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GINSENG, GREEN TEA OR FIBRATE: valid options for nonalcoholic steatohepatitis prevention?

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ABSTRACT - ***Objective*** - *Panax ginseng*, *Camellia sinensis* and bezafibrate were compared for their lipid-lowering, antioxidant and anti-inflammatory properties as potential agents to prevent nonalcoholic fatty liver disease and its progression to nonalcoholic steatohepatitis. ***Methods*** - Fifty Wistar rats were randomized into five groups: G1 (feed with standard diet); G2 (feed with high-fat diet with 58% of energy from fat); G3 (high-fat diet + standardized *Panax ginseng* extract at 100 mg/kg/day); G4 (high-fat diet + standardized *Camellia sinensis* extract at 100 mg/kg/day); and G5 (high-fat diet + bezafibrate at 100 mg/kg/day), given by gavage. The animals were sacrificed eight weeks later and blood was collected for glucose, insulin, cholesterol, triglycerides, AST, ALT, alkaline phosphatase and gamma-glutamyl transferase determinations. The score system for nonalcoholic fatty liver disease was used to analyse the liver samples. ***Results and Conclusions*** - High-fat diet resulted in a significant increase in animal body weight, biochemical changes and enzymatic elevations. Steatosis, inflammation and hepatocellular ballooning scores were significant high in this group. The biochemical and histological variables were statistically similar in the bezafibrate group and control group. Treatment with *Panax ginseng* extract prevented obesity and histological features of nonalcoholic steatohepatitis (steatosis and inflammation) compared to high-fat diet. *Camellia sinensis* showed a less effective biochemical response, with small reduction in steatosis and inflammation but lower ballooning scores.

HEADINGS – *Panax*. *Camellia sinensis*. Liver diseases. Metabolic Syndrome X.

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) has been described in clinical, experimental and epidemiological studies as hepatic manifestation of metabolic syndrome[3, 21], which main components are abdominal obesity, atherogenic dyslipidemia, arterial hypertension, insulin resistance/glucose intolerance, proinflammatory and prothrombotic states[12]. Nowadays, NAFLD is considered the most common hepatic condition associated with increased aminotransferases and cryptogenic cirrhosis[6] and that may progress to hepatocellular carcinoma[11]. Its prevalence has been estimated in 10% to 30% in Western countries[7], and has been documented in 15% of individuals with normal weight and 80% obese[27]. Other risk factors also play a role in the pathogenesis of NAFLD including gender, ethnicity, and genetic and immunological factors[13].

The pathophysiological mechanisms of NAFLD are not yet fully understood but accumulation of triglycerides in hepatocytes resulting from insulin resistance[29] is the first step in the currently accepted pathogenetic model. Oxidative stress resulting from mitochondrial oxidation of fatty acids and expression of inflammatory cytokines have been implicated as secondary causal factors to liver damage, fibrosis and inflammation[8]. NAFLD is associated with a spectrum of morphological variations in disease progression with two distinct forms of presentation: hepatic steatosis (HS) characterized only by fat deposition in hepatocytes; and nonalcoholic steatohepatitis (NASH), a more severe form of disease with varying degrees of necrosis and inflammation[8].

Still now, there is no licensed pharmacological treatment for NAFLD, a number of pharmacological agents have been investigated and tested, as antioxidant therapies and insulin-sensitising agents, however, the current treatment strategy for NAFLD is primarily focused on the control of risk factors including lifestyle changes, weight loss and regular exercise[10, 22, 28].

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People are increasingly willing to use plant based medicinal drugs worldwide but the scientific literature lacks in evidence-based analysis of these compounds, making it difficult to establish its effectiveness. Experimental studies investigating the mechanisms of action of *Panax ginseng* and *Camellia sinensis* have been conducted in recent decades but most of them sought to assess qualitatively and quantitatively crude extracts and active principles alone[24].

The current study was carried out to compare the effectiveness of *Panax ginseng* and *Camellia sinensis* in disease prevention using biochemical and histopathological parameters. *Panax ginseng* is known for its detergent and lipid-lowering properties through the action of ginsenosides, the main components of bioactive saponins[17]. It is thus expected to act on the first phase of the metabolic process characterized by increased intake of fatty acids to the liver. Since oxidative stress plays a central role in the development of steatohepatitis, *Camellia sinensis* was selected as a comparative natural product due to antioxidant effects of its catechins[26]. The positive control selected was a synthetic product, bezafibrate, wich is an agonist of peroxisome proliferator-activated receptors (PPAR) with lipid-lowering effects resulting from increased beta-oxidation of fat in the liver while reducing oxidative stress and directly preventing inflammation[27].

METHODS

The study was approved by animal research Ethics Committee (number 0205/08) of Laboratory of Pharmaceutical Technology at Federal University of Paraíba. It followed the guidelines for the care and use of experimental animals according to Brazilian law n° 11.794 (October, 8, 2008). Fifty Wistar rats aged 2 months weighing 200 to 250 mg, from the Thomas George Vivarium, were submitted to the experiment. The animals were kept in polyethylene cages (five animals per cage) under stable temperature and humidity conditions, free access to water and alternate 12-hour light-dark cycles, the amount of feed consumed by the animals was controlled at the end of each day. Animal weight was recorded the same day, once a week. The animals were divided into five groups: G1 (normal control group receiving standard diet); G2 (control group receiving high-fat diet with 58% of energy from fat, 18% from protein and 24% from carbohydrate); G3 (high-fat diet + standardized *Panax ginseng* extract at a dose of 100 mg/kg/day with 10% ginsenosides); G4 (high-fat diet + *Camellia sinensis* extract at a dose of 100 mg/kg/day with 35% catechins and 15% epigallocatechin-3-gallate [EGCG]); and G5 (high-fat diet + bezafibrate at a dose of 100 mg/kg/day), the medication was given by gavage. The animals were sacrificed eight weeks later, after 8-hour fasting, by cervical dislocation under sedation. Blood was collected, centrifuged and blood glucose, insulin, total cholesterol, HDL cholesterol, VLDL cholesterol, triglycerides, serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and gamma-glutamyl transferase (GGT) were determined in a reference laboratory. The liver was removed and fixed in buffered formalin and prepared according to standard microtechniques[23]. Tissue sections (5 mm) were prepared, fixed and stained with hematoxylin-eosin, picrosirius red and Masson's trichrome and then examined using a histological scoring system, NAFLD activity score (NAS)[5, 19].

Statistical analysis

One-way and two-way analysis of variance (ANOVA), Tukey's multiple comparison tests, Dwass-Steel-Chritchlow-Fligner test and nonparametric Kruskal-Wallis test were used. All tests were analyzed at a 5% significance level (P<0.05). Stata v. 9.0, SPSS for Windows (Statistical Package for Social Sciences) v. 18.0, Statistical Data Analysis R – statistical computing and graphics, v. 2.13.1 (2011-07-08) and MYSTAT – Statistical Analysis Product v. 12.0 were used in the analyses.

RESULTS

See Table 1, 2, 3 and Figure 1.

TABLE 1. Baseline and final weight (g) in the experimental groups

Groups	Baseline weight (g)	Final weight (g)	Average
G1	207±3.57	335.4±11.83	292.2±3.0
G2	241±3.5	405.2±16.45	346.4±7.32
G3	221.3±5.2	350±6.3	296.8±4.73
G4	228.5±5.42	393.1±12.63	324.5±6.81
G5	245.6±1.34	354.6±13.74	311.2±9.36

G1: controls; G2: high-fat diet; G3: *Panax ginseng;* G4: *Camellia sinensis;* G5: bezafibrate. ANOVA, Tuckey, P< 0.05 (G1≠G2,G4; G2≠G3; G3≠G4;G4≠G5).

TABLE 2. Biochemical parameters described as average (±Standard deviation) in the experimental groups

Variables	Experimental groups (mean ± SD)				
	G1	G2	G3	G4	G5
Blood glucose	95.6±28.7	233.6±40.7	131.1±44.6	191.7±54.3	155.0±27.5
Insulin level	1.68±0.07	3.54±0.08	1.74±0.05	2.04±0.13	1.98±0.11
Cholesterol	41.33±8.1	53.20±6.2	48.00±8.2	48.33±15.6	37.80±7.95
HDL	18.8±3.3	15.8±2.3	18.8±1.9	23.4±6.8	19.1±4.1
VLDL	7.1±21.6	22.3±3.5	14.8±4.3	20.9±7.2	9.8±2.3
TGLs	35.6±23.2	111.6±18.9	76.8±23.9	103.8±36.5	49.6±11.7
ALP	165.0±44.1	180.1±58.6	160.1±58.7	158.7±92.3	416.3±112.3
GGT	2.7±0.7	2.3±1.1	1.8± 0.6	2.22±1.3	7.2±6.2
ALT	169.8±62.6	271.5±108.2	201.9±124.8	204.8±101.8	139.5±39.6
AST	49.1±17.6	84.7±101.1	68.78±10.45	63.5±10.9	45.6±9.6

G1: controls; G2: high-fat diet; G3: *Panax ginseng;* G4: *Camellia sinensis;* G5: bezafibrate. Results of Kruskal-Wallis H test of differences in biochemical parameters between the groups. To identify statistically significant differences mentioned above, between the groups of animals, convenient multi-Steel-Dwass Chritchlow-Fligner comparison test was carried out. Glucose (G1≠G2,G4);Insulin (G2≠G3,G4,G5) Cholesterol (G1≠G2;G3≠G5); HDL(G1≠G4); VLDL (G1≠G2,G4; G2≠G3;G5; G4≠G5); TGLs: triglycerides (G1≠G2,G4; G2≠G3,G5; G4≠G5); ALP: alkaline phosphatase (G1≠G5; G2≠G5;G3≠G5; G4≠G5); AST: serum aspartate aminotransferase (G1≠G2,G5;G3≠G5); ALT: alanine aminotransferase (G1≠G2;G3≠G5); GGT: gamma-glutamyl transferase (G1≠G5; G2≠G5;G3≠G5;G4≠G5). *P* value <0.05

TABLE 3. Descriptive measures of histological features (steatosis, inflammation, and ballooning) between the groups at the end of the experiment

		Mean score	Standard deviation (SD)	Standard error (SE)	Confidence interval for the mean	*P*-value >0.05
Steatosis	G1	0.00	0.00	0.00	0.00	0.00
	G2	3	0.42	0.13	2.50	3.10
	G3	1	0.99	0.31	0.19	1.61
	G4	2	0.42	0.13	1.90	2.50
	G5	0.00	0.00	0.00	0.00	0.00
Inflammation	G1	0.00	0.00	0.00	0.00	0.00
	G2	1	0.48	0.15	0.95	1.65
	G3	1	0.92	0.29	0.14	1.46
	G4	1	0.63	0.20	1.35	2.25
	G5	0	0.52	0.16	0.03	0.77
Hepatocellular ballooning	G1	0.00	0.00	0.00	0.00	0.00
	G2	2	0.42	0.13	0.90	1.50
	G3	1	0.00	0.00	1.00	1.00
	G4	1	0.00	0.00	0.00	0.00
	G5	1	0.42	0.13	0.10	0.50
NAS score	G1	0	0.00	0.00	0.00	0.00
	G2	5	0.48	0.15	3.95	4.65
	G3	3	1.87	0.59	0.46	3.14
	G4	4	0.67	0.21	3.52	4.48
	G5	1	0.52	0.16	0.03	0.77

G1: controls; G2: high-fat diet; G3: *Panax ginseng;* G4: *Camellia sinensis;* G5: bezafibrate.

(*) Statistically significant difference, ANOVA ($P < 0.05$). Diagnostic categorization: 0 to 2: not NASH; 3 to 4: probable, borderline NASH; 5 to 8: NASH. For the statistical differences between the groups it was used the multiple comparison test (Tukey). Steatosis (G1≠G2,G3,G4; G2≠G3,G5; G3≠G4,G5; G4xG12); Inflammation (G1≠G2,G4; G2≠G5; G3≠G4; G4≠G5); Hepatocellular ballooning (G1≠G2,G3;G2≠G4,G5; G3≠G4,G5); NAS score (G1≠G2,G3,G4; G2≠G1,G3; G3≠G1,G2,G3,G4; G4≠G1,G3,G5; G5≠G2,G4).

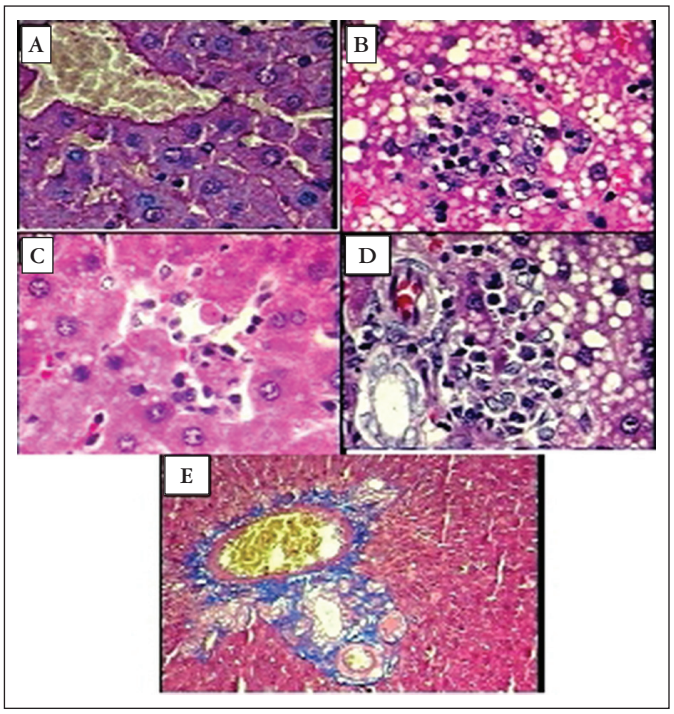

FIGURE 1. Liver: **A)** Control group – parenchyma with trabeculated isomorphic hepatocytes surrounding the terminal hepatic veins that have thin walls permeable to sinusoids. **B)** High-fat diet group – moderate to severe panacinar steatosis, inflammatory cell infiltrate of macrophages and granulocytes with lipogranuloma in zone 2. **C)** *Panax ginseng* group – mild microvesicular steatosis and rare hepatocellular ballooning. **D)** *Camellia sinensis* group – moderate to severe steatosis accompanied with inflammation. **E)** Portal space with vascular-biliary triad (artery, vein and bile duct) within matrix of loose collagen, without septum and no portal-parenchymal interface inflammation (A: picrosirius red, X400; C: hematoxylin and eosin, X100; B, D: hematoxylin and eosin, X400; E: Masson's trichrome, X100).

DISCUSSION

The study results are in agreement with previous studies that used the high-fat diet as an experimental model of obesity[31, 33]. Wang et al.[32] demonstrated that rats undergoing a 7-week high-fat diet (58% of daily calories), high in saturated fat, had greater body fat than those fed on unsaturated fat or low-fat isocaloric diet.

A similar phenotype to the metabolic syndrome (obesity, hyperglycemia, hyperinsulinemia, hypercholesterolemia, hypertriglyceridemia, hyperuricemia, and low levels of HDL cholesterol) has been described in animals fed on a high-fat diet with 58% of energy from fat, 18% from protein and 24% from carbohydrate. In this experimental model of obesity, hepatocellular damage led to increased aminotransferase levels, particularly ALT, with no significant changes in cholestatic enzymes.

The present study animals receiving concomitant treatment with bezafibrate showed a percentage of weight gain similar to that seen in the standard diet group, demonstrating the effectiveness of this synthetic agent in controlling high-fat diet induced weight gain. The recognized agonist effect of bezafibrate on PPARs in the liver, adipose tissue and muscle results in improved control of glucose and lipid metabolism and fat storage as well[1]. Sasaki and colleagues[30] also demonstrated the anti-obesity effect of bezafibrate through inhibition of fat accumulation in mice.

The *Panax ginseng* group showed similar results to the standard diet group, corroborating previous data on the effects of this herbal medicine on obesity management. Its effect has been associated with a probable agonist action on PPARg receptors[15] and inhibition of both hyperinsulinemia and insulin resistance in adipose tissue. Since obesity is an inflammatory condition, *Panax ginseng* effect can be also explained by a proven anti-inflammatory action[20]. In addition to its agonist action on PPARg receptors, *Panax ginseng* has also been proved to regulate the lipoprotein lipase expression in adipose tissue, as well as to play a role in the regulation of glucose transporter 4 (GLUT4) and insulin receptor in skeletal muscle and liver[24].

In the current experiment, one group of rats received *Camellia sinensis* extract (100 mg/kg/day) with 35% of catechins. In the animals of the present study the group receiving green tea extract had a mean body weight lower than that of the high-fat diet group, but this difference was not statistically significant. Bose and colleagues[4] also reported in an experiment under similar conditions a tendency to weight reduction as early as four weeks.

Treatment with bezafibrate prevented an increase in lipid and blood glucose levels with no increased aminotransferase levels, although higher levels of Alkaline phosphatase and GGT were found in this group. Treatment with *Panax ginseng* extract controled weight gain in animals with reduced levels of glucose and lipids. The use of *Camellia sinensis* extract did not either prevent weight gain or significantly reduced blood glucose, cholesterol, triglycerides. On the other hand, it was associated with increased levels of HDL cholesterol.

Although GGT increase has been reported[9] as a sensitive diagnostic marker and predictor of NASH, this finding was not corroborated in high-fat diet group with hepatic necroinflammatory changes in the current study. GGT levels were significantly higher in the bezafibrate group.

Those animals treated with the synthetic product (bezafibrate) showed the best histological and biochemical responses when compared to other groups, with reduction of serum levels of total cholesterol, triglycerides, VLDL cholesterol, ALT and AST as well. It is of note that ALP and GGT levels were increased in the absence of histological signs of liver bile retention. Bertolami[2] argued that bezafibrate may induce hepatocellular damage, which may cause increased aminotransferases, or cholestasis with increased bilirubins (especially direct bilirubin, ALP and GGT). Liver damage induced by lipid lowering agents is mainly hepatocellular, leading to increased AST and ALT. This is usually transient, asymptomatic and resolves with drug discontinuation.

In the present experiment, groups of animals fed on high-fat diet alone and those treated with *Camellia sinensis* showed sta-

tistically significant higher rates of steatosis compared to other groups ($P<0.001$). The extent of steatosis in the bezafibrate group was similar to that in the control group. The animals in the *Panax ginseng* group showed steatosis at an intermediate extent compared to other groups. The *Camellia sinensis* group also showed the highest percentages of inflammation, which were statistically similar to the high-fat diet group. No significant difference was seen between the bezafibrate and the control group, and between the *Panax ginseng* and *Camellia sinensis* groups compared to the high-fat diet group.

The highest percentage of hepatocellular ballooning was found in the high-fat diet group, with statistically higher values compared to other groups, followed by the *Panax ginseng* group. The greatest NAS scores were seen in the high-fat diet and *Camellia sinensis* groups with statistically significant differences compared to other groups. The bezafibrate group showed no statistically significant difference compared to the control group.

In this experimental context, these rats studied did not show any lobular, portal and periportal fibrogenic reactions. Yet, there has been reported that high-fat diet may prevent the development of histologic evidence of NASH, of which fibrosis is a major components[18]. Similarly, Nakamoto and colleagues[26], in a four week experimental study, failed to induce fibrosis associated with high-fat diet alone in rats.

The inflammatory activity associated with NAFLD was studied in the present study based on NAS score[19]. In a recent validation study[14] found sensitivity and specificity over 80% for predicting steatohepatitis, and a score ≥4 was the recommended cutoff for a NASH intervention program.

In this study, the high-fat diet group had higher NASH scores, histologically characterized by steatosis and moderate to severe inflammation, commonly accompanied by ballooning. Treatment with bezafibrate reversed NASH, with an almost overlapping of its morphological features in the liver with those seen in the control group. Ackerman and colleagues[1] experimentally reported significant reduction of macrovesicular hepatic steatosis with bezafibrate but with no change in microvesicular lipid retention, inflammation, or fibrosis score. In mice with NASH, bezafibrate induced a marked reduction, and near elimination, of fat vesicles and improved NAS score[30]. Improvement of steatosis has been attributed to upregulation of PPAR-α receptors in mitochondrial fatty acid beta-oxidation, with increased catabolism of fatty acids and prevention of deposition of hepatocellular triglycerides[16, 25]. The positive effects on PPARγ receptors are expressed predominantly in adipose tissue with involvement in the mechanisms of insulin resistance to upregulate GLU4. This receptor subtype promotes uptake of fatty acids to adipocytes, which induces their differentiation, increases triglyceride accumulation and reduces fatty acid influx to the liver[25].

In this study, *Panax ginseng* induced partial NASH reversion with significant reduction in the final NAS score and histological hepatic features similar to those seen in the control group. *Camellia sinensis* showed a less effective biochemical response, with small reduction in steatosis and inflammation but lower ballooning scores.

CONCLUSIONS

The current study showed that drug-induced weight loss was associated with lower serum biochemical and histological hepatic changes, especially in the groups treated with bezafibrate and *Panax ginseng*. Bezafibrate also prevented histological damage, which was quite similar to that seen in the control group. The animals treated with *Panax ginseng* showed better biochemical response compared to those receiving of *Camellia sinensis*. Their biochemical profile was significantly similar to that seen in the control and bezafibrate groups. The biochemical parameters revealed that *Panax ginseng* was more effective in preventing increased level of blood glucose, cholesterol and VLDL cholesterol. It was also showed a reversal of histological liver damage, confirming other reports that *Panax ginseng* has hypoglycemic and lipid-lowering properties. Nevertheless, randomized clinical studies are needed to investigate its use for prevention of NAFLD.

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RESUMO - ***Objetivo*** - *Panax ginseng, Camellia sinensis* e bezafibrato foram comparados por suas propriedades hipolipemiantes, antioxidantes e anti-inflamatórias, como potenciais agentes capazes de prevenir a doença hepática gordurosa não alcoólica e sua progressão para esteato-hepatite não alcoólica. ***Métodos*** - Cinqüenta ratos Wistar foram distribuídos aleatoriamente em cinco grupos: G1 (alimentados com dieta padrão); G2 (alimentados com dieta hipercalórica com 58% de energia a partir de gordura); G3 (dieta rica em gordura + extrato padronizado *Panax ginseng* em 100 mg / kg / dia); G4 (dieta rica em gordura + extrato de *Camellia sinensis* padronizado a 100 mg / kg / dia); e G5 (dieta rica em gordura + bezafibrato, a 100 mg / kg / dia), administrado via oral. Os animais foram sacrificados após oito semanas e o sangue foi coletado para determinação da glicose, insulina, colesterol, triglicérides, AST, ALT, fosfatase alcalina e gama-glutamil transferase. O sistema NAS de pontuação para doença hepática gordurosa não alcoólica foi utilizado para analisar as amostras de fígado. ***Resultados e Conclusões*** – A dieta hipercalórica resultou em um aumento significativo no peso corporal dos animais, associado a alterações bioquímicas e elevações enzimáticas. Os escores de esteatose, inflamação e balonização hepatocelular foram significativamente mais elevados neste grupo. As variáveis bioquímicas e histológicas foram estatisticamente semelhantes entre os grupos bezafibrato e controle. O uso do extrato do *Panax ginseng* esteve associado a um menor ganho de peso dos animais, em média, bem como a menores índices nos escores de esteato-hepatite (esteatose e inflamação) em comparação com o grupo apenas alimentado com dieta hipercalórica. No grupo ao qual foi administrado o extrato da *Camellia sinensis* uma resposta bioquímica e histológica menos pronunciada foi observada, entretanto, com menores escores de balonização quando comparado ao grupos do *Panax ginseng*.

DESCRITORES – *Panax*. *Camellia sinensis*. Hepatopatias. Síndrome X Metabólica.

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