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Effects of Oral Administration of Aqueous Extract of *Tridax procumbens* Leaves on Some Haematological Variables in Rats

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Abstract

The haematological indices(haemoglobin (Hb), packed cell volume (PCV), mean Corpsular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular volume (MCV), red blood cell count (RBC), white blood cell count (WBC), platelets, neutrophil, lymphocytes, basophil, monocytes and eosinophil) following oral administration of aqueous extract of *Tridax procumbens* leaves at doses of 25 mg/kgbw, 50 mg/kgbw and 100 mg/kgbw in albino rats were evaluated progressively on daily basis for 28 days. The acute toxicity value of oral administration of aqueous extract was greater than 5000 mg/kgbw. Extract administration at 25 mg/kgbw significantly ($P<0.05$) altered Hb, PCV, and MCV counts. The extract had no significant ($P>0.05$) toxic effects on differential counts, RBC, WBC and MCHC. In the first week of treatment with the extract at 25 mg/kgbw and 500 mg/kgbw, feed and fluid intake of rats were not significantly ($P>0.05$) altered while 100 mg/kgbw significantly reduced these parameters when compared with the control. These results indicate that aqueous extract of *T. procumbens* leaves exhibit low toxicity in rats and may be safe for use as therapeutic for managing clinical conditions.

Keywords: Blood metabolites; Relative body weight; Aqueous extract; Tridax procumbens leaves

Introduction

Medicinal plants have been identified and used throughout human history. They are plants used in herbalism and thoughts to have medicinal properties. Awareness of medicinal plant usage is as a result of the many years of struggle against diseases due to which man learnt to obtain drugs in barks, leaves, fruits and seeds. The knowledge and development of ideas led to the usage of these plants in modern medicine [1].

These plants are easily cultivated and harvested and most of them improve nutrition, boost food security, increase full blood count and support sustainable land care. Plants and their phytochemical constituents have been proven to have medicinal effects by rigorous sciences and have been approved by regulatory agencies. As useful as these plants are, however, most of them are toxic to human system. Literature has documented severe toxic effects from the use of herbs on many separate occasions [2]. The potential toxicity of herbs has not been recognized by the general public or by professional groups of traditional medicine practitioners [3]. Patients are often unaware of the important similarities and differences between medicinal plants and medication. Some mistakenly think of herbs as natural alternatives to chemical, failing to recognize that herbs are composed of bioactive chemicals, some of which may be toxic [4]. Systemic toxicity can lead to decreased ability to excrete body wastes, inability to maintain body balance, increased resistance of microorganisms to drugs and decreased synthesis of essential hormones by the body [5].

Tridax procumbens (Asteraceae) is one of the most important plants commonly found in sub-tropical countries. Its common English names are Coat Buttons, Tridax Daisy. The Igbo people of south eastern Nigeria call it Mbuli. Traditionally it is used for the treatment of bronchial catarrh, dysentery, malaria, stomach ache, diarrhea, high blood pressure and to check hemorrhage from cuts, bruises and wounds and to prevent falling of hair. It possesses antiseptic, insecticidal and hepatoprotective properties and has marked depressant actions on respiration [6-8]. It is used as an antidote to arrow poison. Powdered leaves are applied to the wounds [9,10]. In the present study, we investigate the effect of aqueous extract of *T. procumbens* on heamatological parameters, fluid and feed intake of rats with a view of finding any clue to the mechanism of its toxicity role(s).

Materials and Methods

Collection and identification of plant

Plant samples were collected from Ilorin, Kwara State and was identified at the Department of Soil Science, Federal University of Technology, Minna by an ethnobotanist. The plant was identified as *Tridax procumbens*.

Preparation and identification of *Tridax procumbens*

The method recommended by NIPRD (2009) was employed for the extraction of the leaves of *Tridax procumbens* [11]. Briefly, the leaves were plucked from the stem and dried at room temperature 28 ± 2°C for three weeks. The dried leaves were crushed and grinded using blender. Five hundred grams of the grounded powdered sample were extracted in one litre of sterile distilled water by refluxing for six hours.

The mixture was filtered using Whatman filter paper No.1. The filtrate was evaporated to dryness using a steam bath. The extract was transferred to air tight sterile sample bottles and stored at 4°C until required for use.

Phytochemical screening of aqueous extract of *Tridax procumbens*

Qualitative phytochemical screening of aqueous extract was conducted to detect the presence or absence of various secondary metabolites. The method of Habone; Trease and Evans; Medicinal Plant Research and Traditional Medicine Practice, MPR-TMP. were employed [12-14].

Laboratory animals

Thirty-three albino rats were obtained from the Department of Biochemistry, Federal University of Technology, Minna. They were housed in plastic cages bedded with dry clean wood shavings. They were maintained at a temperature of 25°C and observed under 12 hour light/dark cycle in a well ventilated room for one week before the commencement of the experiment. The animals were fed with standard NIPRD formulated feed and water ad libitum. The experimental room were cleaned and disinfected regularly. The animals were housed and cared for in accordance with good laboratory practice (GLP) regulations of WHO [15]. The principles of animal care were also followed throughout the study [16].

Acute toxicity studies (LD50) of aqueous extract of *Tridax procumbens*

The method of Aniagu et al. was employed in the acute toxicity studies (LD50) of the extract [17].

The effect of the extract on heamatology of rats

The method of Aniagu et al. and Salawu et al. were employed in the determination of the effect of the aqueous extract of *Tridax procumbens* on the heamatological parameters of the albino rats [17,18]. Twenty four rats were divided into four groups of six each. Three groups were administered orally 25 mg/kgbw, 50 mg/kgbw and 100 mg/kgbw of the plant extract respectively. The last group served as control and was administered distilled water only. The four groups were observed for any sign of toxicity and mortality for the period of 28 days.

On the 28th day, the rats were sacrificed under chloroform anaethesia. Blood were collected by cardiac puncture after opening the rats surgically in EDTA bottles for estimation of differential cells (basophil, oesinophil, neutrophil, monocytes and lymphocytes count) and packed cell volume (PCV), haemoglobin concentration (HB), red blood cell count (RBC), platelet, white blood cell count (WBC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) using automated haematological machine (Cell DynTM abbot, US).

Statistical analysis

Data generated were expressed as mean value ± standard error of mean (SEM). Comparison within groups were determined by the Analysis of Variance (ANOVA) test. Significant differences at p=0.05 between control and experimental groups were evaluated by Duncan Multiple Range Test (DMRT) using SPSS version 19 (Table 1).

Phytochemical Constituents	Aqueous Extract
Saponins	+
Tannins	-
Terpenes	+
Flavonoids	-
Proteins	+
Balsams	+
Free anthraquinones	-
Alkaloids	+
Steroids	-
Glycosides	+
+: present, -: absent	

Table 1: Phytochemical Screening of aqueous extract of *Tridax procumbens*.

Discussion

Phytochemical screening of aqueous extract of *Tridax procumbens* indicated the presence of saponins, balsams, proteins alkaloids, terpenes and glycosides. The results obtained were similar to that of Dhanobalan et al.; Ikewuchi et al. and Regina et al. [19-21].

The acute toxicological evaluation of aqueous extract of *Tridax procumbens* revealed an oral LD50 value greater than 5000 mg/kgbw. This value is 50 times greater than minimum effective dose of 100 mg/kgbw in rats as reported by Salawu et al. [22]. Reports showed that if the median lethal dose of a test substance is three times greater than minimum effective dose, the substance is considered a good candidate for future studies [18]. Therefore, this medicinal plant can be regarded as relatively non-toxic acutely. This suggests that oral application of *Tridax procumbens* leaf extract may not produce toxic effect at dose lower than 5000 mg/kgbw.

Effects on heamatological parameters

Clinical signs of toxicity: The oral administration of aqueous extract of *T. procumbens* was not accompanied by any sign of physical toxicity in any of the animals throughout the period of study (28 days). There were no changes in the nature of the stool, urine and eye colour of the animals. The animals did not exhibit uncoordinated muscle movements, respiratory stress during the period of the study. However, animals exposed to 50 mg/kgbw and 100 mg/kgbw concentrations of the extract became aggressive after 20 days of exposure.

	Treatments (mg/kg bw)			
Differential*	Control	25	50	100
Neutrophils*	45.00 ± 2.30^{a}	50.40 ± 3.20^{a}	49.40 ± 2.23^{a}	42.80 ± 2.22^{a}
Lymphocytes*	46.00 ± 2.10^{a}	42.00 ± 3.67^{a}	41.80 ± 1.20^{a}	47.00 ± 2.72^{a}
Monocytes*	5.80 ± 1.16^{a}	4.40 ± 0.51^{a}	5.20 ± 1.32^{a}	4.20 ± 0.20^{a}
Eosinophils*	3.00 ± 0.55^{a}	3.20 ± 0.37^{a}	3.00 ± 0.71^{a}	2.80 ± 0.37^{a}
Basophils*	0.20 ± 0.20^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.40 ± 0.25^{a}

Table 2: Effect of aqueous leaf extract of *T. procumbens* on differential counts of rats. Values are means ± S.E.M for n=5. *Mean data on row carrying the same superscript do not differ significantly from each other (P>0.05).

Table 2 shows the differential count indices of rats following oral administration with aqueous leaf extract of *T. procumbens*. Differential leukocyte count are useful indices in assessing the toxicity profiles of plant extracts in living system. Variations in basophils, eosinophils and monocyte imply response to allergic reactions, while variations in lymphocyte and neutrophil counts may signify response to antigen or infection. Results obtained from this study showed that aqueous leaf extract of *T. procumbens* has no significant toxic effect (P>0.05) on differential counts (neutrophils, lymphocytes, monocytes, eosinophils and basophils) in treated groups when compared with the control. This implies that consumption of the medicinal plant is well tolerated by the animal's immune system as the extract did not elicit host immune reaction in response to its consumption. This observation is in agreement with the findings of Aba et al. whose report underscored that the non-significant (p>0.05) variation in differential white blood counts of animals administered *Hibiscus sabdariffa* implied that the extract does not impair the functions of differential leucocytes [23].

	Treatments (mg/kg bw)			
Heamatograms	Control	25	50	100
PCV (%)	41.60 ± 2.25^{b}	34.60 ± 2.04^{a}	40.00 ± 1.58^{ab}	39.00 ± 0.32^{ab}
RBC (x 10^{9}/l)	3.90 ± 0.33^{a}	4.18 ± 0.25^{a}	3.96 ± 0.24^{a}	3.78 ± 0.21^{a}
WBC (x 10^{9}/l)	4.70 ± 0.36^{a}	5.46 ± 0.39^{a}	4.90 ± 0.44^{a}	5.26 ± 0.27^{a}
Hb (g/d1)	13.86 ± 0.75^{b}	11.66 ± 0.61^{a}	12.98 ± 0.63^{ab}	13.76 ± 0.52^{b}
MCV (fL)	10.67 ± 0.54^{b}	8.28 ± 0.40^{a}	10.10 ± 0.45^{b}	10.32 ± 0.49^{b}
MCH (g/dl)	3.55 ± 0.27^{b}	2.79 ± 0.19^{ab}	3.28 ± 0.25^{b}	3.64 ± 0.29^{b}
MCHC (g/dl)	334.20 ± 1.02^{a}	359.20 ± 1.33^{a}	333.80 ± 0.37^{a}	332.80 ± 0.37^{a}

Table 3: Effect of aqueous leaf extract of T. procumbens on haematograms of rats. Values are means ± S.E.M for n=5. *NS: Mean data on row carrying the same superscript do not differ significantly from each other (P>0.05), Hb: Haemoglobin concentration, MCHC: mean cell haemoglobin concentration, PCV: packed cell volume, WBC: white blood cell, RBC: red blood cell.

Table 3 shows haematological indices of rats following oral administration with aqueous leaf extract of *T. procumbens*. Packed cell volume (PCV) is a point of reference of the red blood cell's capability of delivering oxygen to tissues, while hemoglobin (Hb) is the protein molecule in red blood cells that carries oxygen from the lungs to the body's tissues. The results obtained in the present study showed that animals in groups exposed to 50 mg/kgbw and 100 mg/kgbw of aqueous extracts of *T. procumbens* showed reduction in PCV and Hb which did not differ significantly (p>0.05) from the control. However, there was marked reduction (p<0.05) in PCV and Hb levels in animals fed with 25 mg/kgbw of the plant extract. This marked reduction in PCV and Hb suggests that the plant extract at lower concentration may interfere with osmoregulatory system of the blood that can result to anaemia. This observation is supported by Audu et al. whose report acknowledged that significant reduction in PCV and Hb could be indication of severe anaemia caused by destruction of erythrocytes or haemo-dilution, resulting from impaired osmoregulation across the gill epithelium [24].

The results obtained in the present study also showed that there was no significant (p>0.05) difference in red blood cell (RBC) count and mean cell haemoglobin concentration (MCHC) of animals in groups exposed to all the concentrations of the plant extract when compared with the control. While this suggests a broader picture of non-toxicity of the plant extract on RBC, studies conducted by Wolfsthal, posits that decrease in levels of Hb, with or without the concomitant decrease in RBC, can cause anaemia [25]. The results of white blood cell (WBC) count in the present study also showed that exposure of the animals to the various concentrations of the medicinal plant extract has no significant (p>0.05) toxic effect on WBC. This result suggests that the

aqueous leaf extract of *T. procumbens* does not elicit antigenic reactions that can cause variations in WBC count. Findings of Aba et al. substantiates the claim [23].

The results presented in Table 3 also showed that the mean corpuscular volume (MCV) and mean haemoglobin concentration (MCH) of animals exposed to 50 mg/kgbw and 100 mg/kgbw of the plant extract do not differ significantly ($p>0.05$) from the control animals. On the other hand, animals exposed to 25 mg/kgbw showed reduction in MCH which is not significantly ($p>0.05$) different from the control animals, but there was marked reduction in MCV in animal groups exposed to 25 mg/kgbw of the plant extract when compared with the control. MCH reflects the haemoglobin content of the red blood cells while MCV reflects the size of red blood cells. While this suggests that *T. procumbens* may have a modulating effect on red blood cells at lower concentration, these effects were not significant to cause a remarkable variation in total red blood count and normalized with increasing concentration of the plant extract.

	Time (weeks)			
Treatments (mg/kg bw)	1	2	3	4
Control	51.43 ± 1.43^{b}	65.57 ± 2.94^{c}	81.86 ± 0.67^{d}	89.71 ± 1.57^{c}
25	48.14 ± 0.91^{b}	63.14 ± 2.46^{c}	72.86 ± 3.25^{c}	87.14 ± 2.41^{c}
50	46.57 ± 2.38^{b}	53.29 ± 1.38^{b}	49.14 ± 3.69^{b}	80.71 ± 2.02^{b}
100*	41.00 ± 2.44^{a}	44.29 ± 1.70^{a}	41.00 ± 1.31^{a}	31.43 ± 2.10^{a}

Table 4: Daily feed intake of rats following oral administration with aqueous leaf extract of *T. procumbens*. Values are means ± S.E.M for n=5. *Mean data on column carrying the same superscript do not differ significantly from each other (P>0.05).

Table 4 shows daily feed intake of rats following oral administration with aqueous leaf extract of *T. procumbens*. In the first week of treatment, animals administered 25 mg/kgbw and 50 mg/kgbw showed similarity in pattern of feeding that is not significantly ($p>0.05$) different from the control. However, rats administered 100 mg/kgbw showed marked ($p<0.05$) decrease in feed intake when compared with the control ($P<0.05$) animals. This significant reduction in feed intake is an indication that higher concentration of the plant extract (100 mg/kgbw) significantly impacted the palatability perception of the animals in this group to cause a reduction in their feed intake. According to Raal and Matto, the perception of palatability of certain foods or drinks are partially emotional, but Boon and Smith hold a contrasting view that the herbal preparations are believed to be unpalatable, and the sensations of astringency or bitterness may limit their acceptance [26,27]. Week 2: Rats administered 25 mg/kgbw showed no significant ($p>0.05$) difference in feed consumption when compared with control. Furthermore, animals fed 50 mg/kgbw and 100 mg/kgbw showed significant ($p<0.05$) decrease in their feed consumption when compared with the control. It is noteworthy that the reduction in feed intake was dose dependent, with the group fed the highest concentration of plant extract (100 mg/kgbw) showing the most reduction in feed consumption. This suggests that the higher concentration of the plant extract may have impacted the odor or taste perception of the animals subsequently. This is consistent with the assertion of Meyerhof et al. that secondary metabolites do taste bitter and induce aversive reactions [28]. Week 3: There was significant ($p<0.05$) progressive dose dependent decrease in feed consumption between the groups treated with extract and the control. This dose dependent decrease may be substantiated by the fact that the higher concentration of the plant extract contained sufficiently more amounts of the compound that confer bitterness, thereby increasing the intensity of bitterness and invariably resulting in palatability aversion. This argument is in line with the view of Drewnowski and Carneros, which stated that increasing the content of bitter phytonutrients for health may be wholly incompatible with consumer acceptance, hence sensory factors ought to be taken into account when studying phytonutrients and health [29]. Week 4: The feeding behavior of the treated groups in the fourth week showed similarity in feeding pattern with the results obtained in week 2. The animals exposed to 50 mg/kgbw and 100 mg/kgbw showed marked decrease ($p<0.05$) in feed intake when compared with the control, whereas, animals in group administered 25 mg/kgbw did not differ significantly ($p>0.05$) in feeding behavior when compared with the control.

	Weeks			
Treatments (mg/kgbw)	1	2	3	4
Control	228.57 ± 8.57^{b}	297.14 ± 8.37^{d}	415.71 ± 17.30^{d}	491.43 ± 5.53^{d}
25	232.86 ± 5.76^{b}	242.86 ± 7.47^{c}	320.00 ± 8.45^{c}	367.14 ± 15.69^{c}
50	227.14 ± 7.86^{b}	215.00 ± 4.50^{b}	205.71 ± 2.02^{b}	138.57 ± 2.37^{b}
100*	172.86 ± 23.37^{a}	170.00 ± 3.27^{a}	171.71 ± 3.88^{a}	92.86 ± 2.64^{a}

Table 5: Daily fluid intake of rats following oral administration with aqueous leaf extract of *T. procumbens*. Values are means ± S.E.M for n=5. *Mean data on column carrying the same superscript do not differ significantly from each other (P>0.05).

Table 5 shows daily water intake of rats following oral administration with aqueous leaf extract of *T. procumbens*. Week 1: Animals administered 25 mg/kgbw and 50 mg/kgbw showed no significant ($p>0.05$) difference in fluid intake when compared with the control. Whereas, rats treated with 100 mg/kgbw showed marked decrease ($p<0.05$) in fluid intake when compared with the control. This shows a correlation with the results of feed intake obtained in the present study. A recent study by Yadav and Nayak reported *T. procumbens* as having an acrid taste [30]. In another study of chemical composition of *Tridax procumbens*, the medicinal plant was reported as rich source in sodium [31]. Sodium itself have been reported as sole regulator of water content of the body. Hence, it is safe to speculate that the high sodium content coupled with the acrid taste of the medicinal plant may have impacted the fluid consumption of the animals. The results obtained in the present study for subsequent weeks (week 2, 3 and 4) showed a significant ($p<0.05$) dose dependent decrease in fluid intake when compared with the control. This suggests a concentration effect of the plant extract on the fluid intake of the animal groups. The reduction in fluid and feed intake did not affect the animals physically. Suppression of appetite in these animals may have activated compensatory physiological responses to cope with the stress of hunger and thirst [32].

Conclusion

We conclude that the aqueous leaf extract of *Tridax procumbens* significantly altered the Hb, PCV and MCV in rats fed 25 mg/kgbw. The extract (25 mg/kgbw, 50 mg/kgbw, 100 mg/kgbw) did not affect the RBC, WBC, MCH, MCHC in exposed rats. Oral LD50 value for the extract is above 5000 mg/kgbw. Further studies are needed on effect of extract on plasma biochemical parameters and histopathology.

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