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Artigo

Hypotriglyceridemic effect of *Morus alba* L., Moraceae, leaves in hyperlipidemic rats

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RESUMO: "Efeito hipotrigliceridêmico de folhas de *Morus alba* L., Moraceae, em ratos hiperlipidêmicos". As folhas de amoreira branca *Morus alba* L., Moraceae, são utilizadas no Brasil na medicina popular para tratar febre, como protetor do fígado, para baixar a pressão e o colesterol. O extrato aquoso de folhas de *M. alba* foi administrado via oral através de gavagem em ratos hiperlipidêmicos com uma dieta enriquecida com colesterol (1%) com uma dose de 150 mg/kg/dia por quatorze dias que reduziu significativamente em 55,01% o nível de triglicerídios plasmáticos. Desta forma, o tratamento diminuiu o nível de triglicerídios e uma enzima hepática testada, demonstrando que extratos de *Morus alba* que tem sido popularmente utilizados possuem um grande potencial para se tornar um fitomedicamento.

Unitermos: *Morus alba*, ratos hiperlipidêmicos, triglicerídios.

ABSTRACT: Mulberry *Morus alba* L., Moraceae, leaves are used in Brazil medicines against fever, to protect the liver, to lower the blood pressure and cholesterol. The aqueous extract of leaves of *M. alba* given by oral route to hyperlipidemic rats with diet enriched by cholesterol (1% by weight) with dose of 150 mg/kg/day for forteen days reduced significantly by 55,01% the plasma triglycerides level. Therefore, the treatment not only decreased the plasma level of triglycerides but also affected a hepatic enzyme tested supportting the fact that the extracts of *Morus alba* that have been used by folk medicine have a great potential to be further developed into a phytomedicine.

Keywords: *Morus alba,* hyperlipidemic rats, triglycerides.

INTRODUCTION

Hyperlipidemia, resulting from lipidic metabolitc changes, is a major cause of cardiovascular disturbances (Chobanian, 1991). Although a high level of serum cholesterol has been identified as a risk factor for atherosclerosis and coronary heart disease (Kanel et al., 1971), the role of a high level of triglycerides has only recently established as an independent risk factor for such diseases (Cambien et al., 1986; Austin, 1989). The medicinal properties of plants have been investigated in the light of recent scientific developments throughout the world, due to their potent pharmacological activities, low toxicity and economic viability (Sharma et al., 1992).

Mulberry, the plant used in this work, was selected on the basis of the ethnomedical information available. Leaves of *Morus alba* L. were subject to qualitative phytochemical investigation of the phytoconstituents anthocyanins, prenyflavons, flavonoids, stillbenes, 2-arylbenzofurans, carbohydrates, alkaloid and glycoproteins (Yagi et al., 1976; Nomura et al., 1977; Nomura et al., 1980; Hikino et al., 1985; Gerasopoulos & Stavorulakis, 1997; Fukai & Nomura, 1998; Kim et al., 1999; Kaio et al., 2001; Sharma et al., 2001; Jin et al., 2002; Fukai et al., 2003).

The leaves, roots and fruits have a wide range of pharmacological activities and are used in tradicional Chinese medicine as diabetes, expectorant, sedative, analgesic, diuretic, hypotensive, antiepileptic, antiphlogistic and liver protective (Yamatake et al., 1976; Nomura, 1988; Chen et al., 1995; Quer, 1995; Chevalier, 1996). But in literature, there were a few reports regarding the hypolipidemic effect of *M. alba* leaves (Soares et al., 2005) and two with root barks and fruits (Chen et al., 2005; El-Bebshbishy et al., 2006). In this study we investigated the effect of *Morus alba* leaves water extract on experimental hyperlipidemic rats.

MATERIAL AND METHODS

Plant material

The young leaves of *Morus alba* L., Moraceae, were collected in December 2005 from Balneário Camboriú-SC, Brazil. The plant was identified by Dr. Lúcia Sevegnani, Professor of Botany and authenticated

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by comparing with the voucher specimen number 2564 located at Herbário Dr. Miguel Pedro Klein of Universidade Regional de Blumenau (FURB).

Extraction

Aqueous extract of mulberry leaves were prepared at 1.5 g%, as recommended by the traditional healers, boiling the water and after put this one over the fresh and young leaves and cover for 10 min (extract was prepared daily). The average percentage yield of this extract was found to be 20.23% w/w.

Animals

Wistar rats, weighting 200-300 g, threee months old, were bred in the animal center of Universidade Regional de Blumenau. They were housed in standard environmental conditions, fed with diet (Nuvilab®) and water *ad libitum*. For experimental purpose the animals were kept fasting overnight but were allowed free access to water. Animals were handled in accordance with laboratory animal welfare guidelines, experimental protocols and procedures were approved by the Animal Experimentation Ethics Committee/FURB (002/2003).

Experimental design

The experiment was carried out with four groups (I, II, III and IV) of eight rats each: Grupo I: normal, sacrificed to obtain normal biochemical parameters (before the experiment); Group II: normal control, fed with normal diet; Group III: hyperlipidemic treated with 150 mg/kg/day of extract and fed with diet enriched in cholesterol (1% by weight); Grupo IV: hyperlipidemic control, fed with diet enriched in cholesterol (1% by weight). Control rats (Groups II and IV) received orally vehicle (distilled water) only while group III received 150 mg/kg of extract orally, suspended in distilled water. The animals were fed 35 days with a hyperlipidemic diet (Rosa et al., 1998), after this period they were treated with extract of *M. alba* by gavage for 14 days. All biochemical parameters were estimated at the beginning (Group I) and after 49 days of experiment (Groups II, III and IV).

Collection and processing of blood for estimation of biochemical parameters

Blood samples were collected by portal venous under ether anaesthesia in Eppendorff's tubes (1 mL) containing 50 µL of anticoagulant (heparin) and plasma was separated by centrifuging at 6000 rpm for 15 min. Plasma total cholesterol (TC), high density lipoprotein (HDL) and low density lipoprotein (LDL)-cholesterol, triglyceride (TG), glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT) levels were evaluated using a commercial kit. Several hepatic enzymes, such as GOT and GPT, were used as biochemical markers for hepatic damage.

Histological analysis

Animals were sacrificed by ether inhalation and the liver was removed, which was fixed in 10% formalin, dehydrated and included in paraffin wax for 5 µm sections that was stained with hematoxylin and eosin (HE) and observed under light microscope.

Statistical analysis

The data were subjected to the analysis of variance (one way ANOVA) to determine the significance of changes, Student's *t*-test were made to analyse the significance of difference between the experimental groups. *p* values of 0.05 were taken as significant.

RESULTS AND DISCUSSION

The oral supplementation of *M. alba* (150 mg/kg/day) to hyperlipidemic rats resulted in significant declines in plasma TG (55.01%) but not significantly to TC (10.57%), LDL (7.89%) as compared to the hyperlipidemic control (Table 1). The HDL decreased 15.17% but not significantly. Different of negative results obtained in another work with water extract of *M. alba* leaves (Soares et al., 2005). Recent studies suggest that TG itself is independently related to coronary heart disease and most of the antihyperlipidemic drugs do not decrease TG levels (Bainton et al., 1992; El-Hazmi & Warsy, 2001).

Table 1. Effect of administration of feeding the aqueous extract of *Morus alba* L. leaves on TG, TC, HDL and LDL in normal and hyperlipidemic rats for 14 days (mg/dl).

Experimental groups	TG	TC	HDL	LDL
Normal control (group I)	50.30±18.52	78.30±15.18	44.50±9.77[b]	21.80±4.36
Control (group II)	47.75±13.04[a]	82.37±20.92	50.12±12.35	25.37±8.63
Hyperlipidemic treated (group III)	49.75±13.04[a]	87.50±4.036	54.375±4.80	23.75±4.68
Hyperlipidemic control (group IV)	77.12±13.33[a]	96.75±16.98	62.62±59.03[b]	25.62±4.80

Data are expressed as mean± SE, *N*=8.

[a] Statistical significance from normal control and treated as compared to the hyperlipidemic group (P <0.05).

[b] Statistical significance from normal control as compared to the hyperlipidemic group (P <0.05).

The body weight increased significantly in all studied Groups, II, III and IV, respectively, 12.63%, 20.90% and 27.38% (Data not shown). Administration of aqueous Mulberry leaves decreased the plasma GPT 38.86% but increased 7.02% GOT levels in treated rats (Table 2) but not significantly. Although, histological analysis of Group IV liver revealed comparing with Group III, leukocyte aggregations near blood vessels and evident vascular congestion, inflammatory focus was observed in all the liver parenchyma, principally in the perivascular region (Data not shown). Liver cells presented altered metabolic activity, which was also verified by blood GPT analysis, taking these data together is possible suggest hepatocyte death (group IV), according Friedman & Young, 1997; Burtis, & Ashwood, 2001 and reduction of severety damage of liver (group III) provocked by hyperlipidemic diet.

Table 2. Effect of administration of feeding the aqueous extract of *Morus alba* L. leaves on TGO and TGP in normal and hyperlipidemic rats for 14 days (mg/dl).

Experimental groups	TGO	TGP
Normal control (group I)	103.00±18.67	65.10±13.48
Control (group II)	107.25±20.19	52.62±8.97
Hyperlipidemic treated (group III)	126.25±35.21	65.62±31.01
Hyperlipidemic control (group IV)	117.38±34.30	91.12±60.56

Data are expressed as mean± SE, *N*=8.

Mulberry leaves were able to prevent hypertrigliceridemia, this indicate a specific inhibitory effect of *M. alba* on fatty acids synthesis. Such effect has been demonstrated in experiments using *M. alba* root bark or fruit (Chen et al., 2005; El-Bebshbishy et al., 2006) and in other plants (Chan et al., 1999; Wang et al., 2000; Yeh et al., 2003).

In conclusion *M. alba* leaves aqueous extract of leaves was able to decrease plasma tryglicerides and repress progression of liver damage in hyperlipidemic rats and appears to confirm the hypolipidemic and liver protective effects.

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