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Original Article

Acute toxicity studies of locally cultivated *Artemisia annua* leaf extract in Rats

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ABSTRACT

Artemisia annua is a medicinal plant native to China and well known for its antiplasmodial, antirheumatic and anticancer properties. The acute toxicity, phytochemical screening and elemental analysis of hexane extract of *A.annua* were investigated. 1000, 2000, 2500mg/kg of the hexane leaf extract A. *annua* were administered intraperitoneally to the test groups while distilled water was given to the control group. The parameters measured were food and fluid intake, urine and fecal output and hemaetological parameters. The lethal dose (LD_{50})of the extract was 2750mg/kg body weight, carbohydrates, cardiac glycosides, flavonoids andterpenes were the bioactive compounds detected. The elemental contents included sodium, potassium, magnesium, iron, copper, zinc aluminium and silver. The hexane extract of *A. annua* decreased both food and fluid intake but increased urine and fecal output. Significant changes in some of the hemaetological parameters were also elicited by the extract. This study has shown the toxicity characteristics of the hexane extract of the *A. annua* leaves in short time treatment with the extract.

Key words: *Artemisia annua*, Acute toxicity, Phytochemicals, Elemental analysis, Haematology

INTRODUCTION

Artemisia annua or sweet wormwood is an annual, aromatic herb from Asia and has been used in China to treat fevers for more than 2,000 years [1]. It is well known for its antimalarial properties. In traditional Chinese medicine it is often prescribed in combination with other herbs to treat jaundice, headache, dizziness and nosebleeds [2]. *A.annua*is a vigorous growing annual weedy herb, usually single-stemmed, reaching up to 2 to 3 m in height. The plant produces a beautiful port-folio of bioactive compounds. Thus far, the most important of the sesquiterpenoids seems to be artemisinin [3]. The actual challenge associated with the chemotherapy of malaria is drug resistance. Plasmodium parasites soon develop resistance to new drugs, once released into the market. This lead to the introduction of Artemisinin in the middle of the 1990s [4].The introduction of Artemisinin based therapy became an acceptable alternative to Chloroquine and Sulphadoxine/Pyrimethamine, which had become totally ineffective in the face of widespread resistance. Artemisinin based therapy greatly reduced the morbidity and mortality due to acute severe malaria in endemic countries [5], thus prompting the World Health Organization to recommend the use of artemisinin based combination therapy for the treatment of uncomplicated malaria in endemic regions of the world[6]. The toxicity data on *A. annua* botanical drugs are incomplete and studies have also shown that *A.annua* is safe in moderation but large doses are toxic [7], therefore the present study was undertaken to evaluate the toxicity of locally cultivated *A.annua* in rats.

MATERIALS AND METHODS

Plant material: The leaves of *Artemisia annua* were collected from Lang tang South Area of plateau State during the flowering season. It was identified by a taxonomist at the Department of Biotechnology Engineering; University of Jos. A voucher specimen was deposited at the Department of pharmacology herbarium, University of Jos.

Extract preparation: The leaves were pounded to powder in a wooden mortar with pestle and the extract was prepared by maceration of the plant

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material using n-Hexane in a conical flask (50gm of powder in 150ml of n-Hexane) and kept at4oC for 72 hours with intermittent shaking. The extract was finally evaporated to powder in a water bath set at 40oC, the residues was found to be 10.25% w/w and stored in a refrigerator at4oC for further investigation.

Phytochemical tests: The phytochemical screening of hexane extract of *A. annua*leaves was carried out to determine the presence of the following phytochemicals, tannins, alkaloids, flavonoids saponins, glycosides, sterols, terpenes and reducing sugars using standard procedures [8].

Elemental analysis: Elemental composition (Proximate) of the hexane extract of *A. Annua* was determined using the inductively coupled Plasma Optical Emission Spectrometer (ICP – OES) [9].

Animals: Wister albino rats of both sexes (185-200g) obtained from the Animal House Unit of the University of Jos, Nigeria in cages and then acclimatized to laboratory condition for 7 days prior to experiment. These were fed dailywith standardmarsh and water *ad libitum*.

Acute toxicity study: The acute toxicity of the extract was evaluated using Lorkes method [10] with modifications. The animals were divided into six groups designated as A, B, C, D, E, F, each group consists of 6rats. The A, B, C, D, E groups were administered intraperitoneally (IP) with graded doses (10, 100, 1000, 1600 and 2900mg/kg respectively) of the extract. Group F taken as control treated with IP distilled water. The number of deaths in each group within 24 hours was recorded and the LD_{50} values were calculated.

Metabolic cage study: Water, food intake, urine and fecal output were monitored daily for 5days.

Haematological methods: The rats were euthanized in an airtight glass chamber saturated with chloroform and after opening up the rats surgically after 5 days. Blood samples were collected by cardiac puncture into ethylene diamine tetraacetic acid (EDTA) bottles for the analysis of haematological parameters [white blood cell (WBC), red blood cells (RBC) haemoglobin (HGB), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), platelets (PLT) and lymphocytes (LMP)] using Sysmex KX-21N automated hematology analyzer (Sysmex America Inc, USA). The microhaematocrit and cyanmethanemoglobin methods of ReyV ´ azquez and Guerrero [11] were used for the assay.

Statistical analysis: Results were expressed as the mean ± standard error of mean (SEM). Statistical analysis of data was carried out using one-way analysis of variance (ANOVA).

RESULTS

Phytochemical tests: The result of the phytochemical screening of the extract of A.*annua*is presented in Table1. The analysis revealed the presence of reducing sugars; cardiac glycosides, terpenes, flavonoids, terpenes and steroid in the extracts. However alkaloids, saponins and tannin were absent.

Elemental analysis: Table 2 shows the metallic elements detected in hexane extract of *A.annua*. Calcium, lead, nickel and arsenium were not found in the extract.

Acute toxicity test: Table 3 shows the mortality rate induced by different doses of the hexane extract of *A.annua*. The toxicity of the hexane extract administered intraperitoneally was estimated to be 2750 mg/kg body weight. At acute dose of 2900mg/kg bodyweight of the extract and above the animals posed toxic symptoms like twitching, tremor, poor motor coordination and cerebral itching.

Effect of the extract on water and food intake: There were significant decreases in water and food intake observed for all the treatment groups when compared to the control group (Fig 1 and Fig2).

Effect of the extract on urine and fecal output: There were dose dependent reduction in urine and fecal output noted with the extract treated groups compared to the control group (Fig 3 and Fig 4).

Effect of the extract on haematological parameters in rats: There was highly significant ($p < 0.01$) decrease in the white blood cell count in the groups treated 2000 mg extract/kg and a highly significant ($p<0.01$) reduction in the lymphocyte levels in the group treated with 2500mg/kg extract. There was a highly significant ($p < 0.01$) decrease in the haemoglobin in the 2000 mg and 2500mg extract/ kg group and a highly significant ($p < 0.01$) decrease in haematocrit at 2500 mg extract/kg. There was a highly significant ($p < 0.01$) decrease in the platelet count in the 2000 mg group and a significant ($p < 0.05$) decrease at 2500 mg extract/kg. There were no significant changes for all the treated groups in mean corpuscular volume and mean corpuscular haemoglobin (Table 4).

DISCUSSION

The preliminary phytochemical screening of the hexane extract of *A. annua*revealed the presence of terpenes, flavonoids, steroids, cardiac glycosides and reducing compounds. Similar research had also been conducted by some researchers [12] though these results were similar but did not detect the presence of alkaloid, carbohydrate and cardiac glycosides. This difference may be attributed to certain clinical factors, which include time and place of plant collection and extraction technique. In the present studies the technique employed for extraction is maceration, the plant was appropriately obtained from Langtang South area of Plateau State where the plant is commonly used for the treatment of malaria and the plant was harvested in June which is their optimal time of collection [13]. In addition, wild-growing populations of *A. annua* can differ significantly in their chemical composition. Even the dominant secondary metabolites are known to vary from population to population [14]. Elemental analysis of the extract indicated the presence of pharmacologically useful trace metal elements with standard established usefulness various body functions. These elements are used extensively in chemotherapy and are essential in human and animal health [15]. The result of our finding is similar to those ofAlassane*et al*[15], however,Ca, Mn ,Ar and Cd were not detected, the variations may be attributed to the preferential absorbability of a particular plant for the corresponding element and the mineral composition of the soil in which the plant grows, method of analysis as well as its surrounding climatologically conditions [15, 16]. The presence of potential toxic element in extract of *A.annua* as aluminum (Al) constitutes a reason for concern. Al interferes with the uptake, transport and utilization of essential nutrients including Ca, Mg, K, P, Cu, Fe, Mn and Zn [17], the presence of this toxic element may be attributed to fertilizer and soil composition in which the plant grows [15]. The elements Fe, Cu and Zn are essential trace elements (micronutrients) for living organisms and well known for their role against parasitic diseases [18]. Although the acute toxicity (LD_{50}) test has been widely criticized as a parameter for assessing toxicity [19], there are still certain occasions when some useful information could be obtained from such studies. Apart from giving a clue on the range of doses that could be used in subsequent toxicity testing, it could equally reveal the possible clinical signs elicited by the substance under investigation [20]. The results of this study indicated low toxicity in mice, which confirms its lowest side effects [13].

The diets were well affected by the treated rats suggesting that the extract possibly cause alterations in carbohydrate, protein or fat metabolism in these experimental animals. It also shows that the extracts adversely interfere with the nutritional benefits (e.g. weight gain, stability of appetite) expected of animals that are continually supplied with food and water *ad libitum*[20]. Reduction of urine output indicates that the extract has antidiuretic potential, the result of this study is similar to the result of study conducted byRatnasooriya *et al*[21] in which antidiuretic potential of *Ficusracemosa* was evaluated. Inhibition of fecal output by the extract shows that the extract possesses antidiarrheal properties [21]. The antidiarrheal activities of this extract may be attributed to the presence of reducing sugars, terpenes and sterols which are secondary metabolites found in the extract. This result is consistent with previous studies conducted by some researchers [22, 23]. These authors opined that the antidiarrhoeal properties of medicinal plants were due to tannins, alkaloids, saponins, reducing sugars, sterols and triterpenes.

Decrease in WBC and LMP levels showed that the extract affected leukopoeisis[24].The reduction of heamatological parameters such as RBC, HBT and HCT in the extract treated animals indicated that the extract could cause lysis of blood cells and or inhibition in blood cells synthesis [25] which showed that anaemia is as a result of hemolytic phenomenon and or inhibition of blood cell synthesis by active constituents of the extract and decrease in hematological parameters has been associated with anemia [25]. Platelets are responsible for haemostasis —a process aimed at reducing blood loss and repairing vascular injury [26]. The decrease observed in the platelet count may indicate that the extract did not stimulate the biosynthesis of clotting factors by the liver and may therefore not be useful in the treatment of haemorrhage[27].

CONCLUSION

These results suggest that hexane leaf extract of *A. annua*has low toxicity when used acutely in rats. However, this study provides the basis for further study on the detailed toxic and pharmacological effects of the extracts of *A.annua*and its active component(s).

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Table 1: Phytochemical screening of hexane extract of A. *annua*

Chemical compounds	Test methods	Remarks
Alkaloids	Wagner's test Mayer's test Dragendorff's test	- - -
Reducing sugars	Fehling's test	+
Cardiac glycosides	Keller Killiani's test	+
Flavonoids	Ferric chloride test Lead acetate test Sodium hydroxide test	+ + +
Saponins	Liebermann's test Foam test	- -
Tannins	Ferric chloride test Lead acetate test	- -
Terpenes	Salkowski's test	+
Sterols	Liebermann's test	+

(+) = Presence, (−) = Absence

Table 2: Elemental analysis of hexane extract of *A.annua*

Elements	Remarks
Sodium	+
Potassium	+
Calcium	-
Magnesium	+
Manganese	-
Iron	+
Copper	+
Zinc	+
Aluminium	+
Silver	+
Lead	-
Nickel	-
Cadmium	-
Arsenium	-

(+) = Presence, (−) = Absence

Table 3: Percentage mortality in rats given IP injection of hexane extract of *A.annua*

Dose mg/kg	No of animals	No of deaths	Percent mortality
10	6	-	-
100	6	1	16.7
1000	6	1	16.7
1600	6	2	33.3
2900	6	6	100

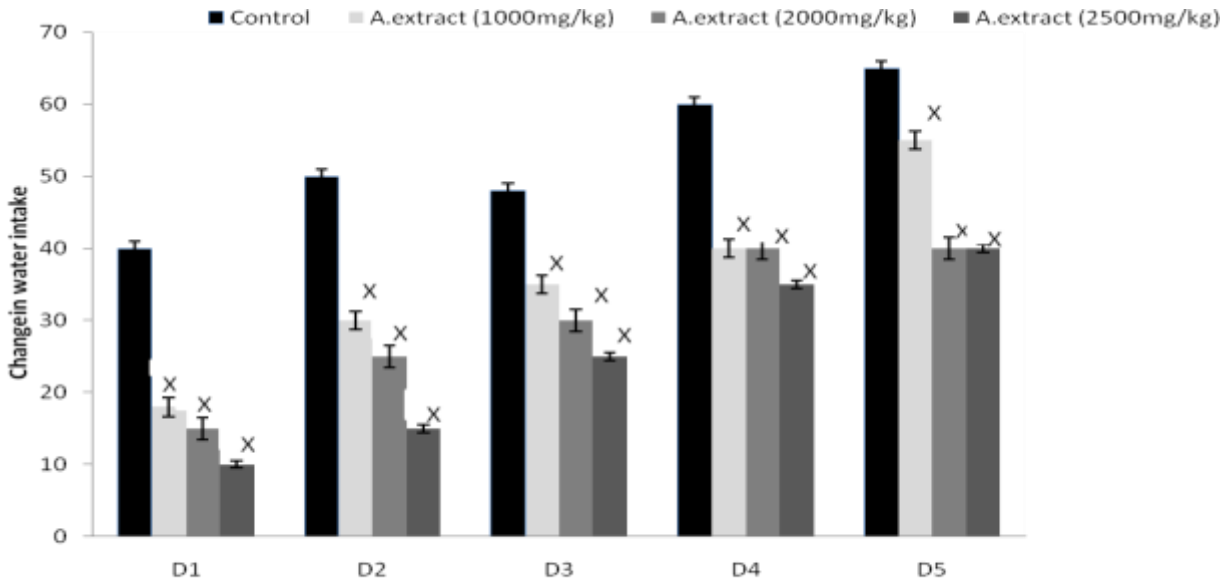

Fig 1 : Effect of hexane extract of A. annua on daily water intake in rats

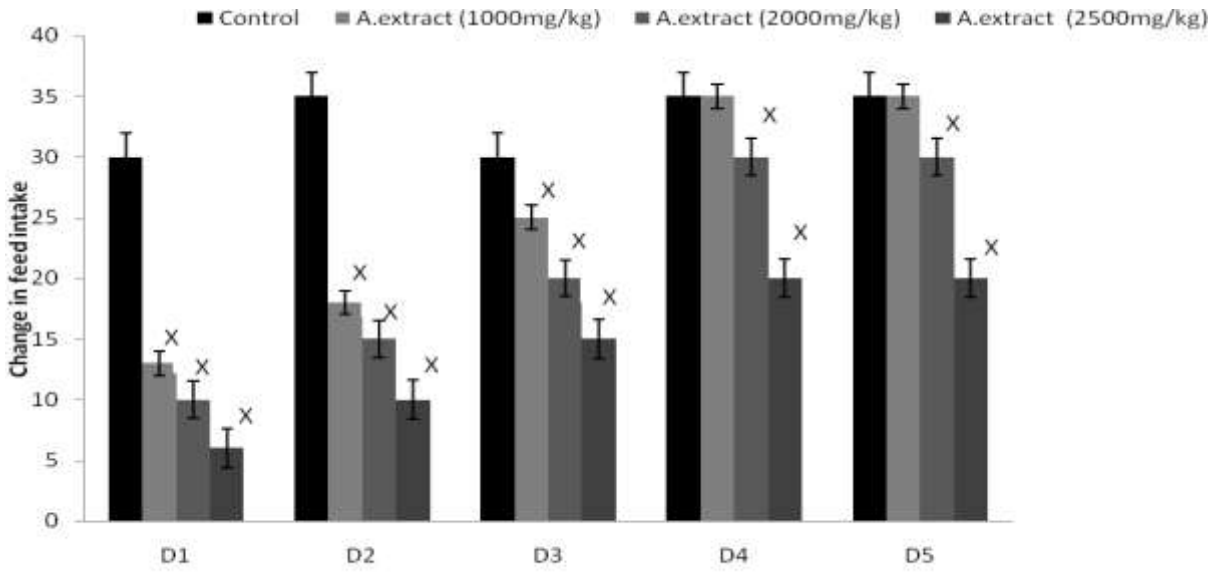

Fig 2 : Effect of hexane extract of *A.annua* on feed intake in rats

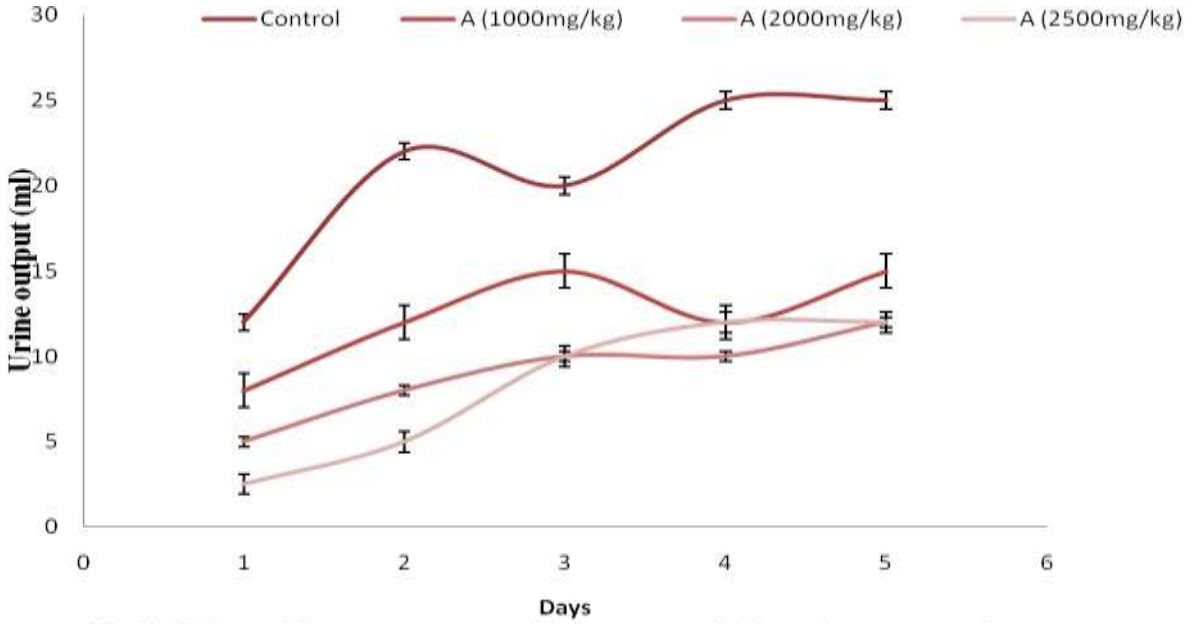

Fig 3: Effect of hexane extract of *A. annua* on daily urine output of rats

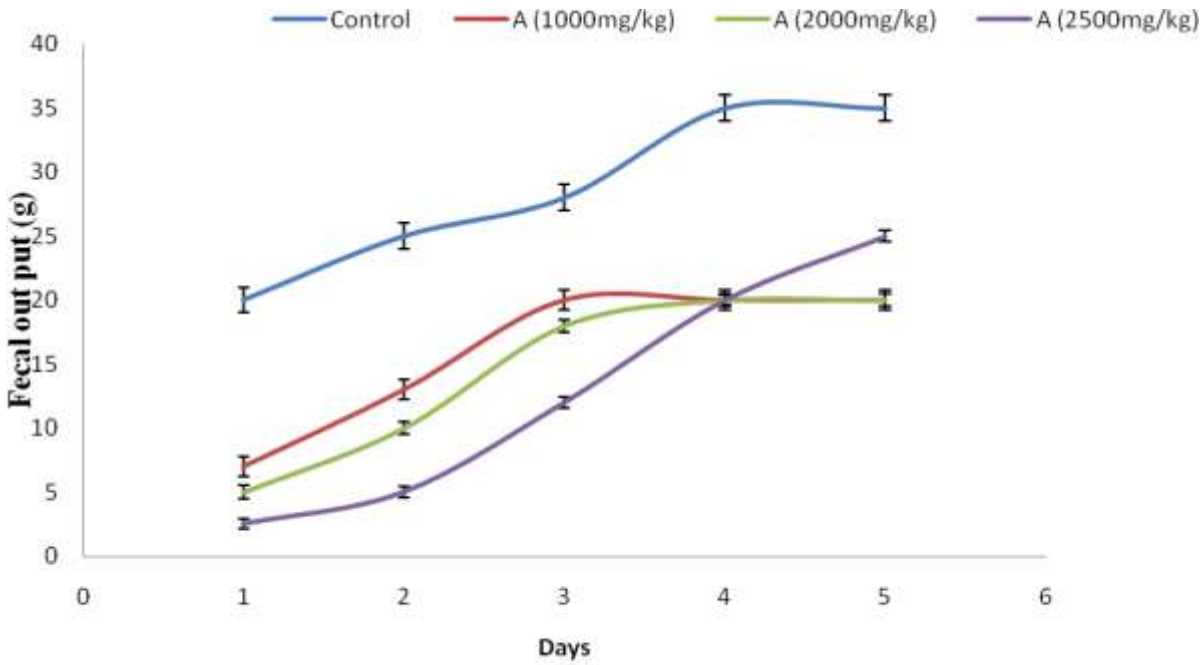

Fig 4 : Effect of hexane extract of *A. annua* on fecal out put of rats

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