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Acute and chronic toxicity of the aqueous extract of *Artemisia afra* in rodents

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Abstract

Artemisia afra (*Jacq. Ex. Willd*), "African Wormwood" is widely used traditionally in South Africa with no literature evidence substantiating its safety. The aim of this study was to investigate the safety of the aqueous extract of *Artemisia afra* by determining its pharmaco-toxicological effects after acute and chronic administration in mice and rats, respectively. The aqueous extract mimicked the traditional decoction dosage form of *Artemisia afra*. In mice, single intraperitoneal injections of *Artemisia afra*-extract (1.5–5.5 g/kg) induced a regular dose-dependent increase in the death rate and incidence of general behaviour adverse effects, while with single oral doses (2–24 g/kg) the increases in incidence of general behaviour adverse effects and mortality rate were dose-independent. The LD_{50s} after acute intraperitoneal and oral doses were 2.45 and 8.96 g/kg, respectively. Rats given oral doses of *Artemisia afra*-extract (0.1 or 1 g/kg/day) survived the 3 months of dosing (i.e. LD_{50} much higher than 1 g/kg), experienced no significant changes in general behaviour and haematological and biochemical parameters, except for transient decrease in AST activity. No significant changes were observed in organ weights, and histopathological results showed normal profile suggesting no morphological alterations. Collectively, the results indicate that *Artemisia afra*-extract is non-toxic when given acutely, has low chronic toxicity potential and, in high doses, may have a hepatoprotective effect.
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Keywords: *Artemisia afra*; Aqueous extract; Plant medicine; Acute and chronic toxicity; Rodents; Traditional dosage form

1. Introduction

Artemisia afra (African wormwood, family Asteraceae) is a medicinal plant commonly found in most areas of Southern Africa, where it has a reputation for its claimed healing properties and use in specific ailments (Watt and Breyer-Brandwijk, 1962; Roberts, 1990; Iwu, 1993). In fact, based on South African indigenous knowledge the plant is traditionally used for a wide range of specific ailments including coughs, colds, sore throat, heartburns, hemorrhoids, fevers, malaria, asthma, diabetes mellitus and other conditions (Roberts, 1990; Iwu, 1993; Hutchings et al., 1996; Dyson, 1998; Van Wyk et al., 2000). A few laboratory studies have shown that *Artemisia afra* materials did exhibit a wide range of biological and pharmacological activities, which may substantiate the therapeutic use of this plant in traditional medicine. For instance, investigations conducted on the aqueous extract of *Artemisia afra* have indicated that the plant has anti-histaminic and narcotic analgesic properties (Hutchings et al., 1996). Other assays carried out on the ethanolic and dichloromethane extracts have shown the plant to have in vitro hypotensive and anti-tuberculosis effects, respectively (MRC and SA Healthinfo, 2004).

The merit of the traditional use of *Artemisia afra* has also been supported by the isolation and identification of several possible active chemical constituents, including flavonoids and volatile oil, the latter containing mainly 1,8-cineole, α-thujone, β-thujone, camphor and borneol (Van Wyk et al., 2000). The plant also contains terpenoids, coumarins and acetylenes but their contribution to the biological activity of *Artemisia afra* is not yet known (Van Wyk et al., 2000).

However, the biological activity of *Artemisia afra* and it constituents may not always be just beneficial. For instance, the volatile oil of *Artemisia afra* as isolated by Van der Lingen (Watt and Breyer-Brandwijk, 1962) is said to be as toxic as the

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oil of Sabine and produced hemorrhagic nephritis, degenerative changes in the liver and pulmonary oedema when orally administered to rabbits and sometimes abortion in pregnant rabbits and guinea pigs (Watt and Breyer-Brandwijk, 1962). The volatile oil also produces hallucinogenic effects that may be attributed to thujone, so that overdoses or continued use over long periods are potentially harmful (Van Wyk et al., 2000), while elsewhere the aqueous extract of *Artemisia afra* has been shown to be cytotoxic to Hela cells in vitro (SA Healthinfo, 2004). Despite the wide spread traditional use of this indigenous medicine in South Africa and the fact that some of its chemical constituents such as the volatile oils can potentially cause significant toxicity, no systematic study has so far been done on the toxicity of *Artemisia afra*, particularly as it is traditionally used.

The purpose of the present study was thus to investigate the safety of the aqueous extract of *Artemisia afra* by determining its behavioural and pharmaco-toxicological effects after acute and chronic administration in mice and rats, respectively. By using the aqueous extract the traditional decoction dosage form of *Artemisia afra* could be mimicked.

2. Materials and methods

2.1. Plant material

Freshly picked *Artemisia afra* plant material (i.e. mature leaves and aerial parts) was collected in Montague garden (Western Cape Province, South Africa) during its flowering period in summer 2004. The material was authenticated as *Artemisia afra* by Mr. Franz Weitz, a botanist in the Department of Botany at the University of the Western Cape (UWC) and a voucher specimen number 6639 was dried and deposited in the herbarium at UWC.

2.2. Preparation of the aqueous extract of Artemisia afra

The wet leaves of *Artemisia afra* plucked from the stalks were washed with distilled water, dried in the oven at 30 °C and slightly crushed (but not powered) by hand. The aqueous extract was prepared in a way that, as much as possible, mimicked the method traditional herbal practitioners use to extract their plant medicines. The dried leaves were suspended in distilled water (50 g dried leaves per 1 l water) and the mixture boiled for 30 min. The decoction obtained was cooled, filtered, frozen at −70 °C, freeze-dried (Virtis™ mobile freeze-dryer, model 125L) and then sterilized by gamma irradiation (Hepro Cape Gamma, Cape Town). The yield of the crude aqueous extract was about 20% (w/w) and the final *Artemisia afra*-extract was stored at −20 °C until further bioassay.

2.3. Acute toxicity study in mice

Healthy BALB/C mice of both sexes, weighing 21–31 g, obtained from the University of Cape Town (Animal Unit), were divided into 16 groups of 6 animals matched for weight and size. These animals were housed 3 mice per sex per cage in a well ventilated room with 12 h cycle of day and night light conditions and temperature maintained at around 25 °C. All animals had free access to tap water and the same type of food throughout the experiment, except for the short fasting period before the oral administration of the single doses of *Artemisia afra*-extract. The *Artemisia afra*-extract was aseptically suspended in normal saline (0.9% NaCl solution) and administered in single intraperitoneal (i.p.) doses of 0, 1500, 2500, 3500, 4500, and 5500 mg/kg or by gavage (p.o.) at single doses of 0, 2, 4, 6, 8, 10, 12, 16, 20, and 24 g/kg. The general behaviour of the mice was continuously monitored for 1 h after dosing, periodically during the first 24 h (with special attention given during the first 4 h) (Hilaly et al., 2004), and then daily thereafter, for a total of 14 days. Changes in the normal activity of mice and their weights were monitored and the time at which signs of toxicity or death appeared recorded. All surviving animals were euthanised with diethyl ether at day 14 and various tissues (brain, kidneys and liver) collected, weighed and visually inspected for any histopathological changes. For each route of administration, the LD_{50} values were determined using the method of Litchfield and Wilcoxon (1949).

The protocol for this study as well as the chronic toxicity study was approved by the UWC Senate Ethics Research Committee.

2.4. Chronic toxicity study in rats

Male Wistar rats, weighing 230–270 g, were housed in 5 (I–V) groups of 6 animals each under the same conditions as described for the mice. The rats were first left for 7 days to acclimatize to laboratory conditions, then baseline readings of their weights were recorded and samples of blood collected from 6 sacrificed individuals (group V). The *Artemisia afra*-extract dispersed in sterile normal saline (0.9% NaCl solution as vehicle) was administered orally, daily for 3 months, to the groups I to VI at treatment doses of 0, 100, 1000 or 1000 mg/kg BW, respectively. Based on the information reported by Roberts (1990), the dose 100 mg is equivalent to double the dose used by traditional healers and from the acute toxicity study 1000 mg was equivalent to 10% of the oral LD_{50} obtained in the mice. In the treated animals, deviations in normal behaviour, coat condition, discharge, movements and mortality of the rats were monitored daily, and body weight changes and food intake were recorded weekly. At the end of the 3 month-experiment, all the animals were anaesthetized with intraperitoneal injection of sodium pentobarbitone 6% solution (40 mg/kg) (Waynforth, 1980) and blood samples collected via cardiac puncture into EDTA, sodium fluoride or gel containing tubes for the hematological and biochemical analyses. In addition, select organs (brain, liver and kidneys) were dissected out for histopathological examination.

2.5. Blood analyses

The hematological and the biochemical analyses were performed at a large nationally accredited commercial laboratory (PathCare, Cape Town, South Africa). Full blood cell counts (red blood cell, hematocrit, hemoglobin, white blood cell and platelets) were determined on a fully automated analyzer

(CELL-DYN 3700, Abbott Laboratories, Santa Clara, CA, USA) using the "Combination laser light scattering and impedance counting analytical method" (on EDTA blood) and the blood chemistry tests were performed on an auto-analyzer (Beckman Coulter CX7, Fullerton, CA, USA) using different methods, viz. a kinetic rate method for the enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST); the modified rate Jaffe method for creatinine; the timed endpoint method for total cholesterol (on blood aliquots in gel tubes) and the oxygen rate method for glucose (blood in sodium fluoride tubes) (Path Care, 2005).

2.6. Histopathological examinations

The wet organs, viz. kidneys, liver and brain, of each rat were isolated, weighed, dissected and then inspected for any histopathological changes.

2.7. Statistical analysis

Results are expressed as mean ± standard deviation. Statistical comparisons between the data for the control and treatment groups were performed using the Student's *t*-test. *P*-values less than 0.05 were set as the level of significance.

3. Results

3.1. Acute toxicity studies in mice

There was a regular dose-dependent increase in mortality and decrease in mortality latency in both sexes of mice after the i.p. administration of *Artemisia afra*-extract. The first mouse died between 72 and 120 h after injection of the 2500 mg/kg dose of *Artemisia afra*-extract, and the maximum frequency of death occurred at 4500 mg/kg and higher doses with the animals dying within 1 to 24 h after the injection. Twenty, i.e. 9 males and 11 females, of the 30 mice treated with single doses of *Artemisia afra*-extract died. In addition to death, the intraperitoneally administered plant extract also induced minor to accentuated hypo activity, piloerection, dizziness, hyperventilation, loss of weight, convulsion and syncope. For this route the no-observed-adverse-effect (NOAEL) dose (Hilaly et al., 2004) for the *Artemisia afra*-extract was 1500 mg/kg, the maximum tolerated dose (MTD: highest dose for which the mice recovered completely from all effects of *Artemisia afra*-extract) assumed to be between 1500 mg and 2500 mg/kg, the minimum lethal dose (MLD: the lowest dose that induced the first mortality in mice) 2500 mg/kg and the single dose LD_{50} 2450 mg/kg (19/20 confidence limits = 1750–3430 mg/kg) (Table 1).

With the oral single doses of *Artemisia afra*-extract, there was an irregular dose-dependent increase in mortality in both sexes of mice. Thirty (15 males and 15 females) of the 54 mice given the plant extract via the oral route died. The mortality latency was relatively constant over all the doses, and the other symptoms of toxicity recorded were similar to those that occurred after i.p. administration, except for salivation as the only additional symptom. Via this route the NOAEL dose was 2000 mg/kg, the MTD 4000 mg/kg, the MLD 6000 mg/kg and the acute oral LD_{50} 8960 mg/kg (19/20 confidence limits = 5490–14,600 mg/kg) (Table 2).

3.2. Chronic toxicity studies in rats

3.2.1. Effect of oral Artemisia afra-extract on the general behaviour of rats

The chronic oral administration of *Artemisia afra*-extract caused no noticeable change in the general behaviour of the rats and, compared to the control group (dose 0 mg/kg), no significant changes in body weight, food intake and utilization of food in the treated mice. Both the control and treated rats appeared uniformly healthy at the end and throughout the 3-month period of study. A few rats receiving the high dose (1000 mg/kg) of extract manifested minor symptoms of toxicity comprising intermittent diarrhea, salivation and partial hypoactivity. No deaths

Table 1
Effects of single doses of *Artemisia afra* aqueous extract administered i.p. in mice

Dose of *AA*-extract (mg/kg)	Mice		Effects	
	Sex	*D/T*	Mortality latency (h)	Symptoms of toxicity
0	Male	0/3	–	None
	Female	0/3	–	None
1500	Male	0/3	–	Hypo activity, loss of appetite, piloerection
	Female	0/3	–	Hypo activity, loss of appetite, piloerection
2500	Male	1/3	>120, <144	Hypo activity, loss of appetite, piloerection
	Female	2/3	>72, <120	Hypo activity, loss of appetite
3500	Male	2/3	>12, <48	Convulsion, dizziness, hyperventilation, syncope
	Female	3/3	>12, <36	Convulsion, dizziness, hyperventilation, syncope
4500	Male	3/3	>12, <24	Convulsion, disorientation, hyperventilation, syncope
	Female	3/3	>12, <24	Convulsion, disorientation, hyperventilation, syncope
5500	Male	3/3	>1, <24	Convulsion, disorientation, hyperventilation, syncope
	Female	3/3	>1, <12	Convulsion, disorientation, hyperventilation, syncope

AA, *Artemisia afra*; *D/T*, number of dead/number of treated mice; (−) no toxic symptoms during the period of observation; mortality latency, time to death (in hours) following the injection.

Table 2
Effects of single doses of *Artemisia afra* aqueous extract administered p.o. in mice

Dose of *AA*-extract (mg/kg)	Mice		Effects	
	Sex	*D*/*T*	Mortality latency (h)	Symptoms of toxicity
0	Male	0/3	–	None
	Female	0/3	–	None
2000	Male	0/3	–	None
	Female	0/3	–	None
4000	Male	0/3	–	Hypo activity, piloerection
	Female	0/3	–	Hypo activity, piloerection
6000	Male	1/3	>12, <24	Convulsion, dizziness, hypo activity, loss of appetite
	Female	2/3	>12, <192	Convulsion, hyperventilation, hypo activity, salivation
8000	Male	1/3	>12, <24	Convulsion, hyperventilation, hypo activity, salivation
	Female	0/3	–	Hypo activity, loss of appetite, salivation
10,000	Male	3/3	>12, <24	Convulsion, dizziness, hyperventilation, hypo activity
	Female	2/3	>12, <24	Convulsion, dizziness, hyperventilation, hypo activity
12,000	Male	1/3	>24, <36	Convulsion, dizziness, hyperventilation, hypo activity
	Female	2/3	>12, <96	Convulsion, hyperventilation, loss of appetite, salivation
16,000	Male	3/3	>12, <24	Convulsion, hyperventilation, salivation, syncope
	Female	3/3	>12, <24	Convulsion, dizziness, hyperventilation, hypo activity
20,000	Male	3/3	>12, <24	Convulsion, dizziness, hyperventilation, salivation, syncope
	Female	3/3	>12, <24	Convulsion, dizziness, hyperventilation, hypo activity
24,000	Male	3/3	>12, <24	Convulsion, dizziness, hyperventilation, syncope
	Female	3/3	>12, <24	Convulsion, dizziness, hyperventilation, hypo activity

however occurred at any of the doses (up to 1000 mg/kg) given indicating that the LD_{50} for chronic p.o. dosing with *Artemisia afra*-extract was much higher than 1000 mg/kg. A summary of the growth and mortality results and gross symptoms of toxicity observed after the chronic oral administration of plant extract to the rats are presented in Fig. 1 and Table 3, respectively.

3.2.2. *Effect of oral Artemisia afra-extract on the haematological and biochemical parameters of rats*

The haematological parameters, hematocrit, hemoglobin concentration, platelets, red and white blood cells in the treated rats did not differ significantly ($P > 0.05$) from that of the control group (Table 4) and all the values remained within normal limits throughout the experimental period. As shown in Table 4, no significant treatment-related changes in the levels of plasma analytes such as cholesterol, creatinine and glucose, and serum ALT activities were observed at the termination of the study. However, there appeared to be a significant increase in the serum

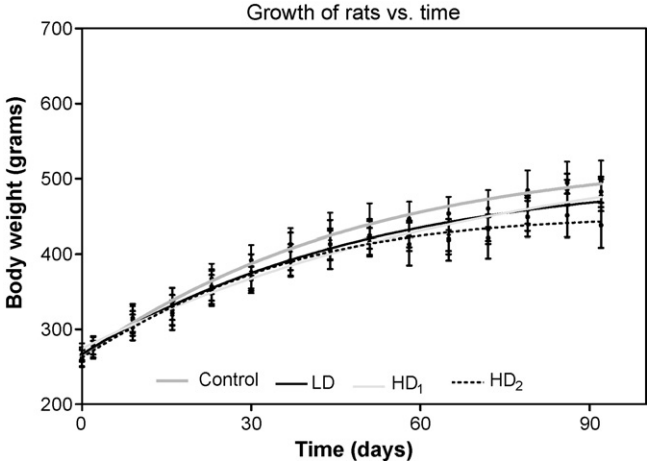

Fig. 1. Mean body weights of groups of rats (control = 0 mg/kg/day; LD = 100 mg/kg/day; HD_1 or HD_2 = 1000 mg/kg/day; $n = 6$) given daily doses of aqueous extract of *Artemisia afra* by gavage for 92 days.

Table 3
Mortality and gross symptoms of toxicity observed after chronic oral administration of plant extract to the rats

Dose of *AA*-extract (mg/kg)	Rats			Effects	
	#/Dose	Sex	*D*/*T*	Mortality latency (day)	Symptoms of toxicity
0	6	Male	0/6	–	None
100 (LD)	6	Male	0/6	–	None
1000 (HD_1)	6	Male	0/6	–	Partial hypo activity, diarrhoea, salivation
1000 (HD_2)	6	Male	0/6	–	Partial hypo activity, diarrhoea, salivation
Total	24		0/26		

AA, *Artemisia afra*; #, number; *D*, number of dead; *T*, number of treated rats; LD: low dose; HD_1: high dose 1; HD_2: high dose 2.

Table 4
The effect of *Artemisia afra*-extract on the haematological and biochemical blood parameters of rats after 3 months daily p.o. dosing

Parameter (units)	Dose group (expressed in mean ± S.D., $n = 6$)				
	Control D0 (0 mg/kg)	Control D92 (0 mg/kg)	LD at D92 (100 mg/kg)	HD_1 at D92 (1000 mg/kg)	HD_2 at D93 (1000 mg/kg)
Haematocrit (l/l)	0.63 ± 0.05	0.68 ± 0.07	0.70 ± 0.04	0.74 ± 0.09	0.71 ± 0.05
Haemoglobin (g/dl)	14.00 ± 1.10	14.73 ± 0.73	14.60 ± 0.68	15.38 ± 1.60	14.93 ± 0.85
Platelet (10^9/l)	490 ± 354	469 ± 273	431 ± 324	391 ± 190	742 ± 312
RBC (10^{12}/l)	7.10 ± 0.69	7.90 ± 0.68	8.30 ± 0.50	8.90 ± 0.91	8.60 ± 0.41
WBC (10^9/l)	4.6 ± 2.2	3.8 ± 1.3	2.8 ± 0.5	3.5 ± 1.5	3.4 ± 1.6
ALT (U/l) 37 °C	80.5 ± 36.6	114.8 ± 33.1	116.0 ± 43.4	88.8 ± 45.8	119.5 ± 33.1
AST (U/l) 37 °C	365.5 ± 202.3	725.5 ± 283.6[a]	613.0 ± 222.2[a]	361.7 ± 178.9[b]	624.3 ± 84.66[a]
Cholesterol (mmol/l)	1.3 ± 0.54	1.2 ± 0.20	1.2 ± 0.20	1.1 ± 0.19	1.1 ± 0.15
Creatinine (μmol/l)	58 ± 1.3	62 ± 6.3	64 ± 4.4	60 ± 2.0	73 ± 9.5
Glucose (mmol/l)	8.5 ± 1.3	6.9 ± 0.81	6.6 ± 0.36	6.1 ± 0.93	6.8 ± 0.34

[a] Statistically significantly different from control at D0 (*t*-test, $P < 0.05$).
[b] Statistically significantly different from control at D92 (*t*-test, $P < 0.05$).

AST activities for the control, low dose (LD) at day 92 and high dose$_2$ (HD_2) at day 93 groups, when compared with that for the control group at day 0 (i.e. Control D92, LD D92 and HD_2 D93 versus Control D0, $P < 0.05$). These differences may be biologically relevant but were not considered treatment-related because they did not occur with any consistent relationship to the dose. The difference in AST levels was even observed in the control group at the termination of the study (control D92) versus control group at the start (Control day D0), suggesting that the AST activity levels increased with age (time). There was however a significant difference in the AST activities between the control and high dose group at day 92, i.e. groups from which blood was taken shortly (1–2 h) after the last dosing. But when the levels were measured 1 day after the last dose (i.e. 25 h later), much higher levels were obtained with no significant difference between the levels in the Control D92 versus HD_2 D93 groups ($P > 0.05$, Table 4). The relevance of this result may be associated with the biological value of the plant *Artemisia afra*.

3.2.3. Organ weights, organ to body weight ratio and histopathological examination

Absolute organ wet weights of rats treated with p.o. *Artemisia afra*-extract are shown in Table 5. There was no statistically significant change in the wet organ weights of the treated rats compared to that of the controls. However, statistically significant increases in liver and kidney to body weight ratios were noted in the second group of rats given the 1000 mg/kg/day dose (HD_2) compared to the ratios for the control group. The magnitudes of these differences were small (<5%) and did not increase in relation to the doses ingested. In addition, there were no histopathological findings that could be attributed to the *Artemisia afra* and no differences in the organ morphology across the dose groups. Therefore, the increases in liver and kidney to body weight ratios found in the rats given the high dose (HD_2) were considered unrelated to treatment.

Table 5
Wet weight of organs of rats chronically dosed with *Artemisia afra*

Dose group	Wet weight (g; mean ± S.D.; $n = 6$)		
	Liver	Kidneys	Brain
Control	14.7 ± 0.49	3.2 ± 0.12	3.2 ± 0.22
Low dose (100 mg/kg)	13.9 ± 0.38	3.18 ± 0.09	2.97 ± 0.19
High dose 1 (1000 mg/kg)	14.4 ± 0.57	3.22 ± 0.10	3.02 ± 0.25
High dose 2 (1000 mg/kg)	13.6 ± 0.59	3.15 ± 0.08	2.88 ± 0.12

4. Discussion

A Word Health Organization survey indicated that about 70–80% of the world's populations rely on non-conventional medicine, mainly of herbal source, in their primary healthcare. This is especially the case in developing countries where the cost of consulting a western style doctor and the price of medication are beyond the means of most people (Dyson, 1998; Chan, 2003). Although medicinal plants may produce several biological activities in humans, generally very little is known about their toxicity and the same applies for *Artemisia afra*. Because safety should be the overriding criterion in the selection of medicinal plants for use in healthcare systems (Tomlinson and Akerele, 1998) one should, in addition to the use of historical documentation on *Artemisia afra*, also have formal toxicological evaluation of this plant to optimize its safe use as a medicine.

The freeze-dried aqueous extract of *Artemisia afra* used in the present study offers several advantages as a form of the *Artemisia afra* medicine. Firstly, it closely resembles the traditional dosage form viz. decoction and infusion (Roberts, 1990). Secondly, the freeze-dried aqueous extract of *Artemisia afra* provides a form that can be standardized in terms of constituents (marker compounds) and physicochemical properties and is easily packaged, stored and/or converted into high quality pharmaceutical dosage forms. Assessment of the toxicity of this form of *Artemisia afra* is thus most relevant, but before such evaluation can be fully justified in humans, the preclinical evaluation of the safety of the *Artemisia afra* aqueous extract is required.

In this study, the aqueous extract of *Artemisia afra* was found to be non-toxic in mice and rats when administered orally in doses up to 2000 mg and 1000 mg/kg, respectively. Mortality and other adverse effects occurred only in the mice receiving relatively higher doses of *Artemisia afra*-extract ($LD_{50} = 8960$ and 2450 mg/kg via p.o. and i.p. routes, respectively). Based on the classification of Loomis and Hayes (1996), viz. that substances with LD_{50} between 500 and 5000 and between 5000

and 15,000 mg/kg bodyweight are regarded as being slightly toxic and practically non-toxic, respectively, the present results suggested that *Artemisia afra* safety falls between these 2 categories. Although intraperitoneal injection is one of the routes of drug administration that occasionally provides a rapid and predictable absorption (Guilhermino et al., 1998), the human exposure to the crude extracts of *Artemisia afra* plant is very unlikely to occur by this route. Therefore, it would be best to use the p.o. results and when doing so the freeze-dried aqueous extract of *Artemisia afra*, based on this animal study, may be described as being practically non-toxic.

In the 3-month chronic toxicity study, the *Artemisia afra*-extract, regardless of dose used, did not appear to affect the bodyweight or the behaviour of the rats and caused no significant changes in their food intake and utilization of food indicating normal metabolism in the animals and suggesting that, at the oral doses administered, *Artemisia afra*-extract did not retard the growth of rats.

After 3 months treatment, there were also no treatment-related changes in the haematological parameters (i.e. hematocrit, hemoglobin concentration, platelets, red and white blood cells) between control and treated groups indicating that the *Artemisia afra*-extract was not toxic to the circulating red cells, nor interfered with their production and that of platelets. Hematopoiesis and leucopoiesis were also not affected even though the haematopoietic system is one of the most sensitive targets for toxic compounds (Harper, 1973) and an important index of physiological and pathological status in man and animals (Adeneye et al., 2006). In addition, most of the biochemical parameters (i.e. ALT, cholesterol, creatinine and glucose) were also unchanged by the ingested *Artemisia afra*-extract regardless of the dose given. The lack of significant alterations in the levels of ALT, and cholesterol and creatinine, good indicators of liver and kidney functions, respectively (Hilaly et al., 2004), suggests that chronic ingestion of *Artemisia afra*-extract did not alter the hepatocytes and kidneys of the rats, and, furthermore, the normal metabolism of the animals.

The transaminases (AST and ALT) are well known enzymes used as biomarkers predicting possible toxicity (Rahman et al., 2001). Generally, damage to the parenchymal liver cells will result in elevations of both these transaminases (Wolf et al., 1972). In this study, the AST activity levels were over time only increased in the control rats and those treated with low dose of *Artemisia afra*, while it was maintained at the pre-dose levels in the rats on the high dose treatment. This suggests that AST levels increased with age, but that ongoing treatment with high dose of *Artemisia afra* removed this elevation, strongly suggesting that *Artemisia afra* may have a liver protecting effect. Indeed, Huang et al have found that, in both male and female rats and similar to our finding, AST levels increase with age (Huang et al., 2005). Also several plants, for instance *Vicia calcarata* and *Laggera alata*, or flavonoids have been shown to lower AST levels or to be liver protecting (Singab et al., 2005; Wu et al., 2006), confirming that a flavonoid-containing plant such as *Artemisia afra* could be liver protecting. However, since the AST levels were back to the post-dose levels 25 h after termination of the *Artemisia afra* high dose treatment, our results also suggest that the liver-protecting or AST-reducing effect of *Artemisia afra* was reversible and most likely dependent on actives from the plant, e.g. flavonoids, etc., being present in the blood stream.

Apart from liver damage, the isolated elevation of AST levels in the presence of normal levels of other cholestatic markers could also be due to the fact that AST is present in a wide variety of tissues including heart, skeletal muscle, kidney, brain and liver, whereas ALT, for instance, is localized primarily in the liver. Moreover, AST can exist as a macroenzyme by forming a complex with an immunoglobulin and the immunoglobulin-complexed macromolecule can cause an elevation in serum AST activity, which may be detected in blood chemistry analysis and erroneously be considered to indicate the presence of liver disease (Litin et al., 1987). It is thus possible that *Artemisia afra* might influence these mechanisms to lower the AST levels rather than act on the liver. Clearly, the possible AST lowering effect of *Artemisia afra* require further investigation.

Finally, chronic oral ingestion of *Artemisia afra* did not adversely affect the morphology of the rat's organs confirming the biochemical parameter results and that this plant, in doses up to 1000 mg/kg/day, was well tolerated by the rats. This dose is substantially higher than the dose of *Artemisia afra* tea traditionally used (i.e. double dose of *Artemisia afra* tea in humans = 100 mg/kg *Artemisia afra* aqueous extract). Extrapolation of the findings of this study to humans thus suggests that, at the doses of tea routinely and chronically taken, the freeze-dried *Artemisia afra* aqueous extract should be relatively safe to use.

Overall, this study provides valuable preliminary data on the toxicity profile of *Artemisia afra* that should be useful for the planning of future pre-clinical and clinical studies of this plant medicine. The *Artemisia afra* aqueous extract appears to be relatively non-toxic, causes no apparent organ damage, and in high doses may have a hepatoprotective effect. Further studies to determine the effects of this plant on the foetus in pregnant animals, on the reproductive capacity of animals, on the genetic system and to determine its potential to produce tumors are needed to complete the safety profile of *Artemisia afra*. Aspects suggesting that it may be liver protecting could be pursued. In the mean time, additional work on the phytochemistry of *Artemisia afra* has been done and shall be reported elsewhere. Collectively the findings of the present study would suggest a very low potential of this plant to produce adverse effects.

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