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Full Length Research Paper

Safety evaluation of the extract from the shoots of *Arctotis arctotoides* in rats and mice

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The aqueous extract from the shoot of *Arctotis arctotoides* (L.f.) O. Hoffm (Asteraceae) was evaluated for its acute toxicity by the oral route in mice and for the sub-acute effect on haematological, biochemical, and histological parameters in rats. In the acute toxicity test, *A. arctotoides* caused no death even up to 3200 mg/kg dose. Oral treatments with this extract at 500, 1,000 and 2,000 mg/kg did not cause any significant change in the red blood cell count, packed cell volume, haemoglobin concentration, mean corpuscular volume, mean corpuscular haemoglobin concentration, and mean corpuscular haemoglobin. It did cause a significance increase in white blood cell count and its differentials. The extract caused a significant decrease in the levels of some liver enzymes, blood urea nitrogen, potassium, total and conjugated bilirubin. Changes were noted in the body and organ weights while variable changes were observed in the levels of some electrolytes. No significant lesions were observed in the organs examined. The result indicated that the plant may be relatively safe for medicinal uses.

Key words: *Arctotis arctotoides*, acute toxicity, sub acute toxicity haematology, serum biochemistry, histopathology.

INTRODUCTION

Arctotis arctotoides (L.f.) O. Hoffm (Asteraceae), a decumbent herb, is widespread throughout the summer rainfall areas of South Africa and Lesotho, usually in disturbed areas like road verges. The rural dwellers of the Eastern Cape Province use the plant for the treatment of epilepsy, indigestion, and catarrh of the stomach (Afolayan, 2003). The leaf juice or a paste is applied topically to treat wounds. Studies have shown that leaf extracts have antibacterial properties (Afolayan, 2003; Sultana and Afolayan, 2003). Bioassay guided fractionation of the hexane extract of the shoot has led to the isolation of sesquiterpene lactones, farnesol derivatives (Sultana and Afolayan, 2003), β-sitosterol, stigmasterol, lupeyl acetate, and abietic acid (Dahmy et al., 1985; Tsichritzis et al., 1990). A comprehensive investigation into the chemical composition of the volatile oil from *A. arctotoides* also showed the occurrence of sesquiterpenes in its root (Oyedeji et al., 2005).

In this paper, we present the results of an evaluation of the safety of fresh aqueous extracts from the shoots of *A. arctotoides*. The findings from this work might add to the overall value of the medicinal potential of the herb.

MATERIALS AND METHODS

Preparation of the aqueous crude extract

The shoot materials of *A. arctotoides* were collected in January 2007 from plants growing in the natural population around the University of Fort Hare campus. The plant has been previously authenticated by Prof. D. S. Grierson of the Department of Botany, University of Fort Hare, when a voucher specimen (Jimoh02-2007) was prepared and deposited at the herbarium of the Department.

The fresh plant material (100 g) was macerated in 200 ml of distilled water for 24 h on an orbital shaker (Digisystem Laboratory, Germany) at room temperature (24ºC). The extract was filtered

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using a Buckner funnel and Whatman No 1 filter paper. Filtrate was concentrated to dryness under reduced pressure at 40ºC. The thick solution was lyophilized using freeze-drying system for biological investigations. The extract yielded 9.8 g. Graded aqueous solutions of the extract was prepared and administered to the rats per os by gavage once daily for 14 days.

Animal care and handling

Sixteen male Wistar rats weighing between 389 and 406 g were used in the sub-acute study, and 30 male albino mice with body weights ranging between 25 and 40 g were used in the acute toxicity study. The animals were procured from the Medical Research Council of South Africa, Johannesburg.

The animals were housed in cages at the Experimental Animal House in the Zoology Building, University of Fort Hare, and were allowed *ad libitum* access to feed and water. They were fed on commercial rat cubes (EPOL Feeds, South Africa Ltd.). All experimental protocols were in compliance with University of Fort Hare Ethics Committee on Research in Animals as well as internationally accepted principles for laboratory animal use and care.

Experimental designs

The rats were divided into four groups of four, which were administered with the plant extract by oral gavage at doses of 500, 1,000 and 2,000 mg/kg body weight respectively. The control groups received distil water instead of the extract. All the animals were weighed at the start, 7^{th} and 14^{th} day of the experiment.

The mice were divided into five groups of six per group and were administered with plant extract by oral gavage at doses of 400, 800, 1,600 and 3,200 mg/kg dose of the extract. The control group received distilled water (3 ml/kg body weight).

Acute toxicity study

The acute toxicity of *A. arctotoides* aqueous extract was determined according to the method of Sawadogo et al. (2006). Mice fasted for 16 h were randomly divided into groups of six per group. Graded doses of the extract (400, 800, 1,600 and 3,200 mg/kg body weight po) were separately administered to the mice in each of the test groups by means of bulbed steel needle. The control group was treated with orally administered distilled water (3 ml/kg po) only. All the animals were then allowed free access to food and water and observed over a period of 48 h for signs of acute toxicity. The number of deaths within this period of time was recorded.

Collection of blood and serum samples

Paired blood samples were collected by cervical decapitation from diethyl ether anaesthetized rats into heparinized and non-heparinized tubes. The heparinised blood was used for haematological evaluation; the non-heparinized blood was allowed to coagulate, and then centrifuged, the serum was separated and analyzed for biochemical parameters.

Determination of haematological and serum biochemical parameters

The haematological and serum biochemical parameters were determined using Beckman DXC 600 (USA) for serum chemistry and Advia 2120 (Bayer, Germany) for haematology. Erythrocytes indices such as mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC) and mean corpuscular haemoglobin (MCH) were determined from values obtained from red blood cell (RBC) count, haemoglobin concentration and packed cell volume (PCV) values (Coles, 1986). The biochemical parameters evaluated were: glucose, creatinine, blood urea nitrogen (BUN), aspartate transaminase (AST), alanine transaminase (ALT), gamma-glutamyl transferase (GGT), alkaline phosphatase (ALP) potassium, sodium, chloride, phosphorus, total bilirubin, total protein, albumin, and globulin.

Weight changes

The weights of all the animals were determined at the onset of the study. Weight changes were determined every week till the end of the study. At necropsy, the following organs were collected and weighed: liver, kidney, heart, spleen, and testes.

Histopathology

The liver, kidney, heart, spleen, and testes of all the animals were fixed in 10% buffered formalin in labeled bottles. Tissues were processed routinely and embedded in paraffin wax. Sections 5 µ thick were cut, stained with haematoxylin and eosin, and examined under the light microscope.

Statistical analysis

Results were expressed as mean ± standard error of mean (S.E.M.). Where applicable, the data were subjected to one way analysis of variance (ANOVA) and differences between samples were determined by Duncan's Multiple Range test using the Statistical Analysis System (SAS, 1999) program. P values at 5% were regarded as significant.

RESULTS

The effects of the graded doses of the aqueous extract of *A. arctotoides* on haematological parameters of Wistar rats are shown on Table 1. The PCV and RBC values of treated groups were within the reference range for rats and were not significantly different from the control group except for the 500 mg/kg dose group. Similarly, there was no significant change in haemoglobin concentration and MCH for all the treated groups compared to the control. The MCV of the group which received a dose of 2,000 mg/kg body weight experienced significant decrease relative to the control. On the other hand, the MCHC for the two groups which received doses of 1,000 and 2,000 mg/kg body weight experienced a significant increase compared to those of the control and the group that received a dose of 500 mg/kg body weight. However, all doses caused a significant difference in the levels of white blood cell count and its differentials. The extract also caused significant decrease in the level of platelets.

There was no significant difference in the level of the total protein for the control and groups that received doses 500 and 1,000 mg/kg body weight, though there was a significant difference between the groups that received 500 and 2,000 mg/kg body weight. There were

Table 1. Effects of the graded doses of the aqueous extract of *A. arctotoides* on haematological parameters of Wistar rats.

Parameter	Control	Extract concentration (mg/kg)		
		500	1000	2000
PCV (%)	49.5 ± 0.7 [b]	56.0 ± 0.4 [a]	49.0 ± 0.6 [b]	50.0 ± 0.7 [b]
Hb (g/L)	13.9 ± 0.3 [b]	16.1 ± 0.3 [a]	15.4 ± 0.3 [a]	15.6 ± 0.3 [a]
RBC ($X10^{12}$g/L)	8.2 ± 0.2 [b]	9.6 ± 0.3 [a]	9.1 ± 0.2 [a]	9.4 ± 0.4 [a]
MCV (fl)	60.6 ± 2.1 [a]	58.8 ± 2.0 [a]	54.0 ± 2.2 [b a]	53.3 ± 2.0 [b]
MCH (pg)	17.1 ± 0.7 [a]	16.7 ± 0.6 [a]	17.1 ± 0.7 [a]	16.7 ± 0.5 [a]
MCHC (%)	28.1 ± 0.5 [b]	28.8 ± 0.6 [b]	31.5 ± 0.5 [a]	31.2 ± 0.4 [a]
WBC ($X10^9$/L)	5.5 ± 0.4 [c]	6.9 ± 0.6 [b]	7.7 ± 0.5 [b]	11.0 ± 0.4 [a]
Lymphocytes ($x10^9$/L)	3.3 ± 0.2 [d]	4.6 ± 0.2 [c]	5.7 ± 0.3 [b]	6.8 ± 0.4 [a]
Neutrophils ($x10^9$/L)	1.2 ± 0.1 [a]	0.6 ± 0.1 [b]	0.7 ± 0.1 [b]	0.6 ± 0.1 [b]
Monocytes ($x10^9$/L)	0.4 ± 0.1 [c]	0.8 ± 0.1 [b]	0.7 ± 0.2 [b]	1.9 ± 0.1 [a]
Eosinophils ($x10^9$/L)	0.1 ± 0.03 [a]	0.02± 0.01 [a]	0.1 ± 0.02 [a]	0.1 ± 0.03 [a]
Large unstained cells ($x10^9$/L)	0.4 ± 0.2 [b]	0.7 ± 0.1 [b]	0.6 ± 0.2 [b]	1.5 ± 0.2 [a]
Basophils ($x10^9$/L)	0.1 ± 0.01 [a]	0.02± 0.01 [c]	0.02 ± 0.01 [c]	0.05 ± 0.01 [b]
Platelets	820.9 ± 3.6 [a]	715.8± 3.7 [b]	703.5 ± 3.8 [c]	693.4 ± 3.2 [c]

Values are mean ± S.E.M.; n = 4.
Means along a row with the same superscripts are not significantly different at P < 0.05

Table 2. Effects of the graded doses of aqueous extract of *A. arctotoides* on the serum biochemical parameters of Wistar rats.

Parameter	Control	Extract concentration (mg/kg)		
		500	1000	2000
Total Protein (g/dL)	59.8 ± 1.3 [a]	63.5 ± 1.3 [a]	62.5 ± 1.2 [a]	58.3 ± 1.2 [a]
Albumin (g/dL)	15.6 ± 0.7 [bc]	17.5 ± 0.7 [ba]	18.5 ± 0.7 [a]	15.3 ± 0.6 [c]
Globulin (g/dL)	44.2 ± 1.3 [a]	46.0 ± 1.2 [a]	44.0 ± 1.3 [a]	43.0 ± 1.2 [a]
ALT (U/L)	60.3 ± 1.2 [a]	39.8 ± 1.3 [b]	37.5 ± 1.3 [b]	36.5 ± 1.3 [b]
AST (U/L)	220.0 ± 2.9 [a]	119.5 ± 2.8 [b]	122.3 ± 2.9 [b]	128.5 ± 2.7 [b]
ALP (U/L)	325.0 ± 12.6 [a]	255.0 ± 12.8 [b]	211.5 ± 12.6 [c]	171.8 ± 12.6 [c]
GGT (U/L)	8.3 ± 0.5 [a]	5.5 ± 0.5 [b]	4.1 ± 0.5 [b]	5.6 ± 0.6 [b]
Total bilirubin (mmol)	15.1 ± 0.7 [a]	8.5 ± 0.7 [b]	7.5 ± 0.6 [b]	6.8 ± 0.5 [b]
Conj. Bilirubin (mmol)	9.0 ± 0.3 [a]	1.3 ± 0.3 [b]	1.3 ± 0.3 [b]	1.3 ± 0.3 [b]
Unconj. Bilirubin (mmol)	6.1 ± 0.5 [a]	7.2 ± 0.5 [a]	6.3 ± 0.5 [a]	5.5 ± 0.4 [a]
Urea (mg/dL)	8.7 ± 0.2 [a]	6.1 ± 0.2 [c]	6.5 ± 0.3 [cb]	6.7 ± 0.2 [b]
Creatinine	58.4 ± 1.8 [a]	55.3 ± 1.7 [a]	55.3 ± 1.3 [a]	57.8 ± 1.8 [a]
Sodium	140.2 ± 0.8 [bc]	144.5 ± 0.8 [a]	142.8 ± 0.8 [ba]	139.3 ± 0.7 [c]
Potassium	6.6 ± 0.3 [a]	4.6 ± 0.4 [b]	5.2 ± 0.3 [b]	4.8 ± 0.3 [b]
Chloride	100.5 ± 1.0 [bc]	99.5 ± 1.1 [c]	103.5 ± 1.0 [ba]	106.5 ± 1.0 [c]
Inorganic Phosphorus	2.5 ± 0.2 [c]	3.2 ± 0.2 [a]	3.1 ± 0.2 [ba]	2.6 ± 0.1 [bc]

Values are mean ± S.E.M.; n = 4.
Means along a row with the same superscripts are not significantly different at P < 0.05.

varied differences in the levels of albumin and globulin for the four groups when these were compared with one another. The levels of ALP, ALT, GGT, and AST of all the experimental animals experienced significant decreases relative to the control. For total, conjugated and unconjugated bilirubin, significant decrease in the levels of these parameters was observed between experimental groups and control but no significant change was observed between the treated groups. The extract caused significant decrease in blood urea nitrogen (BUN) in all the experimental groups when compared to the control group. The effects of the extract on the electrolytes showed varied significant differences between the treated groups. The extract did not cause any significant change in the level of creatinine for the entire treated groups (Table 2).

Table 3. Effects of the graded doses of aqueous extract of *A. arctotoides* on the body weights of rats.

Parameter	Control	Extract concentration (mg/kg)		
		500	1000	2000
Weight before extract administration (g)	350.0 ± 12.6 [a]	372.0 ± 12.7 [a]	380.3 ± 12.6 [a]	372.0 ± 12.6 [a]
Weight after 7days of extract administration	373.8 ± 11.5 [a]	380.1 ± 11.5 [a]	393.5 ± 11.4 [a]	378.7 ± 11.5 [a]
Weight after 14 days	384.1 ± 11.0 [a]	387.0 ± 11.1 [a]	397.3 ± 11.0 [a]	373.5 ± 11.0 [a]

Values are mean ± S.E.M.; n = 4.
Means along a row with the same superscripts are not significantly different at P < 0.05.

Table 4. Effect of graded doses of the aqueous extract of *A. arctotoides* on organ weights of rats.

Parameter	Control	Extract concentration (mg/kg)		
		500	1000	2000
Heart (g)	0.4 ± 0.05 [b]	1.3 ± 0.04[a]	1.1 ± 0.04[a]	1.1 ± 0.04[a]
Spleen (g)	0.2 ± 0.06 [b]	0.8 ± 0.05[a]	0.7 ± 0.05[a]	0.8 ± 0.05[a]
Right kidney (g)	0.4 ± 0.08 [b]	1.1 ± 0.07[a]	1.2 ± 0.07[a]	1.1 ± 0.07[a]
Left kidney (g)	0.4 ± 0.07 [b]	1.2 ± 0.06[a]	1.2 ± 0.06[a]	1.2 ± 0.06[a]
Liver (g)	4.4 ± 0.7 [b]	12.7 ± 0.5[b]	13.2 ± 0.4[b]	12.4 ± 0.7[a]
Right testis (g)	0.7 ± 0.05 [c]	3.4 ± 0.05[a]	3.6 ± 0.05[a]	3.9 ± 0.05[a]
Left testis (g)	0.5 ± 0.1[b]	3.5 ± 0.1[a]	3.6 ± 0.1[a]	3.6 ± 0.1[a]

Values are mean ± S.E.M.; n = 4.
Means along a row with the same superscripts are not significantly different at P < 0.05.

With respect to histopathological changes, there were no significant lesions in all the organs of the rats. However, in one of the animals which received 1,000 mg/kg body weight, its kidney showed hyperplasia of the epithelium of the distal convoluted tubules with adjacent chronic inflammation.

The study showed that the extracts did not affect the body weights of the rats although all the organs of tested group experienced significant increase in weight compared to the control (Tables 3 and 4).

No death was recorded in the mice after 48 h of the administration of the extract. No behavioral changes were also observed in these animals.

DISCUSSION

From this study, it is inferred that the aqueous extract of *A. arctotoides* has no toxic effect on the red blood cell parameters; red blood cell count, haemoglobin concentration, packed cell volume, mean corpuscular volume, mean corpuscular haemoglobin concentration and mean corpuscular haemoglobin. This shows that the plant is safe for use because some plants are known to cause destruction of red blood cells leading to anaemia (Blood and Radostits, 1989; Adedapo, 2002; Adedapo et al., 2004; 2007a).

The extract caused significant changes in the total WBC count and its differentials when compared with those of control. This observation of increase in the levels of these parameters by this plant extract shows that the principal function of phagocytes, which is to defend against invading microorganisms by ingesting and destroying them, thus contributing to cellular inflammatory processes, will be enhanced (Paul, 1993; Swenson and Reece, 1993; Adedapo et al., 2005). It was noted that the plant extract also caused significant increase in the level of neutrophils. *A. arctotoides* contains sesquiterpenes in its shoot (Sultana and Afolayan, 2003). These compounds have been reported to be insect antifeedant, antifungal, cytotoxic, and antitumoral (Wedge et al., 2000; Izevbigie, 2003; Zhang et al., 2005; Erasto et al., 2006). The observation of changes in white blood cell count and its differentials may therefore support the antibacterial activity of this plant (Afolayan, 2003).

Platelets play a major role in the process of haemostasis Interaction of the damaged vascular wall with circulating platelets and coagulation protein leads to haemostasis (Fieldman et al., 1988; Johnson and Morris, 1996). The reduction of the level of platelets in this study might mean that haemostasis may be compromised. Therefore, continued administration of this extract may bring about coagulation deficiency leading to internal and external haemorrhage (Cheeke and Shull, 1985; Searcy and Petrie, 1990; Carlson, 1996). Some plants produce toxic effects by causing extensive haemorrhages into tissues leading to severe blood loss (Macfarlane et al., 2001; Adedapo, 2002).

Aqueous extract of *A. arctotoides* also caused no significant change in the levels of total protein and globu-

lin. The increase in albumin level was noted for the 1,000 mg/kg dose only. Albumin is the protein with the highest concentration in plasma. It transports many small molecules in the blood (for example, bilirubin, calcium, progesterone, and drugs). It also prevents the fluid in the blood from leaking out into the tissues (Duncan et al., 1994).

There was a significant decrease in the level of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and γ-glutamyl transferase (GGT). Serum ALT is known to increase when there is liver disease and it has been used as a tool for measuring hepatic necrosis (Bush, 1991; Duncan et al., 1994). AST is however, not a liver specific enzyme as high levels of AST can also be found in skeletal and cardiac muscles as well as red blood cells (Adedapo et al., 2007b). Increase in serum ALP may be considered as an indicator of cholestasis in early stages or mild circumstances preceding other indicators e.g. hyperbilirubinemia (Adedapo et al., 2007a). GGT has its highest concentration in the kidney and the liver though can be found also in small intestine and pancreas (Bush, 1991). These observations of significant decrease in the levels of the liver enzymes may indicate that the extract of *A. arctotoides* has hepatoprotective effects. This is also corroborated by significant decrease in the serum levels of total and conjugated bilirubin. The effect of the extract on the electrolytes showed that the plant did not cause any impairment on the cardiovascular system.

The extract did not affect body weight gain but there was a significant increase in the weights of the organs. The significant increase in the organ weights in this study is similar to a study in which male rats at the dose of 500 mg/kg/day of genistein experienced increase in the size of the spleen, testes, adrenal gland. It was stated that the increase in the spleen weight was likely due to extramedullary erythropoiesis as a compensatory response to osteopetrosis while changes in testes may be due to hormonal effects of genistein (McClain et al., 2006).

Histopathological studies of the testes, spleen, liver, and heart of all the groups showed no significant lesion. In the case of kidneys, one animal from the group that was administered with 1,000 mg/kg body weight showed hyperplasia of the epithelium in the distal convoluted tubules with adjacent chronic inflammation. This however is not ascribed to the effects of the extract, because other animals in the group did not exhibit similar signs and besides it was a chronic inflammation.

The administration of graded doses of the plant extract to mice did not result in mortality neither was there any behavioral change in the animals. This might suggests that the plant is safe for medicinal uses.

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