



RESEARCH PAPER

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Aqueous flower extract of *Tridax procumbens* on the haematology/serum lipid profile of Wistar Rats

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Abstract

This study is aimed at investigating the acute effects of aqueous flower extracts of *Tridax procumbens* on some haematological and serum lipid parameters at a dose of 100mg/kg/d in Wistar albino rats. Following a 7-day oral administration of the aqueous extract, haematological and serum lipid parameters were evaluated in the 10 Wistar albino rats. The rats weighed 187.7±28.49g and were randomly assigned into two groups (Group A: control and group B: 100mg/kg/d of extract). The evaluation was performed on fully automated analyzers (ERBA diagnostics) immediately after blood sample collection. The 100mg/kg/d of the flower extract caused significant increases in the levels of PCV, HB, MCH, MCHC, MCV and Platelet concentration (41.8±1.64, 13.64±0.41, 30.95±2.57, 32.99±0.67, 92.7±9.51 and 141.2±17.49 respectively) when compared to the controls which received no extract at all. The extract also caused reduction in the serum levels of Total cholesterol, Triglycerides, LDL and VLDL (134.68±5.21, 153.5±5.53, 45.5±7.19, 30.7±1.11 respectively). However, the extract produced a significant elevation in the level of the HDL (58.48±4.11). These changes suggest that flower extract has positive effects on the erythron of wistar rats, hemostatic and lipid lowering/anti-atherogenic properties. In conclusion, the flower extract can be exploited as a potential haematinic agent, haemostatic agent (for wound healing activity) and for its anti-hyperlipidaemic/anti-atherogenic properties and thus can be useful for development of therapeutic agents in the prevention or management of diseases associated with haematopoietic and lipid disorders (such as anaemia, thrombocytopenia; obesity, type 2 diabetes mellitus).

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Introduction

The plant kingdom has served as one of man's most important and oldest sources of useful drugs. Plant extracts, either as pure compounds or as standard extracts, provide unlimited opportunities for new drug discovery, and there has been an increasing interest in the pharmaceutical industry to develop new drugs from plant sources (Burton *et al.*, 1983).

A host of plants have now found application either in part or as a whole in the management, treatment or prevention of many diseases. Active components of these plants are now being investigated, extracted and developed into drugs with fewer negative effects or contraindications (Oluyemi *et al.*, 2007). Many indigenous plants are used in herbal medicine to cure diseases and in the treatment of injuries. *Tridax procumbens* is one of such plants.

Tridax procumbens Linn is a plant that belongs to the family Asteraceae. The extracts of the plant have been used commonly as native or indigenous medicine for a number of disease conditions. In the Indian ayurvedic medicine, it has been used extensively for the treatment of wounds, and a number of skin infections (antifungal), as an insect repellent and also anticoagulant. It was also used in diarrhea and dysentery. Leaf extracts are used to treat infectious skin diseases in folk medicines (Mir *et al.*, 2017). In Nigeria, *T. procumbens* widely used as feed in poultry and rabbitary and also as a medicinal plant (Olukunle and Abatan, 2008). *T. procumbens* has been reported by several studies to possess anti-inflammatory, antimicrobial and antiseptic, hepatoprotective, hypoglycemic, hypotensive, wound healing and immunomodulatory properties (Durgacharan *et al.*, 2008; Pareek *et al.*, 2009), and a number of secondary plant metabolites have been reported including anthraquinones, catechol, tannins, flavonoids, isoflavonoids, glycosides, lipids, phenols, phospholipids, reducing sugars, resins, saponins, steroids, and sterols (Manjamalia *et al.*, 2010).

Haematology, and serum biochemical evaluations (such as the serum lipid profile) are relevant tools in

the pathological and physiological status of mammals because they serve to provide information for the proper diagnosis of diseases, making a prognosis, assessing the efficacy of established therapy, and toxic effects of drugs and chemicals (Stockham and Scott, 2008). Haematological parameters of utmost clinical importance include the Red blood cell (RBC) counts, packed cell volume (PCV), Haemoglobin count (HB), Mean corpuscular values (MCV), white blood cell (WBC) counts and differential blood counts (Thrall and Weiser, 2002). Some of these parameters can serve as indicators of the immune status of the organism and can thus provide relevant information regarding the health status of the organism. Determining the lipid profile provides a means of assessing risks of cardiac disorders hence this study will test the activity of the extracts on lipid profile which are known risk factors of cardiac disorders. The important Lipid parameters include Serum total cholesterol, triglycerides, and High-Density Lipoprotein (HDL) and Low-density Lipoprotein (LDL) cholesterol levels. Since the haematological and lipid parameters serve as basic indicators for disease diagnosis, and a deviation from normal values of these parameters usually implies a deviation from the normal healthy status, substances which can modify these parameters towards normal can thus be exploited in the restoration of normal health conditions or in the prevention/ treatment of diseases implicated by these factors. A number of medicinal plants have been reported to possess activities on several haematological parameters either at high or low concentrations (Garima and Goyal, 2007; Nwinuka *et al.*, 2008; Ikpu and Nku, 2008; Ajeigbe *et al.*, 2013; Odesanmi *et al.*, 2010; Oyedemi *et al.*, 2011). The effect of leaf extract of *T. procumbens* has also been reported (Olokunle and Abatan, 2008) but very little is known about *Tridax procumbens* flowers in this regard.

Due to its wide usage as animal feed and also as a medicinal herb, the present study is designed to evaluate the acute haematological and serum lipid effects of extract of the flowers of *T. procumbens* in Wistar albino rats.

Materials and methods

Plant material

Flowers of *Tridax procumbens* sufficient for the study were collected by handpicking from fields within the environment of the study location i.e. Delta state university, Abraka. Identification and authentication of the plant was done in the Department of Pharmacognosy, Faculty of Pharmacy, Delta State University.

Extraction of plant

The flowers were air-dried and reduced to coarse powder using a mechanical blender. For the aqueous maceration, 200g of the powdered flower was soaked in about three Liters of distilled water for a period of 5 days. The obtained extract was filtered over a muslin cloth and the filtrate collected which was concentrated by evaporating to dryness over a hot water-bath at 70°C. The resulting concentrate was then reconstituted with sterile distilled water aseptically at a concentration of 20mg/ml into sterile McCartney bottles and stored in the refrigerator at 4°C for subsequent use

Experimental Design

Ten (10) male albino rats (weighing 187.7±28.49g) were obtained from the animal house of the Department of Pharmacology, Delta State University, Abraka. They were aged between eight to twelve weeks. The rats were weighed and sorted into two groups of five animals each, so that their average weights were approximately equal (Table 1). The animals were housed in well ventilated plastic cages under naturally illuminated environment with proper hygienic conditions, at 25±2°C, and a relative humidity of 45-50%. The rats were fed on a commercially available diet obtained from a market in Abraka, twice daily and tap water *ad libitum*. Prior to commencement of administration of the flower extract of *T. procumbens*, the rats were allowed to acclimatize in the new environment with standard 12-hour light: dark cycle, for a period of 7 days, and were treated for 7 days with a 100mg/kg/d of the plant extract. The extract was administered orally. Oral gavages using a rubber oropharyngeal canula and calibrated hypodermic syringe as was described by ACF

(2000) were used to administer the extract to the animals once daily for 7 days from a stock preparation of 20mg/ml. Before sacrificing, the animals were starved of food overnight and sacrificed by cervical dislocation as was described by Ochei and Kolhatkar (2006).

Table 1. Experimental design.

S/N	ID	Treatment
1	Group A: Control group	Distilled water (received no extract at all).
2	Group B: Treatment 1	100mg/kg/d aqueous extract of <i>Tridax procumbens</i> flower.

Blood Sample Collection

Blood samples for the haematological and serum lipid profile determinations were collected from the superior and inferior vena cava of the 10 albino wistar rats. The blood samples were collected between the hours of 8 and 11 in the morning on the same day. Blood samples for the haematological determination (2ml) were collected into sample bottles containing ethylenediamine tetra-acetic acid (EDTA bottles) while the blood samples (4ml) for serum lipid profile analysis were collected into Lithium heparin bottles while maintain aseptic conditions. The haematological and serum lipid profile determinations were carried out immediately on blood collection.

Haematological and Serum Lipid Determination

The analysis was conducted at the Emma-Maria Research Laboratory and consultancy, Abraka. The haematological analysis was performed on a fully automated hematology analyzer using ERBA's Elite 3 from ERBA diagnostics (Transasia Bio-medicals Ltd, Germany) which utilizes only three reagents (Erba Diluent-Diff, Erba Lyse-Diff, Erba Cleaner), which are environmentally friendly and cyanide free. Whole blood was used for the determination of the different parameters. The parameters determined are red blood cell count (RBC), haemoglobin (HGB), Total white blood cell count (TWBC), Packed cell volume (PCV), Mean corpuscular volume (MCV), Mean corpuscular haemoglobin (MCH), Mean corpuscular haemoglobin

(MCHC), Platelets (PLT), Lymphocytes, Eosinophils and Neutrophils. The automated analysis was done following the manufacturer’s operational guidelines. All the samples were analyzed within thirty minutes of collection.

Lipid analysis was also performed on a fully automated analyzer based on spectrophotometric principle using kits obtained from ERBA diagnostics (Transasia Bio-medicals Ltd, Germany). The serum lipid profile was analyzed on the same day of the collection of blood samples. Three laboratory measures were determined: Total cholesterol; HDL; Triglycerides. From these three data LDL and VLDL may be calculated. According to Friedewald's equation (Friedewald *et al.*, 1972)

$$LDL = \text{Total cholesterol} - HDL - \text{Triglycerides}/5$$

VLDL may be defined as the total cholesterol that is neither HDL nor LDL. Then Friedewald's equation mentioned above yields:

$$VLDL = \text{Triglycerides}/5$$

The blood samples were centrifuged at 3000rpm for five minutes to obtain the serum which was then analyzed for the lipid profile. The analysis of the different samples was performed using the auto-analyzer according to the manufacturer’s operational guidelines. Three different reagents were employed in the determination of the lipids: TC reagent for Total cholesterol, TGL reagent for Triglyceride and HDL precipitating reagent for High density lipoprotein

determination. All the samples were analyzed within thirty minutes of collection.

Statistical analysis of data

All the values are reported as the mean ± standard deviation (S.D). The values of the variable were analyzed for statistically significant differences using the Students *t*-test, with GraphPad Instat. P<0.05 was considered to be significant. Graphs were drawn using Microsoft Office Excel, 2007 software.

Results

Table 2 shows the effect of an aqueous extract of the flowers of *T. procumbens* on the haematological parameters of the treated rats. The 100mg/kg/d treatment produced an increase in the values of PCV, haemoglobin (HB) concentration, platelet concentration, MCH, MCHC and MCV. The flower extract also caused an elevation in the level of circulating neutrophils but there was no significant effect on the level of blood WBC, eosinophils and lymphocytes. Table 3 shows the effect of an aqueous crude flower extract of *T. procumbens* on the serum lipid parameters of the treated rats.

The 100mg/kg/d treatment caused significant reduction in the serum levels of total cholesterol, Triglycerides, Low-density lipoproteins as well as in the level of serum very low-density lipoproteins. The 100mg/kg/d of the flower extract also resulted in a significant elevation in the level of the High-density lipoprotein.

Table 2. Mean ± SD haematological indices of experimental animals after 7 day treatment with aqueous flower extracts of *Tridax procumbens* flowers as compared to control.

Haematological parameter (unit)	Group A		Group B	
	Mean	SD	Mean	SD
PCV (%)	33.60	2.302	41.80	1.643
HB (g/dl)	10.184	0.7268	13.6386	0.4121
RBC (X10 ⁶ /mm ³)	5.242	0.08198	4.466	0.2703
Platelet (X10 ⁶ /mm ³)	122.318	2.718	141.204	17.498
TWBC (mm ³)	10.426	0.3211	9.392	0.3599
LYM (%)	21.61	0.6655	22.232	0.185
EOS (%)	3.80	0.4472	4.00	0.00
NEU (%)	37.60	4.336	51.00	8.944
MCH (pg)	20.342	1.569	30.952	2.575
MCHC (g/L)	30.792	0.87	32.986	0.6719
MCV (ft)	63.302	5.039	92.702	9.511

Table 3. Mean \pm SD serum lipid indices of experimental animals after a 7 day treatment with aqueous flower extracts of *Tridax procumbens* flowers as compared to controls.

Lipid parameter assessed	Group	Mean (mg/dl)	SD
Total cholesterol (CHO)	I.Group A	154.90	7.451
	II.Group B	134.68	5.211
Triglycerides (TGL)	I.Group A	184.52	13.037
	II.Group B	153.50	5.530
High density lipoprotein (HDL)	I.Group A	49.826	3.807
	II.Group B	58.48	4.111
Low-density lipoprotein (LDL)	I.Group A	68.17	8.632
	II.Group B	45.50	7.188
Very low-density lipoprotein (VLDL)	I.Group A	36.904	2.607
	II.Group B	30.70	1.106

Discussion

Blood can function as pathological and physiological indicator of the health of animals (Jorum *et al.*, 2016). The utility of haematological analysis or determination is immense with each parameter serving as a tool or means to aid in disease diagnosis and also to monitor disease progression. In this study, the increase in haemoglobin concentration (HB), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) caused by the aqueous crude extract of *T. procumbens* flower may suggest that the aqueous crude extract of *T. procumbens* flower has positive effects on the erythron of Wistar rats. These RBC indices are reflective of the size (MCV) and level of haemoglobin content (MCH and MCHC) of Red blood cells and can aid in diagnosis of the cause of anaemia (Kanu *et al.*, 2016).

Hence an increase in value of these indices following treatment with the extract may suggest that the extract may possess haematinic properties. Packed cell volume (PCV) was significantly higher in the rats treated with the aqueous crude extract of *T. procumbens* flower at 100mg/kg/d and this is indicative of higher cellularity of the blood. The increase in the concentration of platelets caused by the aqueous crude extract of *T. procumbens* flower may suggest that the aqueous crude extract of *T. procumbens* flower may possess anti-coagulant and wound healing activity and can be exploited as a potential haemostatic agent. This haemostatic activity have also been reported in the leaves of *T. procumbens* (Kale *et al.*, 2008; Ikese *et al.*, 2015).

Cholesterol and triglycerides are important lipid components of cells and they perform a number of vital physiological functions. Cholesterol is important in the maintenance of cellular integrity both in terms of structure and function, in all biological membranes. Cholesterol is obtainable from either the diet or may be biosynthesized in the body. Lipoprotein particles play major roles in the cellular uptake and transport of cholesterol and other lipid molecules present in the circulation.

Cholesterol and triglycerides are usually packaged into lipoproteins in order to be transported into cells. Chylomicrons are responsible for the transport of triglycerides derived from the gut while Low-density lipoproteins and Very low-density lipoprotein molecules distribute liver-derived triglycerides and cholesterol to peripheral tissues. The high-density lipoprotein particles are responsible for the transport of excess cholesterol from the peripheral tissues to the liver for excretion from the body (Otis *et al.*, 2011; Prabhudas *et al.*, 2004).

The reduction in the serum levels of cholesterol, triglycerides, low-density lipoprotein (LDL) as well as the very low-density lipoprotein (VLDL) and an elevation in the level of the circulating high density lipoprotein (HDL) may suggest that the aqueous crude extract of *T. procumbens* flower may possess anti-hyperlipidaemic activity and thus can be useful in the prevention or management of disorders associated with lipid metabolism such as in obesity, and also in management of complications associated with diabetes mellitus (Petchi *et al.*, 2013; Bharathi *et al.*, 2011).

Table 2 shows the weight of the Wistar albino rats before and after treatment with the aqueous crude extract of *T. procumbens* flower. There was a significant reduction in the weight of the rats following the treatment with the aqueous crude extract of *T. procumbens* flower when compared to the control group.

This effect may be due to the reduction in the level of circulating fatty molecules in the body as reported in Table 3. This effect can thus provide further support on the potential of the plant extract to be a good drug candidate for weight reduction and thus can be effective as an anti-obesity agent.

Conclusion

The acute effects of aqueous flower extracts of *T. procumbens* on haematological and serum lipid parameters in wistar albino rats was investigated in the study. The flower extract had significant haematinic/ blood forming and haemostatic activities. The extract was also found to increase serum levels of good cholesterol and reduce the levels of bad cholesterol. Therefore, aqueous extracts from the flowers of *T. procumbens* can be exploited for such haematinic, haemostatic and anti-hyperlipidaemic properties which could be useful in the prevention, treatment and/or management of several disease conditions such as anaemia, bleeding, dyslipidaemia, obesity etc.

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