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RESEARCH ARTICLE

EFFECTS OF *LEPTADENIA HASTATA* EXTRACT ON *ARTEMIA SALINA* AND DOSE RATE ON ORGANS OF ULCER INDUCED RATS

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ABSTRACT

Leptadenia hastata is often used in traditional medicine and it is generally agreed that medicinal plants and their products are naturally safer than their synthetic counterpart drugs. In the present study, *Leptadenia hastata*; a crawling perennial plant was used to determine the cytotocity and the effect of increasing dose rates of five different concentration of hexane extracts on organ weights of ulcer induced rats. A total of 40 white albino rats (male sexes) weighing 150-190 g/kg/bwt of two-month-oldand brine shrimps were used in this study. The rats were stabilized for 1 week in their separate cages of 5 rats each of eight groups A, B, C, D E,F, G and H; an increasing dose rates of aqueous extract of *Leptadenia hastata* viz., 100 200, 300, 400 and 500mg/kg bwt were administered orally daily for 20 days to groups C, D, E, F, G and H respectively. Control group A was given normal saline, B (negative control) was given Indomethacin while groups C (Positive control) Omeprazole for the same experimental period. The rats were sacrificed on day 21 and organ weights were measured using Mettler balance. Cytotocity was evaluated in terms of LC_{50} (Lethality concentration) ten nauplii were added into three replicate of each concentration of the extract. After 24hrs the surviving shrimp larvae were counted and LC_{50} was assessed. The results of the organs revealed a slight change in the mean weight of some organs (1.51±0.01 brain) with little or no significant treat with increase in concertation of the extracts when compared with the control (1.50±0.03). While the cytotoxicity potent of the hexane extract exhibited low potent activity with LC_{50} values9421.49µg/mL.

INTRODUCTION

Historically medicinal plants have been provided a good source of inspiration for novel therapeutic drugs which has made a large contribution to health and well-being of humans. It has been used over the years to as curative agents against many infections and have been exploited in the traditional medicine with their curative potentials well documented (Ibrahim *et al.,* 2011, Dubey *et al.,* 2004 and Ghisalberti *et al.,* 1993). Most plants are capable of synthesizing some chemicals compounds that's are able to perform physiological action in the body. These chemicals compounds are known as phytochemicals. *Leptadenia hastata* (Pers.) Decne, which belongs to the family Asclepiadaceae*,* is a wild plant used as vegetable by many African populations and medicine due to its nutritive and therapeutic properties for the treatment of wounds and stomach upset conditions in children (Aliero *et al.,* 2001; Tamboura *et al.,* 2005). The plant *Leptadenia hastata* is an edible non-domesticated valuable herb with creeping latex stems, glabescent leaves, glomerulus and recemes flowers as well as follicle fruits. It is typically grown in tropical dry land in sand soil (Betti *et al.,* 2011). The plant is commonly used in the north Nigeria as spicesand used as sauces (Ibrahim *et al.,* 2012).Local healers also use the plant for hypertension, catarrh and skin diseases (Danbatta and Aliyu, 2011). It is commonly used as a vegetable and is considered as a famine food in Niger republic due to its high content of valuable nutrients rich in various types of amino acids, fatty acids, terpenes, carotenes, luteines and poly-oxy pregnane (Aquino *et al.,* 1996; Freiberger *et al.,* 1998; Nikiema *et al.,* 2001; Sena *et al.,* 1998).In certain areas of West Africa, breeders claimed the antifertility effect of their animals after consumption of *Leptadenia hastata* leaf stems (Berhaut, 1979; Arbonnier, 2000). The findings in this study may contribute to the present literature in understanding the

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nutritive value of the crawling plant and its potential health implications on prolonged consumption. It is generally agreed that medicinal plants and their products are naturally safer than their synthetic counterpart drugs (Gamaniel, 2000; Mahima *et al.,* 2012). However, assumption of this safety should not always be made since a plant may prove efficacious but could have low therapeutic index (Agaie *et al.,* 2007). The LD_{50} of *Leptadenia hastata* has earlier been reported to be 2320 mg/kg using white albino rats by some workers (Maurice *et al.,* 2011). Bayala *et al.* (2011) also reported the non-toxic effect of the leave extracts of the plant in male albino rat. Though, rat weight gains and adrenals weight did not have significant change,the findings in this study may contribute to the present literature in understanding the medicinal value of the crawling plant and its potential health implications on prolonged intake.

MATERIALS AND METHODS

***Leptadenia hastata* leaves**: freshly leaves of *Leptadenia hastata* were collected from the uncultivated farm land of the Federal University Wukari Taraba State, Nigeria and was authenticated at Ahmadu Bello University Zaria and Voucher No PU: 2 ABU Herbarium No 900220. The plant *Leptadenia hastata* (yadiya) was dried under room temperature.

Extract Preparation: Fresh leaves of the plant *Leptadenia hastata* were washed with distilled water to remove the soil and dust particles they were thoroughly air dried and powdered using laboratory grinder machine (FGR-350, Quest Scientific) extraction using hexane by placing 150g of the powdered samples into an Erlenmeyer flask and hexane three times the weight of the extracts was added, the solution was covered and shaken at an interval of an hour and then allowed at room temperature to stand for 7days, the mixture were then filtered using Whatman filter paper No.4 and the solvent was evaporated using a rotary evaporator (Heidolph Laborato 400) under reduced pressure below 50EC. It was then stored under a frozen condition until required.

Breeding of animals: Albino rats (140-180g) body mass were obtained from the animal farm, National Research Institute VOM, Jos Plateau State Nigeria. They were put in cages at room temperature (20-27^{o}c) under 12/12 night/dark. They were maintained on a standard animal pellets (vital feeds, Grands cereals and oil meal Jos) and water ad libitum.

Experimental protocol: The albino rats were randomly divided into eight groups, this include normal group, negative and positive control group while five groups for extracts dosage, except the normal all the groups were induced with ulcer, extracts were introduced not exceeding the LD_{50} of the plant (Oral limit dose). The animals were starved from food 24hours and water 2hours before the commencement of the experiment.

- Group 1: Normal control (diet/water)
- Group 2: Rats (induced ulcer indomethacin 25mg/kg/bwt +diet /water)
- Group 3: Rats (induced ulcer indomethacin 25mg/kg/bwt +diet/water + Omeprazole)
- Group 4: Rats (induced ulcer indomethacin 25mg/kg/bwt +diet /water +100mg/kg/bwt extracts)
- Group 5: Rats (induced ulcer indomethacin 25mg/kg/bwt +diet /water +200mg/kg/bwt extracts)
- Group 6: Rats (induced ulcer indomethacin 25mg/kg/bwt +diet /water +300mg/kg/bwt extracts)
- Group 7: Rats (induced ulcer indomethacin 25mg/kg/bwt +diet /water +400mg/kg/bwt extracts)
- Group 8: Rats (induced ulcer indomethacin 25mg/kg/bwt +diet /water +500mg/kg/bwt extracts)

Determination of ulcer indices: The drug Indomethacin was administered intragastrically via the aid of an orogastric cannula. Four hours later, the rats were sacrificed using chloroform anesthesia; the stomach was removed and opened along the greater curvature. The tissue was fix with 10% formaldehyde in saline, microscopic examination was carried out and scored the presence of lesion using the method of Nwafor *et al.,* 2000. Ulcer index, organs weight of the rats was assessed and calculated.

RESULTS AND DISCUSSION

It is assumed that a drug or leaf extract is safe when the LD_{50} is above the therapeutic index, however, this assumption should be carried with caution. Especially when some drugs or herbs may tend to increase or decrease the size of body organs, which may consequently affect its functions (Agaie *et al.,* 2007). However, in this present study,*Leptadenia hastata* crude extracts from the leaves, have been tested for its cytotoxicity activities. The lethality concentration of LC_{50} was assessed at 95% confidence using probit analysis. it has been observed LC_{50} value of less than 1000µg/mL is toxic while LC_{50} value of greater than 1000µg/mL is non-toxic (Meyer *et al.,* 1982). Table 2 show the average death of Artemia salina at different concentration of five crude extracts. In this study thymol was used as positive standard which the LC_{50} value against brine shrimp was 1.16µg/mL. Therefore, the average death of *Artemia salina* at different concentration of hexane crude extract of *Leptadenia hastata* Leafcrude extracts showed cytotocity against brine shrimp with LC_{50} values of 9421.49. The average death of brine shrimp in hexane crude extract for leaf was slightly higher with LC_{50} value of 9421.49 at higher concentration of 500µg/mL the leaves caused a few 2.67±0.58 death of the brine shrimp or an average of 26.7%. It was observed that the concentration dependent increment of hexane extracts of the plant parts mortality rate of brine shrimp provides a proof of non-toxic of the hexane extract of the plant *Leptadenia hastata*. This agrees to the fact that there was no alarming increase in the weight of the organs of the ulcer rats as claimed of its steady increase in mean weight of the liver and kidneys as well as marginal increase in mean weight of the brain of the albino rats treated with *Leptadenia hastata*by Hassan *et al.,*(2007). The probable reason for his claim could be due to prolong administration and over dosage which tend to increase their sizes as well asthe liver and kidneys, being two organs most involved in detoxification processes; therefore with increasing dosage or over dosage of the *L. hastata*, there will be more activities in detoxification processes which exponentially increased their weight. Inthis study as observed with the increasing dosage from 200 mg to 500 mg/kg/bwt of the ulcer induced rats the weight of the organs seems to decrease apart from the brain which showed a slight increase at dose of 400mg/kg/bwt (1.51±0.01b),unlike the testes which showed a significant increase with increase in the dose rate of the extract from300-500mg/kg/bwt as shown in the Table 1. However, the increase in mean weight of the brain with increasing dosage was observed only in dose rate

Table 1. Effect of *Leptadenia hastata*hexane leafextract on organs weight in ulcer induced white albino rats

Group	Dose mg/kg/bwt	Liver (g)	Kidney (g)	Brain (g)	Testes (g)	Ulcer indices
A	Control	7.30±1.25	1.20±0.01	1.50±0.02	1.10±1.30	0.00±0.00
B	-ve (control)	7.38±0.14	1.16±1.01	1.49±1.23a	1.05±0.20	2.93±0.70
C	+ve (control)	7.26±0.01	1.19±1.13	1.50±0.03	1.03±0.01	0.11±0.05[a]
D	100mg/kg/bwt	7.26±0.23a	1.18±2.03a	1.50±1.02	1.00±2.03c	2.67±0.46[a]
E	200mg/kg/bwt	7.28±1.12a	1.20±0.01	1.50±0.01	1.00±1.02	2.02±0.68[a]
F	300mg/kg/bwt	7.28±0.12	1.21±0.02a	1.50±0.03	0.98±0.23c	1.53±0.28[a]
G	400mg/kg/bwt	7.29±1.13a	1.20±1.13a	1.51±0.01b	0.98±1.13c	1.20±0.52[a]
H	500mg/kg/bwt	7.29±2.04a	1.20±1.05	1.50±0.02	0.97±0.03c	0.75±0.21[ab]

Values are Mean ± SD (n = 5)
[ac]Significantly (p> 0.05) decreased compared to negative control
[b]Significantly (p> 0.05) increased compared to different extract concentration on each rat pergroup.
[ab]Significantly (p> 0.05) decreased compared to different extract concentration on each rat per group.

Table 2. Average death of Artemia salina at different concentration of hexane crude extract *of Leptadenia hastata* Leaf

Hexane Crude extract	Average death of Artemia salina Concentration (µg/mL)						LC50 (µg/mL)
	1	10	25	50	100	500	
Leaves	0.33±0.58	1.00±0.00	1.33±0.58	2.00±1.00	2.00±1.00	2.67±0.58	9421.49
(-ve control)	0	0	0	0	0	0	-
(+ve control)	5±0.57	7±0.58	10±0.00	10±0.00	10±0.00	10±0.00	1.16

400mg/kg/bwt as when compared with the control table1, which may be due to different in weight and sizes of the rats as well as individual responds to the effect of indomethacin or the blood-brain-barrier factor. This probably limited the clearance of tannins and other compounds into the brain hence reducing or slowing the effect. However, this study agrees with Bayala *et al.* (2011), they did not find any significant change in weight of rats but a decrease in weight of accessory sex organs after treatment with *Leptadenia hastata*, the probable reasons of this discrepancy in the results reported by Hassan *et al.,* (2007) with this present study it could be because of induced ulcer rats was used which could probably affect their physiology. Hence, it should be noted that LD_{50} of *Leptadenia hastata* has been determined to be as high as 2320 mg/kg/bwt (Maurice *et al.,* 2011). Despite of this, the leaf extract of *Leptadenia hastata* is safe to be used as a medicinal plant. Though, caution on excessive usage should be maintained as breeders claimed the antifertility effect of their animals after consumption of *L. hastata* leaf, stems (Berhaut, 1979; Arbonnier, 2000). The findings in this study may contribute to the present literature in understanding the safety value of the crawling plant and its potential to health.

Recommendation: Based on these findings the plant extract is non-toxic and reference to the size of the organs it is safe. However further research needs to be done on different solvent extracts and made a comparative study.

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