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RESEARCH ARTICLE

PHYTOCHEMICAL SCREENING, PROXIMATE ANALYSIS AND ACUTE TOXICITY STUDY OF ETHANOLIC EXTRACT OF *LAUNAEA TARAXACIFOLIA* ON VISCERAL ORGANS OF ALBINO RATS

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ABSTRACT

Despite increase in the acceptance of herbs for the treatment of certain diseases, there are limited information on the effectiveness, safety and toxicity of herbs. In this study, proximate analysis, Phytochemical screening and acute toxicity studies on the herbal recipe, *Launaea taraxacifolia* were carried out. Constituents of *Launaea taraxacifolia* were extracted using ethanol. Phytochemical screening and proximate analysis were carried out on the extract. Fifteen (15) albino rats, divided into 5 groups were used for the toxicity study. The controls (group1) were fed with normal feed without extract while varied concentrations of the extract of *Launaea taraxacifolia were administered orally into other groups* (groupII=500mg/kg, groupIII=1000mg/kg, group IV=2000mg/kg and group V=3000mg/kg) were used for this study. The albino rats were then monitored for signs of toxicity for 24 hours. The result ofphytochemical screening showed that the ethanolic extract contained flavonoids, saponins, terpenoids, steroids, cardiac glycosides and tannin. In the proximate analysis of the extracts, the ethanolic extract showed high calorific value (280.70± 0.80 Kcal/100g), total carbohydrate (18.59 ± 1.33%), crude protein (17.67 ± 1.20 %), crude fibre (16. 06 ± 0.05 %), total ash (21.50 ± 0.07 %), moisture content (23.14 ± 0.50%) and crude fat (4.70 ± 0.03%).After administered into the albino rats, the body and organ weight (liver and kidney) were not affected by the administered extracts of *Launaea taraxacifolia*. None of the albino rats died throughout the period of acute toxicity studies and up till 7 days of further examination. Histopathology tests on liver and kidney showed visible morphological changes and altered affinity for Haematoxylin and Eosin stain. Morphological changes and altered affinity for Haematoxylin and Eosin stain observed in liver and kidneys of albino rats treated with ethanolic extract of *Launaea taraxacifolia* could be due to the concentration of the extract in the organs for excretion and metabolism.

INTRODUCTION

Launaea taraxacifolia occurs mainly in the tropics from Senegal East to Ethiopia and Tanzania. The Ethiopian highlands have been suggested as the origin, from where it spread as a weed to other parts of Africa. *Launaea taraxacifolia* has been domesticated as a leafy vegetable in Nigeria, Senegal and Benin (Adebisi, 2004). It is known as 'yanrin' among the Yorubas of the South-Western part of Nigeria. As it is being used as edible and readily available vegetable, *L. taraxacifolia* leaves has been reported by Burkill in 1985 to produce multiple births in sheep and goats when mixed with natron and increase milk output when fed to lactating cows in northern part of Nigeria. Studies of medicinal plants using scientific approaches showed that various biological components of medicinal plants exhibit a variety of properties and can be used to treat various ailments. Recent years have witnessed a renewed interest in *L. taraxacifolia* since biologically-active molecules have been isolated for pharmaceutical use (Peter, 1995). In Nigeria and Ghana, the leaves are rubbed on the limbs of children to make them walk. The leaves are also mixed with ashes to cure yaws (Ayensu, 1978). In animal, evaluation has shown that it has cholesterol lowering effect (Adebisi, 2004). A previous study by Okafor in 1983 reported that *L. taraxacifolia is a* good source of phytochemicals. Obi *et al*. (2006) reported that the

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consumption of *L. taraxacifolia* could to a large extent prevent infection or further replication of the measles virus. Some of the diseases curable by *L. taraxacifolia* include high blood pressure, heart attack, stroke and other cardiovascular diseases (Williamson *et al*., 1997, Liu, 2004). A number of studies have reported the toxic effects of herbal medicines (Gamble, 1957; Calixto, 2000; Jaound *et al*., 2004; Taziebou *et al*., 2008), but none of them was on *Launaea taraxacifolia.* Severe liver injury, including acute and chronic abnormalities and even cirrhotic transformation and liver failure, have been described after the ingestion of a wide range of herbal products such asmushrooms, germander (Teucriumchamaedrys), chaparral (Larrea tridentate) etc (Stickel *et al.,*2000). Hence, investigations on phytochemical screening, proximate and histopathological changes in acute oral toxicity effect using extracts of *L. taraxacifolia.* Therefore, the present study investigated the status of phytochemical content and toxic effects of ethanolic extracts of *Launaea taraxacifolia* leaves.

Experimental Procedures

Plant Collection: Fresh plant leaves were collected from the Obafemi Awolowo University, Ile-Ife farms in June, 2017 and authenticated by Botany Department of the University. The fresh plant material was then washed under running tap water, air dried, grounded into a fine powder and stored in air-tight containers at 4°C.

Plant Extraction

About 100 g of pulverized air dried leaves of *L. taraxacifolia*, was mixed with 500 mL of 70% ethanol in a conical flask, plugged with cotton and then kept on a shaker for 72 h. The mixture was then filtered and the solvent was evaporated using rotary vacuum pump. The crude extract obtained was stored in an air-tight desiccator for further analysis.

Phytochemical Screening

Phytochemical tests on the leaves of *L. taraxacifolia* were carried out on the crude ethanolic extracts using standard procedures as described by A.O.A.C in 1984.

Proximate analysis

The estimation of the various food parameters such as moisture content, total ash, crude fat, crude fibre, crude protein and total carbohydrate on dry matter basis were carried out according to standard procedures using 2g of dried powdered sample. In crude protein determination, Nitrogen was determined by Kjeldahl method (Pearson, 1976) and converted to protein by multiplying by a factor of 6.25. Moisture content, crude fat, crude fibre and total ash were determined by AOAC (1984) and to determine the total carbohydrate, the method of James (1995) based on difference was employed using the equation:
Total carbohydrate = 100-[% crude protein+ %crude fat+ %crude fibre+ %crude total ash]. Determination of energy or calorific value is the total energy value in the leaves of *L. taraxacifolia* in kcal/100 g determined by the method described by FAO (2003).

Calculation

Energy value = [% crude protein multiplied by 4.0] + [% crude fat multiplied by 9.0] + [% carbohydrate multiplied by 4.0].

Housing and feeding of albino rats

Experimental animals

Experiments were performed using mixed sex healthy young adult albino rats and female among them were nulliparous, non-pregnant and weighing 55-130 g. The animals were housed in polypropylene cages (55 x 32.7 x 19 cm), with sawdust litter in a temperature controlled environment (23 ± 2ºC). The rats were divided into five groups each containing three rats for ethanolic extracts. Each group in the extract used was labeled I, II, III, IV and V to ease the observation. Lighting was controlled to supply 12 h of light and 12 h of dark for each 24-h period. Each cage was identified by a card that contained the cage number, number of albino rats,their weights and group. The animals were fed with standard laboratory animal food pellets with water.

Acute toxicity study using the method described by Lalitha *et al*., 2012

The test substance was administered in a single dose by gavage using specially designed rat oral needle. Animals were fasted 3 h prior to dosing. Following the period of fasting, animals were weighed and test substance was administered orally at a dose of 0 mg/kg, 500mg/kg, 1000 mg/kg, 2000 mg/kg and 3000 mg/kg. After the administration of test substance, foods for the rats were withheld for 4 hours. The extract was prepared by dissolving 500 mg-3000 mg of dried powder of*L*. Taraxacifolia leaves in 1 ml of distilled water. The volume of extract to be administered was determined based on body weight and given to the rat once. The toxicological effects were observed in terms of mortality expressed as $LD_{50.}$ Animals were observed individually after at least once during the first 30 min, hourly during the first 24 h, and daily thereafter, for a total of 7 days. All the rats were observed at least twice daily with the purpose of recording any symptoms of ill-health or behavioral changes.

Tissue processing and histological examination

Rats were sacrificed and dissected to remove liver and kidney. The organs were weighed and fixed in 10% formal saline prior to processing for histological examination.

Histological technique

Liver and kidney were washed thoroughly in normal saline, trimmed, embedded in paraffin and sectioned at a thickness of 4-5 µm. Mayer's Acid-Alum-Haematoxyl in and eosin was adopted (Beaker *et al*., 1998) and photomicrograph taken accordingly.

Statistical analysis

The SPSS (Statistical Package for Social Sciences) software package version 16 was used for statistical analysis. Results were presented as mean ± standard error (Mean ± S.E). The statistical significance between the control and each of the treated groups were determined by Dunnett's t- test. The level of significance was set at $P< 0.05$.

RESULTS

As presented in Table 1,thequalitative phytochemical screening of the ethanolic extract of *Launaea taraxacifolia* leaf showed

the presence of flavonoids, saponins, terpenoids, steroids, cardiac glycosides and tannin. In Table 2 the proximate analysis of the extracts including the calorific value, concentrations (percentage) of total carbohydrate, crude protein, crude fibre, total ash, crude fat and moisture content of leaf of *Launaea taraxacifolia* are demonstrated.

Table 3 shows that the LD_{50} was above 3000 mg/kg in the treated rats. The administered varied doses of the ethanolic extract did not result in killing over the 24 hour period. No death or obvious sign of toxicity was observed in the albino rats after keeping them for extra 7 days. Table 4 Shows differences in body weight after the administration of varied concentration

Table 1. Phytochemical screening of ethanolic extract of *Launaeataraxacifolia*

Phytochemical constituents	Inference
Alkaloid(mayer's reagent)	-
Flavonoids	+
Saponins	+
Terpenoids	+
Steroids	+
Cardiac glycosides	+
Tannins	+
Anthraquinones	-

+: positive.
-: negative.

Table 2. Proximate composition of dry *Launaeataraxacifolia* leaves

Parameters	values (mean ± SEM)
Total Carbohydrate	18.59 ± 1.33(%)
Crude Protein	17.67 ± 1.20(%)
Total Ash	21.50 ± 0.07(%)
Crude Fibre	16. 06 ± 0.05(%)
Crude Fat	4.70 ± 0.03(%)
Moisture content	23.14 ± 0.50(%)
Calorific value	280.70± 0.80(Kcal/100g)

Table 3. LD_{50} after the administration of ethanolic extracts of *Launaeataraxacifolia* leaves to rats

S/N	Response	Group observation				
		I (0mg/kg)	II (500mg/kg)	III (1000mg/kg)	IV (2000mg/kg)	V (3000mg/kg)
1.	Alertness	Yes	Yes	Yes	No	No
2.	Grooming	Normal	Normal	Normal	Normal	Normal
3.	Touch response	Yes	No	No	No	No
4.	Torch response	Yes	Yes	Yes	Yes	Yes
5.	Tremor	No	No	No	No	No
6.	Convulsion	No	No	No	No	No
7.	Gripping strength	Normal	Reduced	Reduced	Reduced	Reduced
8.	Response to food	Yes Normal	No	No	No	No
9.	Pupils		Normal	Normal	Normal	Normal
10.	Urination	Normal	Normal	Normal	Normal	Normal
11.	Salivation	No Normal	No Reduced	No	No	No
12.	Hyperactivity			Reduced	Reduced	Reduced
13.	Skin colour	Normal	Normal	Normal	Normal	Normal
14.	Corneal reflux	NormalNormal	NormalNormal	NormalNormal	NormalNormal	NormalNormal
15.	Pinna reflux					
16	Sound response	Normal	Normal	Normal	Normal	Normal

Source: Lalitha et al., 2012. Asian Journal of Pharmaceutical and Clinical Research

Table 4. Body and organ weight on 7th day after the administration of ethanolic extracts of *Launaeataraxacifolia* leaves to rats

Groups	Conc. of plant extract (mg/kg)	Body weight(g)		Difference in weight (g)	Liver weight(g)	Kidney weight(g)
		Final	initial			
I	0	95.10±26.45	94.26±26.16	0.84±0.28	3.67±0.86	0.50±0.15
II	500	78.60±16.69	78.56±18.10	0.04±1.41	3.27±0.25	0.61±0.19
III	1000	82.95±14.92	77.56±8.90	5.39±6.01	4.10±0.14	0.67±0.04
IV	2000	64.65±3.04	54.76±12.45	9.89±9.40	4.04±0.06	0.60±0.14
V	3000	71.67±3.86	70.16±4.53	1.51±0.66	3.95±0.07	0.67±0.04
	pa-value	0.550	0.560	0.515	0 .587	0.618
	pb-value	0.641	0.527	0.478	0.560	0.270
	pc-value	0.348	0.242	0.403	0.604	0.580
	pd-value	0.341	0.328	0.320	0.695	0.270

pb-value-level of significance between group I and III
pc-value-level of significance between group I and IV
pd-value-level of significance between group I and V
*p-value significant at 0.05(<0.05)

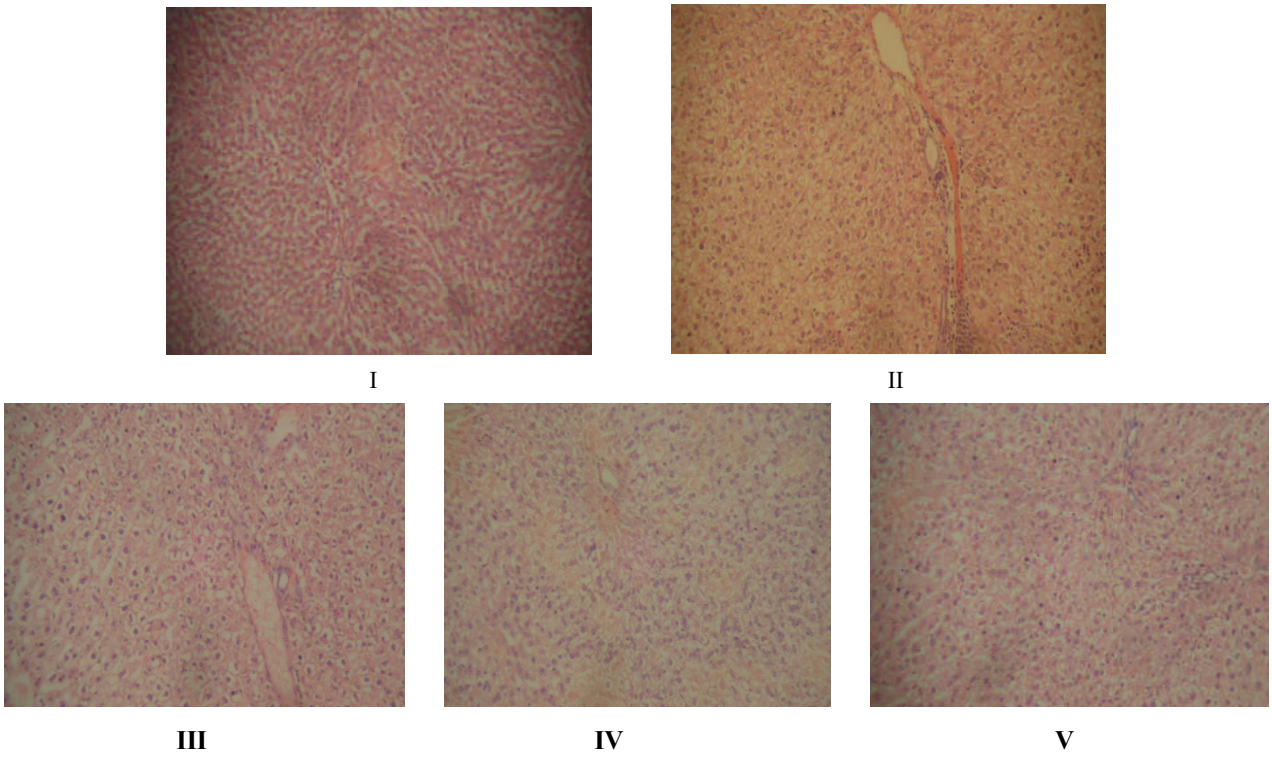

Figure 1. Photomicrographs of rat liver x 400;H&E- (I) control group, (II, III, IV, V) are rats received 500, 1000, 2000, 3000 mg/kg *Launaeataraxacifolia*extract respectively. I. No visible lesionII.There is severe diffuse vacuolar degeneration of hepatocytes. There is mild periportal cellular infiltration (CI) III. There is a severe portal congestion (PC)with diffuse vacuolar degeneration of hepatocytes . IV. There is a moderate peri portal cellular infiltration (CI), with diffuse vacuolardegeneration of hepatocytes. V. There is severe portal congestion with diffuse vacoular degeneration of the hepatocytes

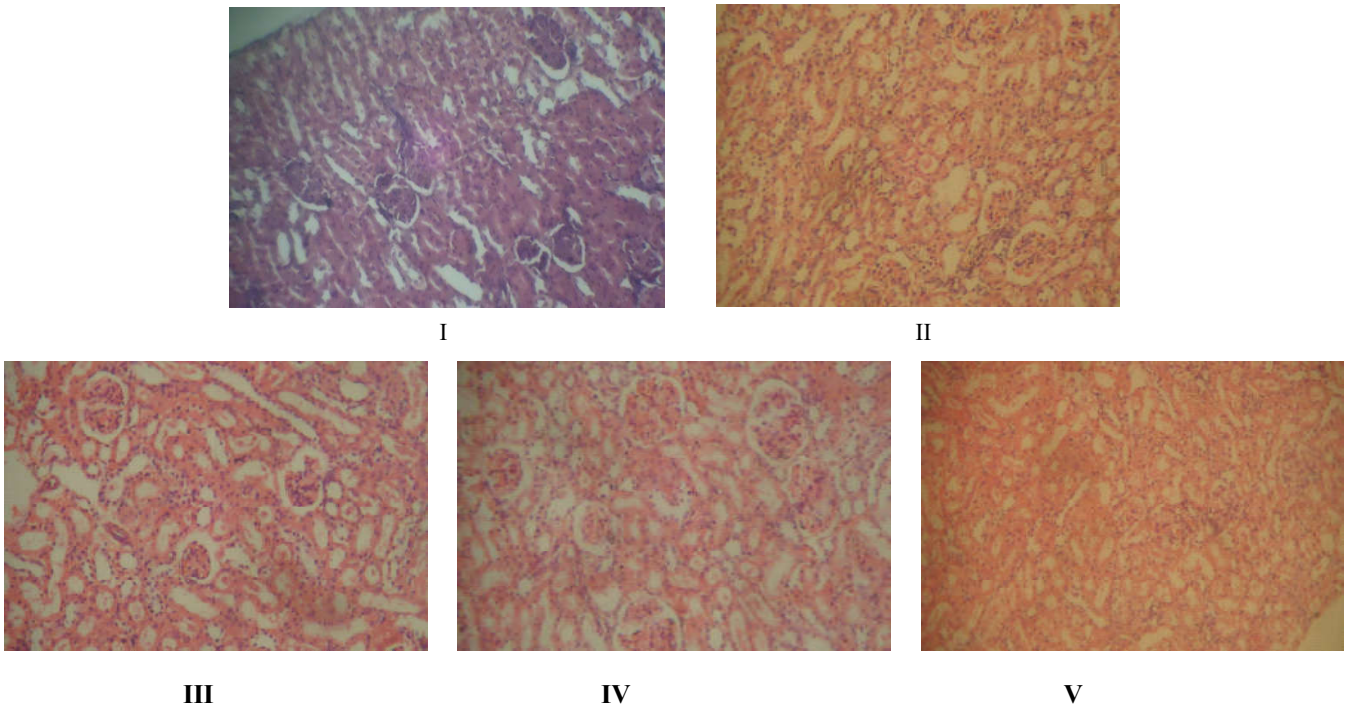

Figure 2. Photomicrographs of rat kidney X 400; H&E - (I) control group, (II, III, IV, V) were rats received 500, 1000, 2000, 3000 mg/kg *Launaeataraxacifolia*extract respectively.I. No visible lesion. II. Many of the tubules have pink staining casts in the lumen. There is mild interstitial cellular infiltration (CI) III. Many of the tubules have pink staining casts lumen. There is a mild interstitial cellular infiltration. IV. Many of the tubules have pink staining casts in the lumen

of ethanolic extracts of *Launaea taraxacifolia* leaves to rats and comparison of organ weight to the control after sacrifice. Figures 1and 2 showed the photomicrographs of the liver and kidney respectively after being administered with varied concentrations of ethanolic extract of the leaf.

DISCUSSION

Launaea taraxacifolia has been used for herbal remedy and food for both humans and animals without prior assessment of the acute or chronic toxicity of the constituents.

The present study shows the presence of phytochemicals liketannins, saponins, steroids, flavonoids, cardiac glycosides and terpenoids. These phytoconstituents of *L. taraxacifolia* leaves may therefore contribute to its medicinal value. Absence of potential toxic matters such as cyanogenic glycosides and anthraquinones in the leaves of *L. taraxacifolia* have been reported earlier by Adinortey *et al.* (2012).The proximate analysis of *L. taraxacifolia* leaves revealed that it contains an appreciably higher amount of moisture (23.14%), crude protein, total ash (21.50%), fibre (16.06%),total carbohydrate (18.59%) and caloric value when compared with values in studies by(Hassan and Umar, 2006), (Ladan *et al.,* 1996), (Oduro*et al.,* 2008), (Asibey-Berko and Tayie, 1999), (Nwaogu*et al.*, 2000) (Hassan *et al.,* 2004).The proximate analysis andphytoconstituents revealed that *L. taraxacifolia*contains nutritional and antioxidant potentials.

In this study, no significant differences noticed in body weightand organ (liver and kidney) of rats after the administration of ethanolic extracts of *Launaea taraxacifolia.* This could indicate that had no effect on the carbohydrate, lipid and protein metabolism of the albino rats used for the study. From the proximal characteristics of theethanolic extracts of*Launaea taraxacifolia,* total carbohydrate and crude fat which might result in regulation in weight balance were in moderate concentrations.

Adverse interactions of many plants extracts with major organs have been reported. Devaki *et al*., 2012 reported that adverse effect of plant extracts manifest as cellular constriction, inflammation and significant weight difference after administration. In this study, no significant differences were found in the organ to body ratio and this supports the non-toxic nature of the extract. The effect of the extract on the liver of albino rats treated with ethanolic extract at graded doses demonstrated degree of structural changes of lesions ranging from mild to severe diffuse degeneration of the hepatocytes with periportal cellular infiltration and presence of multiple foci of caseous necrosis of the hepatic. From our clinical experiences, these effects on the liver may be associated to the pathologic effect of ethanol. Our result seems to corroborate the findings of previous workers on certain herbal constituents, Stickel *et al.,*2000 reported severe liver injury, cirrhotic transformation and liver failure after the ingestion of a wide range of herbal products such as mushrooms, germander (Teucriumchamaedrys) and chaparral (Larrea tridentate) etc (Stickel *et al.,*2000). The extract did not have significant effect on the cellular architecture of the kidney except on the cellular affinity for the stain. These changes in the affinity of the organs for stain could be due to concentration ability of the constituents of the extract in the kidney and liver of the albino rats for metabolism and excretion.

Conclusion

It could be concluded from the histological result that ethanolic extract of *Launaea taraxacifolia* graded doses above what used in this study could induce toxicity effects on the liver and kidney, though no death of animal was recorded and caution should therefore be exercised in its use for medicinal purposes.. Phytochemicals and proximate contents showed that some nutritional content of these vegetables have potentials in reducing some diseases in man. Considering the structural changes effects of the plant extract on organ architectures, lower dosage could not be injurious to the organ under study. Pharmaceutical dosage should be sought in relation to the beneficial effect of the plant on man.

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