquiste, Batista et al. 2012, Batista, Lenquiste et al. 2013). However, FJP intake for 8 weeks increased HDL-cholesterol in obese animals (Lenquiste, Batista et al. 2012) and reduced serum saturated lipids (SFA), which are associated with atherosclerosis, inflammatory process and insulin resistance (Batista, Lenquiste et al. 2014). Recent study showed that streptozotocin (STZ) induced diabetic rats that received a jaboticaba peel powder dispersed in water for 40 days by gavage reduced triglycerides and total cholesterol, but not increased HDL-cholesterol (Alezandro, Granato et al. 2013). Thus, jaboticaba peel intake present positive effects on lipid profile, but these effects may differ according to the experimental model and supplementation type and time.

Adipose tissue hypertrophy, especially in abdominal area as in obesity, is related to occurrence of metabolic disorders, such as insulin resistance and cardiovascular disease (Poulos, Hausman et al. 2010). Notable emphasis has been given to the role of hormones produced in adipose tissue in obesity and insulin resistance (Vazquez-Vela, Torres et al. 2008, Lago, Gomez et al. 2009). Leptin has an important role in the regulation of metabolism by stimulus of energy expenditure, inhibiting food intake and restoring euglycemia, however, in most obesity cases, leptin resistance limits its biological effectiveness. In contrast to leptin, adiponectin secretion is generally decreased in obesity. Adiponectin acts increasing insulin sensitivity, oxidation of fatty acids as well as decreasing energy reserves and glucose production by the liver (Galic, Oakhill et al. 2010, Poulos, Hausman et al. 2010). Animals fed

with HFF diet showed reduced levels of these hormones compared to control animals. FJP and jaboticaba tea (JE) supplementation was not able to modulate these serum adipokines. Similar results were showed by Lenquist, Batista et al. (2012) and Dragano, Marques et al. (2013) wherein FJP supplementation in high-fat diet did not modulate serum adiponectin and leptin levels in *Sprague-Dawley* rats and serum leptin levels in obese mice, respectively.

Impairment in glucose and insulin metabolism is a central problem in obesity pathology, increasing diabetes and cardiovascular risk (Qatanani and Lazar 2007). The pathogenesis of glucose intolerance is complex and is mainly a function of the interplay between insulin sensitivity and endocrine pancreatic function (Bavenholm, Pigon et al. 2001). In our study, animals that received FJP and jaboticaba tea (JE) by 6 and 12 weeks showed a reversal of hyperglycemia and hyperinsulinemia caused by HFF diet. However, iGTT did not demonstrate improvement in supplemented groups compared to HFF group. In κ ITT the groups supplemented with FJP and JE for 6 weeks presented best results than groups supplemented for 12 weeks, but it may be due to the large variation in response among animals that give a high standard deviation. Total insulin content in pancreatic islets agrees with serum insulin results, showing that FJP and jaboticaba tea (JE) supplementation reduced insulin secretion, as well as pancreatic islet insulin secretion by glucose stimulus. Thus, our study demonstrate that animals that received FJP and jaboticaba tea (JE) for 12 or 6 weeks were protected against insulin resistance based on a significant improvement in fasting serum glucose, hyperinsulinemia and pancreatic islet insulin secretion presented by these animals. These finding are in accordance with previous studies that showed improvement in hyperinsulinemia and HOMA-IR index in obese rats supplemented with 2% FJP in high-fat diet (Lenquist, Batista et al. 2012) and significant increase in κ ITT and improve in insulin signaling by increase the insulin receptor/insulin receptor substrate-1/Akt/forkhead box protein pathway (Dragano, Marques et al. 2013).

Considered as the hepatic manifestation of the metabolic syndrome, nonalcoholic fatty liver disease (NAFLD) is a clinico-histopathological condition characterized by fat accumulation in hepatocytes, associated or not with inflammatory process. Visceral adiposity and insulin resistance play an initial key role for the onset and perpetuation of liver steatosis (Krawczyk, Bonfrate et al. 2010). Certain dietary sugars, particularly fructose, seem to contribute more than fatty acids intake to the development of NAFLD and its progression (Vos and Lavine 2013). Weight liver and liver fat were lower in FJP and JE groups when compared to HFF groups, independent to supplementation time. Histological analysis of hepatic tissue showed that HFF diet induced liver steatosis. FJP and jaboticaba tea (JE) supplementation for 12 weeks was able to reverse this condition, but supplementation for 6 weeks did not show the same effect.

Some studies have shown positive effects of phenolic compounds intake on liver fat accumulation. Bujanda, Hijona et al. (2008) evaluated the effect of resveratrol supplementation in *Wistar* rats with liver steatosis induced by high carbohydrate-fat free modified diet for 4 weeks. Histological analyses showed that resveratrol supplementation reversed liver steatosis condition and lipid peroxidation in the hepatic tissue. Recent study showed that quercetin treatment in rats fed with high-carbohydrate or high-fat diets attenuated liver fat accumulation and inflammatory process in hepatic tissue. The authors attribute these effects to mechanisms related to decreased oxidative stress and inflammation in tissue (Panchal, Poudyal et al. 2012).

In addition, these findings indicate that phenolic compounds and anthocyanins are responsible for positive effects presented by jaboticaba peel intake, considering that similar results were found with supplementation of FJP, which is also a great source of fibers, and with jaboticaba tea (JE).

5 CONCLUSION

In summary, the present study shows that a daily consumption of FJP and jaboticaba tea reduces weight gain and adiposity. FJP and jaboticaba tea supplementation by 12 weeks increases HDL-cholesterol. Total cholesterol and serum leptin and adiponectin were not affected by FJP and jaboticaba tea supplementation. Insulin resistance was positively modulated by FJP and jaboticaba tea supplementation by 6 weeks. In addition, FJP and jaboticaba tea supplementation for 12 weeks reverse liver steatosis. Our results therefore suggest that FJP and jaboticaba tea could serve as a functional food and may be a dietary strategy to prevent or control obesity and related diseases. The long term supplementation (12 weeks) showed positive effects in most important parameters of obesity and insulin metabolism. However, future studies are needed to evaluate the mechanisms by which FJP and jaboticaba tea promote these effects.

6 ACKNOWLEDGEMENTS

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4. DISCUSSÃO GERAL

A casca da jabuticaba possui grande potencial como alimento funcional e como corante natural para a indústria de alimentos (DESSIMONI-PINTO et al., 2011). Adicionalmente, a casca de jabuticaba pode ser utilizada para o preparo de chás, como o formulado no presente estudo. A proposta de um chá de casca de jabuticaba mostra-se como uma alternativa viável de consumo desta fração convencionalmente não consumida desta fruta. Estudos apontam o chá como a segunda bebida mais popular no mundo. Além disso, infusões de frutas contém grande quantidade de flavonoides, substâncias da classe dos compostos fenólicos, aos quais são atribuídas diversas propriedades benéficas à saúde. (EL-BESHBISHY, 2005).

Nossos resultados mostram que a casca de jabuticaba, bem como o chá obtido a partir dela, possui elevada concentração de antocianinas, flavonoides e compostos fenólicos totais, assemelhando-se aos resultados apontados por ALEZANDRO; DUBE; et al. (2013), porém em concentrações inferiores às apontadas por BATISTA et al. (2014). Alguns compostos fenólicos específicos e antocianinas foram dosados por cromatografia líquida de alta eficiência (HPLC) no extrato metanólico da casca de jabuticaba liofilizada (CJL) e no chá da casca de jabuticaba liofilizada (CJL-chá). As concentrações encontradas de ácido gálico e ácido elágico na CJL foram menores que o demonstrado por WU et al. (2013) e similares aos obtido por ABE; LAJOLO; GENOVESE (2012). A concentração de cianidina-3-O-glicosídeo encontrada na CJL foi menor em relação ao CJL-chá, porém ambos os produtos mostraram níveis desta antocianina superiores aos obtidos em estudos anteriores (LEITE et al., 2011; WU et al., 2013).

A comparação dos teores de compostos bioativos e da capacidade antioxidante entre plantas é um difícil trabalho. As diferenças nas concentrações de compostos fenólicos e antocianinas entre os estudos que avaliaram estes compostos na casca de jabuticaba podem ser decorrentes de diversos fatores, desde as diferenças de safra, a qual será influenciada pelo solo, condições climáticas, disponibilidade de chuva, até o tipo de solvente e método de extração utilizado nos ensaios (JAAKOLA; HOHTOLA, 2010). As antocianinas são mais solúveis em água que em metanol, além disso, o aquecimento aumenta a extração de alguns compostos, através da quebra de pontes de hidrogênio, o que pode justificar a maior concentração desta antocianina no chá de jabuticaba que na CJL (AZSMIR et al., 2013).

A capacidade antioxidante da CJL e do CJL-chá foi mensurada pelos ensaios de DPPH, FRAP, ABTS e ORAC. Nos ensaios de DPPH e ORAC, o extrato metanólico de CJL mostrou maior capacidade antioxidante. O ensaio de ABTS mostrou o CJL-chá como tendo capacidade antioxidante maior que o extrato metanólico da CJL. Estudos anteriores com extratos metanólicos da casca de jabuticaba mostraram menores valores para os ensaios de DPPH, FRAP e ABTS (ABE et al., 2012; ALEZANDRO; DUBE; et al., 2013; LEITE-LEGATTI et al., 2012). Porém, para o ensaio de ORAC, maior capacidade antioxidante da casca de jabuticaba foi apresentada por LEITE-LEGATTI et al. (2012) e BATISTA et al. (2014). Assim, a casca de jabuticaba utilizada neste estudo, bem como o chá obtido a partir dela mostram elevada concentração de compostos fenólicos e antocianinas, bem como alto potencial antioxidante *in vitro*, quando comparada a outros estudos que avaliaram esses parâmetros.

A prevenção e combate do dano oxidativo é um dos principais objetos de estudo quando se fala em compostos bioativos (HAN, C. H. et al., 2007). Em nosso estudo foi avaliado o potencial antioxidante no plasma e fígado dos ratos por meio do ensaio FRAP, porém não encontramos modificação deste parâmetro com a suplementação com CJL e CJL-chá. Contrapondo-se ao nosso resultado, LEITE et al. (2011) demonstrou em ratos eutróficos suplementados com CJL nas concentrações de 1 e 2% melhora do potencial antioxidante plasmático evidenciada pelo ensaio de ABTS. Em estudo similar, BATISTA et al. (2014) avaliou o impacto da suplementação com 1, 2 e 4% de CJL adicionada à dieta hiperlipídica oferecida a ratos no potencial antioxidante do plasma e alguns tecidos. Nesse estudo, observou-se significativo aumento do potencial antioxidante plasmático nos animais alimentados com 2 e 4% de CJL por meio de ensaio TEAC, mas não pelos ensaios FRAP e ORAC. No fígado dos animais destes mesmos grupos (2 e 4%) também houve melhora do potencial antioxidante evidenciada pelos ensaios de ORAC. O ensaio de FRAP foi utilizado por ALEZANDRO; GRANATO; GENOVESE (2013) para avaliar o estresse oxidativo em ratos com diabetes induzida por estreptozotocina suplementados com um extrato de CJL. Neste estudo os animais que receberam o extrato de CJL mostraram aumento de 2 a 2,5 vezes do potencial antioxidante plasmático após 40 dias de experimento.

A determinação das espécies reativas ao ácido tiobarbitúrico (TBARS), dentre as quais se destaca o malondialdeído (MDA) como um importante marcador biológico, é um dos métodos mais utilizados para avaliar a peroxidação lipídica (DENG et al., 2010). Os níveis

plasmáticos e hepáticos de MDA foram mensurados nos animais experimentais. A dieta HFF mostrou induzir a peroxidação lipídica no plasma e no fígado dos animais. Esse dano oxidativo foi revertido pela suplementação com a CJL e com CJL-chá, evidenciando melhora da peroxidação lipídica induzida pela dieta. Em concordância com nossos resultados, diversos estudos mostraram melhora da peroxidação lipídica no plasma e fígado mediado pelo consumo de casca de jabuticaba e de outras fontes de antocianinas (ALEZANDRO; DUBE; et al., 2013; BATISTA et al., 2014; HAN, K. H. et al., 2007; WU et al., 2013).

O aumento do estresse oxidativo é relacionado com a maior produção de radicais livres ou a deficiência de algum dos sistemas antioxidantes endógenos (HABIB; IBRAHIM, 2011). A proteína glutathione é a principal fonte de poder redutor nos organismos. Ela é mantida em sua forma reduzida (GR) na qual atua em conjunto com NADPH. Assim, o aumento nos níveis de GSH associado a um aumento ou manutenção dos níveis de GR pode indicar uma melhora no sistema antioxidante mediado pelo ciclo das glutathiones (SZACHOWICZ-PETELSKA et al., 2012). WU et al. (2015) mostrou aumento na atividade de GPx e SOD no plasma de camundongos suplementados com um extrato rico em flavonoides. Em estudo recente, foi observado que a suplementação com CJL em animais com obesidade induzida por dieta promoveu aumento na atividade de SOD e catalase no plasma, bem como de SOD, CAT e GPx no fígado dos animais (BATISTA et al., 2014). Resultados similares foram encontrados por ALEZANDRO et al., 2013, que mostraram aumentos dessas enzimas em plasma, rins, cérebro e fígado de ratos diabéticos suplementados com um extrato de CJL por gavage.

Diversos estudos *in vivo* têm mostrado que a suplementação com alimentos ricos em compostos fenólicos melhoram a atividade das enzimas antioxidantes (HAN, K. H. et al., 2007; SUWANNAPHET et al., 2010; TEDESCO et al., 2001). Evidências científicas mostram que os compostos fenólicos, especialmente as antocianinas, possuem importante potencial antioxidante *in vitro* e *in vivo*, contudo ainda são necessários estudos que mostrem resultados mais consistentes e desvendem os mecanismos biológicos de ação desses compostos.

Estudos recentes têm mostrado que dietas hiperlipídicas e hiperglicídicas, especialmente as ricas em frutose, são capazes de induzir obesidade, dislipidemias, esteatose hepática, inflamação, além de prejudicar a tolerância à glicose e sensibilidade à insulina, assemelhando-se à síndrome metabólica observada em humanos (POUDYAL et al., 2012; SHAPIRO et al., 2011). A ingestão de frutose é associada a efeitos no metabolismo de carboidratos e lipídios, induzindo anormalidades metabólicas em humanos e animais

(TAPPY; LE, 2010; VOS; LAVINE, 2013). Em nosso estudo, a dieta HFF induziu a obesidade, resistência à insulina e esteatose hepática em ratos *Wistar*.

Os efeitos dos compostos fenólicos no ganho de peso e adiposidade têm sido estudados, contudo os resultados ainda são controversos. Nossos resultados mostram que o consumo de CJL e CJL-chá por 12 ou 6 semanas foi capaz de reduzir o ganho de peso e o acúmulo de tecido adiposo. Esses achados são concordantes com estudos que avaliaram o efeito da suplementação com antocianinas purificadas em dietas hiperlipídicas no desenvolvimento da obesidade. JAYAPRAKASAM et al. (2006) e PRIOR et al. (2008) mostraram que a suplementação de cianidina-3-O-glicosídeo extraída de diferentes fontes em dietas hiperlipídicas reduziu o ganho de peso de camundongos. ESPOSITO et al. (2015) mostraram que o consumo diário de 1% de extrato de cássis (32% de antocianinas) por 8 semanas reduziu o ganho de peso em camundongos obesos. Contrapondo-se a isso, estudos mostram que o consumo de alimentos fonte de antocianinas liofilizados adicionados à dietas hiperlipídicas não teve efeito em parâmetros de ganho de peso e composição corporal de modelos animais (DEFURIA et al., 2009; DRAGANO et al., 2013; LENQUISTE et al., 2012; PRIOR et al., 2010). Tais resultados conflitantes sobre o efeito das antocianinas no ganho de peso e composição corporal podem ser devido às diferentes fontes alimentares usadas, modelos experimentais diferenciados, bem como da dieta utilizada para induzir a obesidade nos animais.

A obesidade é associada com diversas complicações, as quais tem grande impacto sobre a saúde. O perfil lipídico é uma das mais importantes variáveis alteradas na obesidade. Elevados níveis de colesterol total, LDL-colesterol e triglicerídeos, além de baixos níveis de HDL-colesterol são associados com maior incidência de doenças cardiovasculares, como aterosclerose, e diabetes (BASTARD et al., 2006; SASAKI et al., 2007).

No presente trabalho, observamos aumento de 37 e 42% do HDL-colesterol nos animais que receberam CJL ou CJL-chá por 12 semanas, respectivamente, em relação ao grupo HFF. Os grupos suplementados por 6 semanas apresentaram aumento do HDL-colesterol, porém este não foi significativo estatisticamente. Como não houve redução nos níveis séricos de colesterol total, acredita-se que esse aumento no HDL-colesterol seja um reflexo na diminuição do LDL-colesterol. Assim, sugere-se que o consumo prolongado de CJL e CJL-chá pode ter efeito protetivo contra as doenças cardiovasculares, possivelmente por meio da redução do LDL-colesterol e aumento do HDL-colesterol. Estudos prévios com

animais obesos alimentados com CJL mostraram resultados bastante similares, com aumento do HDL-colesterol (LENQUISTE et al., 2012), porém sem alteração do colesterol total (BATISTA et al., 2013; LENQUISTE et al., 2012). Estudo recente de BATISTA et al. (2014) mostrou ainda que o consumo de CJL por ratos com obesidade induzida por dieta reduziu os níveis circulatórios de ácidos graxos saturados, os quais são associados com aterosclerose, processo inflamatório e resistência à insulina.

A hipertrofia do tecido adiposo, especialmente na região abdominal, é relacionada com a ocorrência de desordens metabólicas, como resistência à insulina e doenças cardiovasculares (POULOS; HAUSMAN; HAUSMAN, 2010). Grande ênfase tem sido dada ao papel dos hormônios produzidos pelo tecido adiposo na obesidade e resistência à insulina (LAGO et al., 2009; VAZQUEZ-VELA; TORRES; TOVAR, 2008). A leptina é um hormônio produzido pelo tecido adiposo responsável pelo controle do apetite e do gasto energético, inibindo o consumo alimentar e restaurando a euglicemia. Porém, em muitos indivíduos obesos, é observada uma resistência à ação deste hormônio, o que limita sua eficiência biológica. Ao contrário da leptina, a secreção de adiponectina é geralmente diminuída na obesidade. A adiponectina age aumentando a sensibilidade à insulina, a oxidação de lipídios, além de diminuir as reservas energéticas e a produção hepática de glicose (GALIC; OAKHILL; STEINBERG, 2010; POULOS et al., 2010). Como esperado, os animais alimentados com a dieta HFF mostraram menores níveis desses hormônios quando comparados ao controle, porém a suplementação com CJL e CJL-chá não modulou os níveis séricos dessas adipocinas. Resultados similares foram demonstrados por LENQUISTE et al. (2012) e DRAGANO et al. (2013) em que a suplementação com CJL na dieta hiperlipídica não modulou os níveis séricos de adiponectina e leptina em ratos *Sprague-Dawley* e de leptina em camundongos *Swiss*, respectivamente.

O prejuízo no metabolismo de glicose e insulina é um problema central na patologia da obesidade, aumentando o risco de diabetes e doenças cardiovasculares (QATANANI; LAZAR, 2007). A patogênese da intolerância à glicose é complexa e depende da interação entre a sensibilidade à insulina e função pancreática endócrina (BAVENHOLM et al., 2001). Em nosso estudo, os animais que receberam CJL e CJL-chá por 6 ou 12 semanas mostraram reversão da hiperglicemia e hiperinsulinemia induzidas pela dieta. Contudo o teste de tolerância à glicose (GTT) não mostrou melhora comparado ao grupo HFF. No teste de tolerância à insulina (ITT) os grupos suplementados por 6 semanas mostraram significativa

melhora da resistência à insulina comparados ao grupo HFF, enquanto que os suplementados por 12 semanas não mostraram diferença estatística do grupo HFF. Contudo, tal fato pode ser decorrente da grande variação que houve entre os animais, provocando um alto desvio padrão.

O conteúdo total de insulina nas ilhotas pancreática está de acordo com os resultados de insulina plasmática, evidenciando que a suplementação com CJL e CJL-chá reduziu a secreção de insulina, bem como a secreção de insulina estimulada por glicose. Assim, nosso estudo demonstra que a suplementação com CJL e CJL-chá protegeu os animais da resistência à insulina, por meio da melhora da glicemia sérica de jejum, redução da hiperinsulinemia e da secreção de insulina pelas ilhotas pancreáticas, com ou sem estímulo de glicose. Esses achados estão em concordância com estudos anteriores que mostraram melhora da hiperinsulinemia e índice HOMA-IR em ratos obesos suplementados com 2% de CJL (LENQUISTE et al., 2012), bem como significativo aumento na velocidade de decaimento da glicose no ITT (k_{ITT}) e melhora da sinalização de insulina por meio do aumento da expressão do receptor de insulina e da proteína fosforilada AKT (DRAGANO et al., 2013). Diante do exposto, acredita-se que a casca de jabuticaba pode atuar positivamente no controle da resistência à insulina e prevenção do diabetes mellitus tipo 2.

Considerada como uma manifestação hepática da síndrome metabólica, a doença hepática gordurosa não alcoólica (DHGNA) é uma condição clínico-histopatológica caracterizada pelo excessivo acúmulo de gordura no fígado, associada ou não a processo inflamatório. A adiposidade visceral e a resistência à insulina possuem papel chave na iniciação e perpetuação da esteatose hepática (KRAWCZYK; BONFRATE; PORTINCASA, 2010). A ingestão dietética de frutose parece contribuir mais expressivamente para o desenvolvimento de DHGNA do que os ácidos graxos da dieta (VOS; LAVINE, 2013). O peso do fígado, bem como o percentual de gordura no tecido hepático foi maior nos animais do grupo HFF comparado ao AIN-93M. A suplementação de CJL e CJL-chá por 6 e 12 semanas mostrou reverter o acúmulo de gorduras no tecido. A análise histológica do tecido hepático mostra que o grupo HFF desenvolveu esteatose hepática e que a suplementação por 12 semanas com CJL e CJL-cha reverteu esse processo patológico, porém 6 semanas de suplementação não foram suficientes para mostrar os mesmos resultados.

Alguns estudos avaliaram os efeitos da ingestão dietética de compostos fenólicos na esteatose hepática. BUJANDA et al. (2008) avaliaram o efeito da suplementação de resveratrol por 4 semanas em ratos *Wistar* com esteatose hepática induzida por dieta rica em

frutose. As análises histológicas mostraram que a suplementação com resveratrol reverteu a esteatose hepática e a peroxidação lipídica no fígado. Estudo recente mostrou que o tratamento com quercetina em ratos alimentados com dieta hiperlipídica ou hiperglicídica atenuou a acumulação de gordura no fígado e o processo inflamatório neste tecido. Os autores deste trabalho atribuem os efeitos à ação antioxidante da quercetina no tecido hepático (PANCHAL; POUDYAL; BROWN, 2012).

Nossos achados a cerca do potencial antiobesogênico da CJL e CJL-chá indicam que os compostos fenólicos e antocianinas são os responsáveis pelos efeitos positivos da casca de jabuticaba, tendo em vista que resultados semelhantes foram obtidos com a suplementação da CJL e com o chá de jabuticaba. Assim, acreditamos que atribuir os efeitos encontrados com a suplementação da casca de jabuticaba às fibras presentes nessa fração do alimento seria errôneo, uma vez que o chá se apresenta isento desses componentes da casca.

5. CONCLUSÃO GERAL

A casca de jabuticaba (CJL) e o chá da casca de jabuticaba liofilizada (CJL-chá) mostraram elevadas concentrações de compostos bioativos, especialmente antocianinas e ácido elágico. O chá da casca de jabuticaba pode ser uma alternativa de viabilizar o consumo dessa fruta, independentemente da sazonalidade e perecibilidade. Além disso, a CJL e CJL-chá mostraram alto potencial antioxidante *in vitro*.

No estudo do potencial antioxidante *in vivo*, alguns biomarcadores foram positivamente modulados pela CJL e CJL-chá, tais como TBARS, GSH e atividade da enzima catalase, porém outros marcadores, como FRAP, SOD, GPx e GR, não responderam positivamente à suplementação com casca de jabuticaba.

No que diz respeito à obesidade, a suplementação por 6 ou 12 semanas com CJL e CJL-chá reduziu o ganho de peso e a adiposidade visceral, bem como o percentual de gordura no fígado. A suplementação com CJL e CJL-chá por 12 semanas também elevou os níveis de HDL-colesterol, porém não houve modulação dos níveis de colesterol total, leptina e adiponectina com 6 ou 12 semanas de suplementação.

A resistência à insulina foi positivamente modulada pela suplementação com CJL e CJL-chá por 6 e 12 semanas. A esteatose hepática foi revertida nos animais suplementados por 12 semanas, mas não nos animais suplementados por 6 semanas, mostrando que a suplementação com casca de jabuticaba por mais longo período pode promover melhores resultados.

Nossos resultados sugerem que a casca de jabuticaba e o chá da casca de jabuticaba possuem grande potencial funcional, podendo ser utilizados como estratégia nutricional na prevenção e combate ao dano oxidativo, obesidade, resistência à insulina e esteatose hepática.

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7. ANEXOS



CEUA/Unicamp

Comissão de Ética no Uso de Animais
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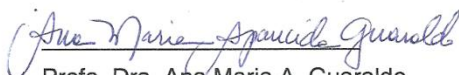
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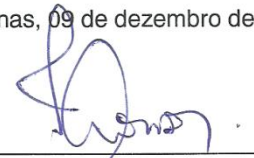
Certificamos que o projeto "Avaliação do potencial da casca de jabuticaba liofilizada e do seu extrato homogeneizado a alta pressão na prevenção e tratamento da obesidade e comorbidades induzida por dieta hiperlipídica e hiperglicídica em ratos Wistar" (protocolo nº 3272-1), sob a responsabilidade de Prof. Dr. Mário Roberto Maróstica Júnior / Sabrina Alves Lenquiste, está de acordo com os **Princípios Éticos na Experimentação Animal** adotados pela **Sociedade Brasileira de Ciência em Animais de Laboratório (SBCAL)** e com a legislação vigente, **LEI Nº 11.794, DE 8 DE OUTUBRO DE 2008**, que estabelece procedimentos para o uso científico de animais, e o **DECRETO Nº 6.899, DE 15 DE JULHO DE 2009**.

A aprovação pela CEUA/UNICAMP não dispensa autorização prévia junto ao **IBAMA, SISBIO ou CIBio**.

O projeto foi aprovado pela Comissão de Ética no Uso de Animais da Universidade Estadual de Campinas - CEUA/UNICAMP - em 09 de dezembro de 2013.

Campinas, 09 de dezembro de 2013.


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