

## Investigation of Liver Tissue and Biochemical Parameters of Adult Wistar Rats treated with *Arctium lappa* L.

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### ABSTRACT

*This study was carried out to evaluate the effects of Arctium lappa L. (burdock) on the liver of adult male Wistar rats as measured by light microscopy and biochemical parameters. The rats received the extract in water bottles at doses of 10 or 20 g/L daily for 40 days. There were no significant changes in the plasma levels of albumin, aspartate transaminase (AST), alanine transaminase (ALT), gamma glutamyl transferase (GGT), total protein, total cholesterol, urea, uric acid, triacylglycerol, calcium, phosphorus, chlorine and direct bilirubin. The morphological analysis did not reveal histopathological alterations in liver tissue. Both biochemical and morphological data did not indicate A. lappa toxicity.*

**Key words:** *Arctium lappa*; Burdock; Plasma components; Liver

### INTRODUCTION

*Arctium lappa* L. (burdock) is a plant brought from Japan and acclimated in Brazil, which is widely used in popular medicine all over the world (Pereira et al., 2005). This plant has long been cultivated as a vegetable for dietary use and is also used as a folk medicine, as a diuretic, antipyretic tea (Chen et al., 2004) as well as for hypertension, gout, hepatitis and other inflammatory disorders (Lin et al., 2002). Several studies have reported that the burdock possesses various pharmaceutical activities including an antibacterial activity (Pereira et al., 2005; Chen et al., 2004), antifungal activity (Pereira et al., 2005), anti-platelet-aggregating effect (Pereira et al., 2005; Lin et al., 2002), an antioxidant (Chen et al., 2004; Pereira et al., 2005; Leonard, et al., 2006), hepatoprotective efficacy (Lin et al., 2000; Lin et

al., 2002) and anti-inflammatory activity (Lin et al., 1996; Chen et al., 2004). Pereira et al. (2005) have demonstrated that *A. Lappa* exhibits antimicrobial activity against oral microorganisms. In recent years, the crude water extract of *A. lappa*, called *A. lappa* tea, has become a promising and important beverage, because it has ample therapeutic action (Lin et al., 2002). It has been pointed out by various researchers that the extensive use of herbal medicines is not as safe, as is frequently claimed to be, and this medicinal plant may not be an exception. It can be harmful to take herbal medicines without being aware of their potential adverse effects (Capasso et al., 2000). In order to establish the therapeutic efficiency of these plants, scientific studies are essential. Since, the definition of hepatotoxicity is based on biochemical parameters (elevation of ALT, AST and GGT) or histological abnormality (Ozcanli,

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2006), an investigation was carried out in controlled conditions to evaluate the possible effect of *A. lappa* on the liver and on biochemical parameters.

## MATERIALS AND METHODS

### Preparation of *A. lappa* aqueous extract

*A. lappa* leaves were obtained from the Entre Folhas Group of the Federal University of Viçosa, Minas Gerais, Brazil in July 2001. The plant material was identified and a voucher specimen was deposited in the VIC Herbarium of the Department of Vegetal Biology, Federal University of Viçosa, Minas Gerais, Brazil, bearing the number 28960. Two aqueous extracts were obtained from the plant material by infusion prepared in the concentrations of 10 and 20 g of dried leaves/L of water. The aqueous extract submitted to lyophilization yielded 0.21 and 0.42%, respectively.

### Animals

The study was carried out on 100-day-old adult male Wistar rats, weighing between 220 and 275 g. They were obtained from the Department of Health and Nutrition (Federal University of Viçosa, Viçosa, MG, Brazil) and were maintained in individual cages in 12hL:12hD conditions. They were provided with feed *ad libitum* for 40 days. These animals were handled and euthanatized in accordance to Ethical Principles for Animal Research established by the Brazilian College for Animal Experimentation (COBEA).

### Treatment

Thirty rats were randomly divided in three groups of ten animals each. All the animals received water and the extract in water bottles attached to the outside of the cage. The control group received only water. Group A and Group B received daily 200mL of leaf extract of *A. lappa* at a dose of 10 and 20g/L, respectively (Franco and Santana, 2002). The infusion was prepared daily from dry leaves.

### Plasma and biochemical analysis

The blood samples were collected and centrifuged at 7100 x G for 15 minutes. The plasma was removed to be assayed for albumin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), calcium, chlorine, creatinine, direct

bilirubin, gamma glutamyl transpeptidase (GGT), phosphorus, total cholesterol, total protein, triacylglycerol, urea and uric acid. All assays were performed using Biolab Diagnostic kits.

### Tissue Preparation

Rats were anesthetized with ether and the thoracic cavity was opened for whole body perfusion-fixation with 2.5% glutaraldehyde in 0.1M phosphate buffer. The liver was post-fixed with the same fixative for 24 h. Blocks of liver tissue were taken from the median lobe and prepared for embedding in glycol methacrylate (Historesin, Leica) using standard techniques. Subsequently, sections of 4 µm thickness were obtained and stained with toluidin blue for microscopic assessment.

### Morphometry of liver and histopathological examination

The histological sections were examined under a light microscope using a 121 intersection grid placed at the ocular lens, coupled to a 100X objective. The hepatic parenchyma was classified as one of the following: hepatocyte cytoplasm or nucleus, hepatic sinusoids, degenerative hepatocytes, central vein, portal space, fatty deposition and inflammatory infiltrate. One thousand points were counted for each animal.

### Statistical analysis

All data were represented as mean ± standard mean error (S.E.M.) or as percentages. Statistical evaluation of significant difference between the means was performed with one way analysis of variance (ANOVA), followed by Duncan test  $p < 0.05$  was regarded as significant.

## RESULTS

*A. lappa* administration at the doses of 10 and 20g/L had no apparent effect on liver tissue and biochemical parameters. There were no significant alterations ( $p > 0.05$ ) in the plasma levels of albumin, ALT, AST, calcium, chlorine, direct bilirubin, GGT, phosphorus, total cholesterol, total protein, triacylglycerol, urea and uric acid between the control and treated groups. The creatinine level increased 11.9% ( $p < 0.05$ ) in group A, whereas in group B, no significant difference was observed (Table 1). Degenerative hepatocytes were

observed and they represent 0.1% in both, A and B groups, and were not observed in the control group. These data was not statistically significant

(Table 2). High degree of fatty deposition was found in the liver of one animal of group A and one in group B.

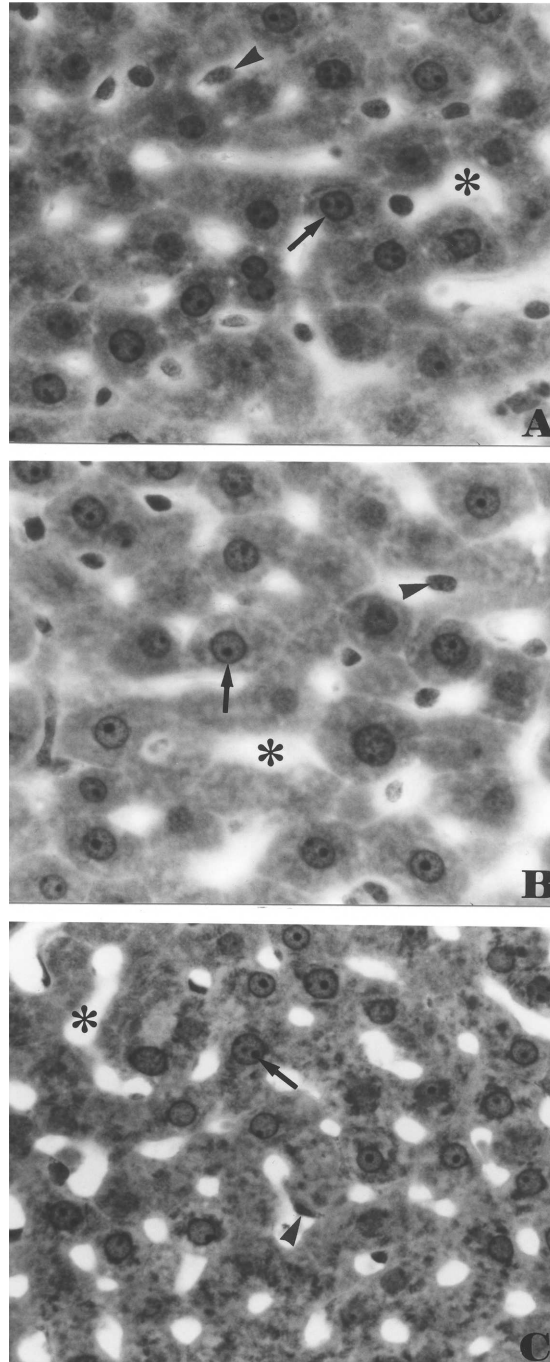
**Table 1-** Biochemical parameters of rats treated with different concentrations of *A. lappa* aqueous extract (mean  $\pm$  SEM).

Parameter	Control	Group A	Group B
Albumin (g/dL)	4.24 $\pm$ 0.37	4.02 $\pm$ 0.27	3.95 $\pm$ 0.32
ALT (UI)	203.20 $\pm$ 70.98	145.40 $\pm$ 22.41	158.25 $\pm$ 44.84
AST (UI)	184.20 $\pm$ 2.76	107.83 $\pm$ 17.15	159.80 $\pm$ 40.20
Calcium (mg/L)	8.65 $\pm$ 0.61	10.47 $\pm$ 0.94	11.22 $\pm$ 1.30
Chlorine(mmol/L)	163.13 $\pm$ 14.06	159.16 $\pm$ 17.60	151.13 $\pm$ 14.42
Creatinine (mg/dL)	0.67 $\pm$ 0.03	0.75 $\pm$ 0.02*	0.68 $\pm$ 0.02
Direct bilirubin (mg/dL)	0.33 $\pm$ 0.07	0.29 $\pm$ 0.05	0.21 $\pm$ 0.05
GAMMA GT (UI)	4.00 $\pm$ 0.42	4.25 $\pm$ 00.66	3.63 $\pm$ 0.26
Phosphorus (mg/L)	12.96 $\pm$ 2.82	11.98 $\pm$ 1.20	9.38 $\pm$ 0.81
Total cholesterol (mg/dL)	86.48 $\pm$ 3.23	81.30 $\pm$ 2.59	82.08 $\pm$ 4.20
Total proteins (g/L)	63.99 $\pm$ 0.75	63.15 $\pm$ 1.21	63.61 $\pm$ 1.06
Triacylglycerol (mg/dL)	45.39 $\pm$ 5.61	39.99 $\pm$ 1.94	41.53 $\pm$ 3.33
Urea (mg/dL)	49.84 $\pm$ 1.39	49.92 $\pm$ 1.32	47.06 $\pm$ 1.19
Uric acid (mg/dL)	1.29 $\pm$ 0.13	1.43 $\pm$ 0.16	1.39 $\pm$ 0.16

\*p<0.05

**Table 2-** Proportion (%) of hepatic constituents of rats treated with different concentrations of *A. lappa* aqueous extract (mean  $\pm$  SEM).

Parameters	Control	Group A	Group B
Hepatocyte nucleus	11.9	10.2	10.7
Hepatocyte cytoplasm	58.2	55.4	58.1
Hepatic sinusoids	18.9	20.6	21.6
Central vein	2.8	2.6	1.8
Portal space	6.5	8.1	7.1
Fatty deposition	1.6	2.6	0.5
Inflammatory infiltration	0.1	0.4	0.1
Degenerative hepatocytes	0.0	0.1	0.1



**Figure 1-** Hepatic tissue of rats treated with different concentrations of *A. lappa* aqueous extract. **A-** Group A. **B-** Group B. **C-** Control Group. Arrow head: Kupffer cell. Arrow: hepatocytes. Asterisc: Hepatic sinusoids. Toluidin blue/ 1% sodium borate. X 540.

## DISCUSSION

In recent years, the crude water extract of *A. lappa*, called *A. lappa* tea, has become a promising and important beverage, because it has ample therapeutic activity (Lin et al., 2002). Therefore,

this study was designed to study possible effects of this plant on biochemical parameters and the liver tissue. Plasma levels of transaminases (AST and ALT) and GGT, and liver histopathological and morphometrical changes were used as indicators of hepatotoxicity (Ozcanli, 2006). The increase of the parameters of transaminases and GGT has

been used as an indicator of damage to liver structural integrity, because these enzymes are cytoplasmic in location and released into the circulating blood only after structural damage (Janbaz and Gilani, 2000; Hagar, 2004). Moreover, alterations of the other biochemical parameters also are positively correlated with toxicity to the animal (Hagar, 2004). However, this study did not show alteration of the plasma levels of these enzymes and other biochemical parameters, when treated and control groups were compared, with the exception of the creatinine plasma level in group A ( $p < 0.05$ ). This alteration could not be attributed to the treatment, since group A received a lower concentration of extract than group B, in which the plasma level was not altered. The morphological examination of the liver also supported the above results, suggesting that the tea did not damage the hepatocytes. The high degree of fatty deposition found in the liver of one animal of group A and one in group B, was not considered to be a consequence of the treatment, since it occurred in few individuals only. The results supported the concept that hepatoprotective effects could be attributed to *A. lappa* in relation to liver injuries induced by chronic ethanol consumption, or chronic ethanol consumption potentiated by carbon tetrachloride (Lin et al., 2002), tetrachloride and acetaminophen (Lin et al., 2000). However, this study in animals suggested that caution should be taken in using *A. lappa* to treat human patients with kidney stones and gout, since the plasmatic levels of uric acid were somewhat increased in the treated groups. This could occur due to its diuretic property, remembering that some diuretics were known to decrease the rate of uric acid excretion (Iwaki and Yonetani, 1984). Thus, the administration of *A. lappa* extract in the dosage and during the period of time used did not show signs of toxicity, according to the biochemical parameters and the lack of alterations in the liver tissue.

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#### RESUMO

A bardana (*Arctium lappa* L) é uma planta trazida do Japão e aclimatada no Brasil, e é extensamente utilizada na medicina popular em todo mundo. Este estudo foi realizado para avaliar os possíveis efeitos da *A. lappa* no fígado e nos parâmetros bioquímicos plasmáticos em ratos Wistar adultos. Estes receberam a infusão de bardana nas doses de 10 ou 20 g de folhas secas /L de água, por 40 dias. Não houve alteração significativa nos níveis plasmáticos de albumina, aspartato transaminase (AST), alanina transaminase (ALT), gamma glutamil transferase (GGT), proteínas totais, colesterol total, uréia, ácido úrico, triglicérides, cálcio, fósforo, bilirrubina direta e cloro. A análise morfológica não revelou alterações histopatológicas no fígado. Os dados bioquímicos e morfológicos não indicaram a toxicidade da bardana.

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