

# Sub-acute Toxicological Evaluation of Methanol Whole Plant Extract of *Tapinanthus dodoneifolius* (D.C) Danser in Wistar Rats

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## Abstract

*Tapinanthus dodoneifolius* (Loranthaceae), has diverse ethnomedicinal uses which include the management of mental illness, epilepsy, diarrhoea, malaria, pain and hypertension. Despite these uses and many validated pharmacological properties, the safety of *T. dodoneifolius* is yet to be evaluated. This study therefore, aimed to evaluate the sub-acute toxicity profile of methanol whole plant extract of *T. dodoneifolius* in rats. Phytochemical screening of the plant extract was carried out according to standard protocols. Oral median lethal dose (LD<sub>50</sub>) was estimated using the Organisation for Economic Cooperation and Development (OECD) 423 limit test. Doses of 250, 500 and 1000 mg/kg of the extract were administered for 28 consecutive days using the OECD 407 guideline. The body weights of animals were taken at 7 days interval within 28 days period. Thereafter, animals were sacrificed and blood samples collected for haematological, biochemical and electrolyte analysis. The spleen, heart, liver, kidney, lungs and stomach were also collected for histological examinations. The extract contains alkaloids, cardiac glycosides, saponins, tannins, flavonoids, steroids, terpenoids and carbohydrate. The LD<sub>50</sub> was found to be  $\geq 5000$  mg/kg. The extract showed significant ( $p \leq 0.05$ ) increase in body weight and decrease in alkaline phosphatase (ALP). There was no significant difference in the mean organ weight. The histological studies did not reveal any serious abnormalities in the organs examined. The methanol whole plant extract of *T. dodoneifolius* does not possess the toxic effects that could affect its ethnomedicinal uses.

**Keywords:** *Tapinanthus dodoneifolius*; toxicity; body weight; organ weight; haematological indices; biochemical parameters

## 1. INTRODUCTION

The increasing use of medicinal plants in the treatment of many ailments necessitates the toxicological screening to ascertain their safety among users [1]. Toxicity is a term that describes the level of an adverse effect caused by the interaction of a toxicant and cell(s) of a living organism [2]. Medicinal plants are essential part of healthcare delivery system in resource poor areas of the world. About 80% percent of the

world's population uses herbal medicine in their healthcare delivery system [3]. The greater demands for resources and time in evaluating the safety and standardizing the use of medicinal plants have led to much emphasis of screening them for efficacy based on traditional claims rather than safety. Toxicological evaluation of medicinal plants is as important as pharmacological validation of ethnomedicinal claims [4]. Other reason that may have led to the shift of attention more on pharmacological validation may

stem from the general belief of safety and acceptability of medicinal plants.

*Tapinanthus dodoneifolius* is a bushy parasitic plant that grows on a wide range of trees around the savannah zone. The plant belongs to the family Loranthaceae. *T. dodoneifolius* is an important plant use in the treatment of many diseases such as stomach ache, diarrhoea, dysentery, diabetes, epilepsy, hepatitis, hypertension, wounds, cancer etc among the Hausa and Fulani tribes of northern Nigeria. The local names of the plants among different tribes in Nigeria are Etu-lonchi (Nupe), Elozie (Ibo), Kauchi (Hausa) and Afomu Igba (Yoruba) [5]. Other ethnomedicinal uses of *Tapinanthus dodoneifolius* are treatment of inflammation, fever, infection, dizziness, energy loss, irritability, vertigo and headache. The has a broad spectrum of activity against multidrug resistant bacteria and fungal isolates of farm animals [6, 5]. *T. dodoneifolius* is also reported to have larvicidal and molluscidal effects. Related Africa mistotle *Agelanthus dodoneifolius* has been reported to have antiplasmodial activity [7]. Despite the dependence on medicinal plants most especially in resource poor areas, there is still sketchy information about their safety [8]. *Tapinanthus dodoneifolius* is one of such plant. This study was undertaken to evaluate sub-acute toxicity profile of methanol whole extract of *Tapinanthus dodoneifolius* in Wistar rats.

## 2. MATERIALS AND METHODS

### Collection of Plant

The whole plant of *Tapinanthus dodoneifolius* was collected from the bush in Sabon Gari Local Government Area of Kaduna State, Nigeria in the month of June, 2018. It was identified and authenticated by Mr. Namadi Sanusi of the Department of Botany, Ahmadu Bello University, Zaria. A voucher number; 0370 was obtained by comparing with existing specimen.

### Preparation of Plant Extract

The whole plant of *Tapinanthus dodoneifolius* was washed, air-dried under shade after which it was grounded to coarse powder using pestle and mortar. About 900 g of the powdered plant material was extracted with 7 L of 70 %<sup>v/v</sup> methanol for three days using cold maceration with intermittent shaking. The mixture was then filtered using Whatman filter No 1. The filtrate was concentrated and subjected to air drying in crucible. Percentage yield of the methanol extract was calculated and the obtained extract was stored in a desiccator and subsequently referred to as

Methanol Extract of *Tapinanthus dodoneifolius* (METD). Fresh concentrations of the extract were prepared on each day of the experiment by reconstituting in distilled water.

### Experimental Animals

Wistar rats of both sexes were obtained from the Animal House Facility of the Department of Pharmacology and Therapeutics Ahmadu Bello University, Zaria. Animals were housed in cages with rodent diet and water *ad libitum*. Ethical approval with an approval number: (ABUCAUC/2019/006) was obtained from the Ahmadu Bello University Committee on Animal Use and Care (ABUCAUC).

### Phytochemical Screening

Preliminary phytochemical screening of the methanol whole extract of *Tapinanthus dodoneifolius* was carried out according to methods described by Trease and Evans and Ayoola [9, 10]

### Acute Toxicity Study

The acute toxicity study was carried out according to the Organization for Economic Co-operation and Development (OECD) guidelines 423[11] and fixed dose studies was adopted with 5000 mg/kg body weight as the limit dose. Three rats were fasted for 3-4 hours, and each rat was administered 5000 mg/kg of methanol whole plant extract of *Tapinanthus dodoneifolius*. Food was withheld for 1 hour post drug administration. Each rat was observed individually for the first 30 minutes and periodically during the first 4 hours and then daily for 14 days for any sign of toxicity, such as tremor, convulsion, salivation, lacrimation, diarrhea, lethargy, sleep, respiratory, behavior pattern, onset and recovery from toxicity and death.

### Sub-acute Oral Toxicity Study

The method described by the Organization for Economic Co-operation and Development (OECD) test guideline 407 was adopted for the study [12]. Twenty-four rats (12 males and 12 females) were weighed and grouped into 4 of 3 male and 3 female rats in each group (the males were separated from the females). Group 1, 2, 3 and 4 received 10 mL/kg distilled water, 250, 500 and 1000 mg/kg per oral of the extract respectively for 28 consecutive days. Animals were sacrificed under chloroform anaesthesia after being deprived of pellet but had free access to water for 24 hours. Blood samples were collected through cardiac puncture into EDTA and non-heparinized containers for haematological and biochemical analysis respectively. The liver, heart, spleen, stomach, kidney

and lungs were collected, weighed and stored in 20 mL of 10 % formal saline sample bottles (Axiom, Zhanjiang Gong Jong medical technology Co. Ltd, China) and thereafter processed for histopathological studies.

#### **Body Weight**

The body weights of the animals were taken at weekly interval during the course of the 28-days treatment. The weight changes were calculated in respect to the initial body weights on day zero.

#### **Organ Weight Index**

The heart, lungs, stomach, liver, spleen and kidney were collected, freed of connective tissues and rinsed in normal saline, blotted with filter paper and weighed. The relative organ body weight (ROW) was calculated as:  $ROW = (\text{organ weight} / \text{total body weight}) \times 100$ .

#### **Haematological Analysis**

Blood samples were collected and assayed. The parameters assayed include haematocrit (HCT), Hemoglobin (HB), red blood cell (RBC) count, platelet count (PLT), white blood cell (WBC) count, monocytes (MON), lymphocytes (LYMPH), granulocytes (GRAN) using automated haematology analyzer (Mythic 18 by Orphee, Switzerland).

#### **Biochemical Analysis**

Serum blood samples were analyzed for alkaline phosphatase (ALP), alanine transaminase (ALT), aspartate transaminase (AST), albumin (Alb), total protein (TP), urea (Bu), creatinine (Crea) using (Gesam Chem 200, USA) automated machine.

#### **Histopathology**

The kidney, liver, heart, lungs, stomach, and spleen were placed into 10 % formalin fixative for histological examinations. Tissue slides were viewed at a magnification of x250 and photomicrographs of the tissues were obtained with the aid of a consultant histopathologist.

#### **Statistical Analysis:**

Data were expressed as mean  $\pm$  SEM. One-way and split plot analysis of variance (ANOVA) were performed appropriately to compare the differences between means followed by Bonferoni post hoc tests using SPSS version 20.0. A mean difference was considered significant when  $p \leq 0.05$ .

### **3. RESULTS**

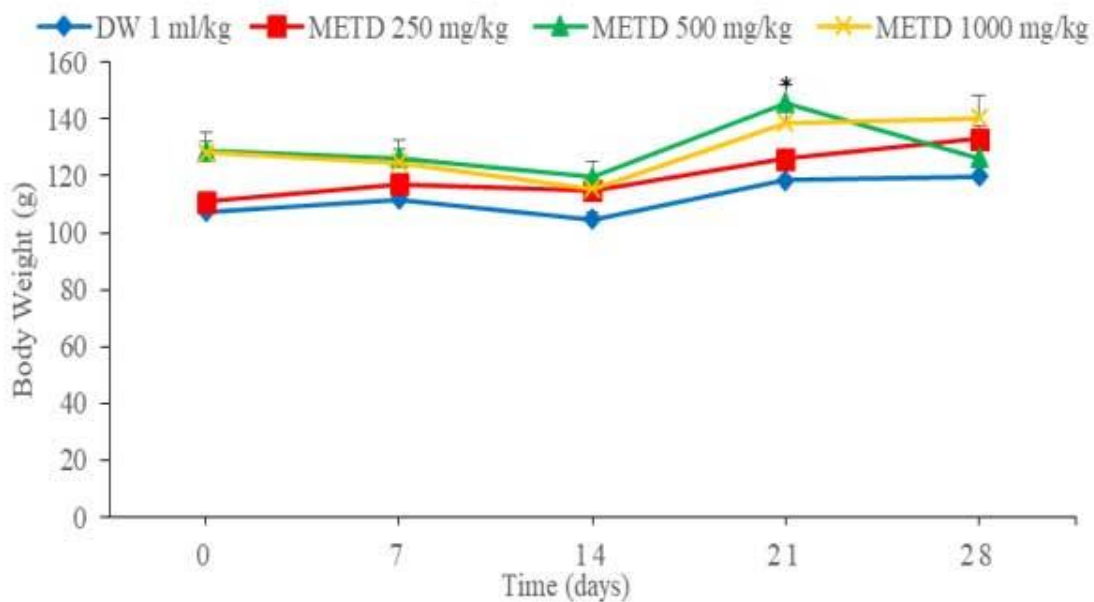
The percentage yield of the powdered plant was 14.4%.

#### **Phytochemical Constituents**

The preliminary phytochemical screening of METD revealed the presence of alkaloids, cardiac glycosides, saponins, tannins, flavonoids, steroids/triterpenes, terpenoids and carbohydrates. However, anthraquinones was absent.

#### **Acute Toxicity Study**

The methanol whole plant extract produced no adverse effect in rats at the dose of 5000 mg/kg. There were no changes in behavior, nature of stool and saliva. There was no mortality observed in the different groups of rats after 7 days. The oral median lethal dose (LD<sub>50</sub>) of the plant was estimated to be  $\geq 5000$  mg/kg.



**Figure 1: Effect of 28 days Administration of Methanol Extract of *Tapinanthus dodoneifolius* on Body Weight of Wistar Rats**

Values are expressed as Mean  $\pm$  S.E.M; \* $p \leq 0.05$  compared to control; Split plot ANOVA followed by Bonferroni post hoc test, n=6, METD = Methanol Extract of *Tapinanthus dodoneifolius*

**Table 1: Effect of 28 days Administration of Methanol Extract of *Tapinanthus dodoneifolius* on Some Haematological Parameters in Wistar Rats**

Parameters	Treatment Groups			
	Control	250 mg/kg	500 mg/kg	1000 mg/kg
WBC ( $\times 10^3/\mu\text{L}$ )	6.72 $\pm$ 0.91	6.10 $\pm$ 0.77	5.27 $\pm$ 0.89	5.74 $\pm$ 0.71
Hb (g/dL)	13.11 $\pm$ 1.11	13.33 $\pm$ 0.46	13.03 $\pm$ 0.69	14.49 $\pm$ 0.59
RBC ( $\times 10^6/\mu\text{L}$ )	4.85 $\pm$ 0.08	4.65 $\pm$ 0.14	4.65 $\pm$ 0.14	4.65 $\pm$ 0.14
PLT ( $\times 10^3/\mu\text{L}$ )	306.40 $\pm$ 31.09	400.67 $\pm$ 25.72	323.47 $\pm$ 34.93	205.80 $\pm$ 21.20
HCT (%)	40.72 $\pm$ 3.75	41.52 $\pm$ 1.05	39.98 $\pm$ 2.09	43.34 $\pm$ 1.66
MON (%)	11.00 $\pm$ 0.95	9.13 $\pm$ 1.24	7.18 $\pm$ 1.67	5.16 $\pm$ 0.72*
LYMPH (%)	33.54 $\pm$ 2.14	36.92 $\pm$ 2.51	32.47 $\pm$ 1.93	32.36 $\pm$ 2.59
GRAN (%)	55.62 $\pm$ 2.41	53.15 $\pm$ 2.36	44.85 $\pm$ 2.83*	44.64 $\pm$ 1.52*

Values are expressed as Mean  $\pm$  S.E.M., \* $p \leq 0.05$  compared to control - One way ANOVA followed by Bonferroni post hoc test, n=6, METD: Methanol extract of *Tapinanthus dodoneifolius*, WBC: White blood Cell, RBC: Red blood cells, HGB: Haemoglobin, HCT: Haematocrit, PLT: Platelets, LYMP: Lymphocytes, MON: Monocytes, GRAN: Granulocytes

**Table 2: Effect of 28 days Administration of Methanol Extract of *Tapinanthus dodoneifolius* on Serum Biochemical Parameters in Wistar Rats**

Parameters	Treatment Groups			
	Control	250 mg/kg	500 mg/kg	1000 mg/kg
ALT (IU/L)	13.00 $\pm$ 3.08	9.00 $\pm$ 1.53	9.50 $\pm$ 1.53	15.60 $\pm$ 1.36
AST (IU/L)	143.20 $\pm$ 18.83	95.00 $\pm$ 13.95	156.17 $\pm$ 8.45	148.20 $\pm$ 6.42
ALP (IU/L)	35.24 $\pm$ 4.77	27.62 $\pm$ 3.86	26.03 $\pm$ 2.52	20.48 $\pm$ 1.96*
TP (g/dL)	9.22 $\pm$ 0.68	10.04 $\pm$ 0.78	12.33 $\pm$ 1.01	12.05 $\pm$ 0.88
ALB (g/dL)	3.16 $\pm$ 0.18	2.26 $\pm$ 0.24	2.90 $\pm$ 0.09	3.02 $\pm$ 0.23

Values are expressed as Mean  $\pm$  S.E.M., \* $p \leq 0.05$  compared to control; One way ANOVA followed by Bonferroni post hoc test, n=6, ALT: Alanine amino transferase, AST: Aspartate amino transferase, ALP: Alkaline Phosphatase, TP: Total protein, ALB: Albumin, METD: Methanol extract of *Tapinanthus dodoneifolius*, IU: International unit

**Table 3: Effect of 28 days Administration of Methanol Extract of *Tapinanthus dodoneifolius* on Kidney Function in Wistar Rats**

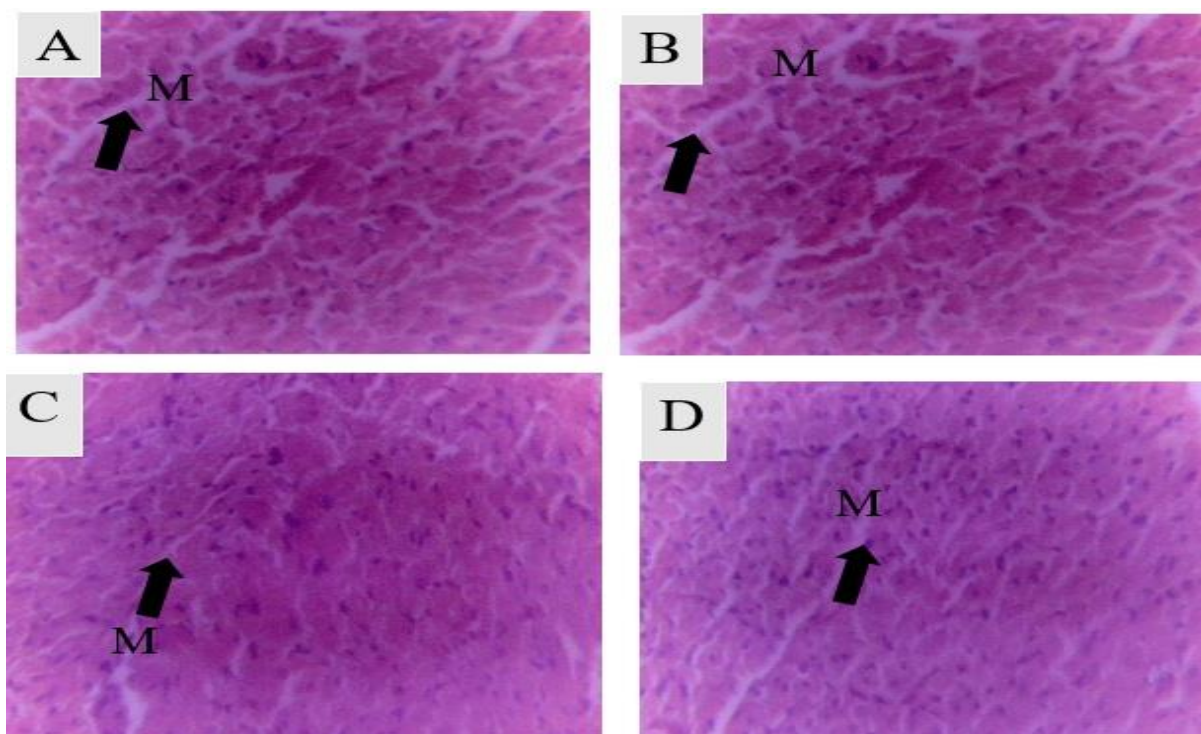
Parameters	Treatment Group			
	Control	250 mg/kg	500 mg/kg	1000 mg/kg
Urea (mg/dL)	46.08±1.28	48.25±2.67	44.05±1.62	49.96±0.95
Sodium (mmol/L)	114.54±5.17	121.95±4.47	123.55±3.08	106.32±5.12
Potassium (mmol/L)	16.82±2.42	11.82±0.76	17.00±1.06	13.96±1.22
Creatinine (mEq/L)	0.94±0.07	0.92±0.06	0.88±0.07	0.82±0.05
Chloride (mEq/L)	21.80±1.39	22.40±1.54	25.33±1.91	25.33±1.9
Bicarbonate (mg/dL)	88.20±3.77	84.00±7.31	85.83±2.40	85.40±5.95

Values are expressed as Mean ± S.E.M; One way ANOVA followed by Bonferroni post hoc test, n=6, METD: Methanol extract of *Tapinanthus dodoneifolius*

**Table 4: Effect of 28 days Administration of Methanol Extract of *Tapinanthus dodoneifolius* on Mean Organ Weight in Wistar rats**

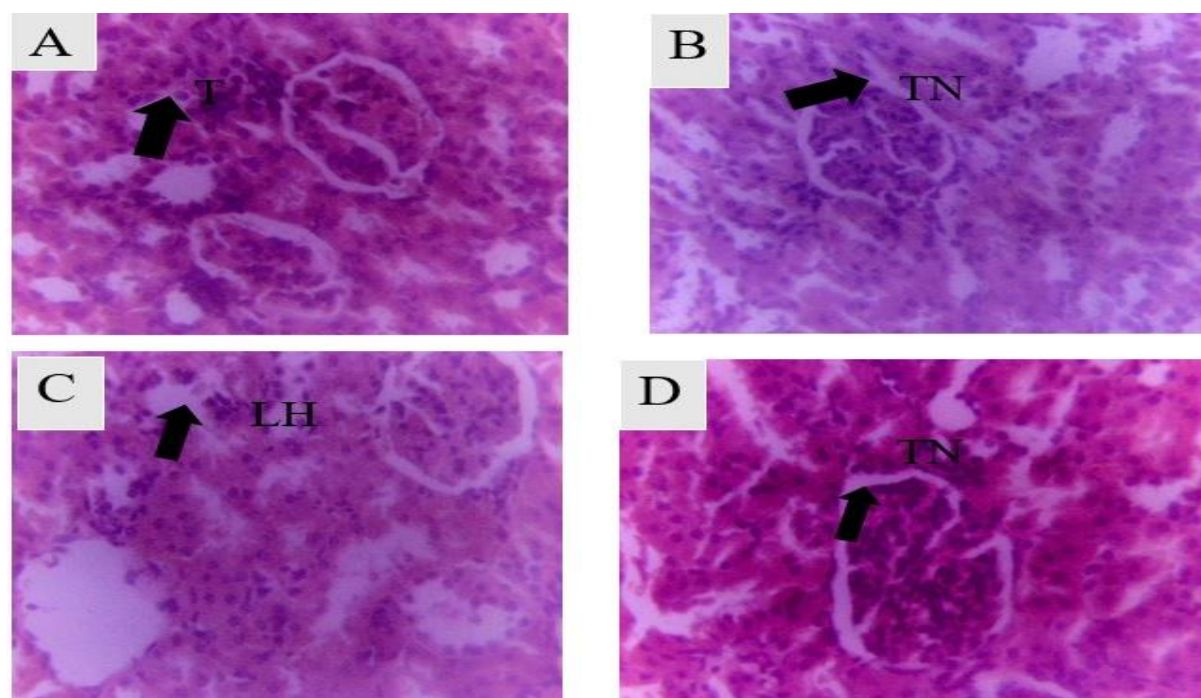
Organs	Treatment Groups			
	Control	250 mg/kg	500 mg/kg	1000 mg/kg
Liver	3.78±0.14	3.22±0.12	3.41±0.13	3.73±0.14
Kidney	0.74±0.05	0.56±0.05	0.63±0.04	0.66±0.05
Lungs	0.97±0.19	0.71±0.11	0.69±0.06	0.69±0.06
Heart	0.42±0.04	0.36±0.06	0.36±0.02	0.41±0.02
Spleen	0.55±0.10	0.39±0.02	0.45±0.03	0.49±0.06
Stomach	1.28±0.12	1.10±0.05	1.21±0.10	1.18±0.07

Values are expressed as Mean ± S.E.M; One way ANOVA followed by Bonferroni post hoc test, n=6, METD = Methanol extract of *Tapinanthus dodoneifolius*



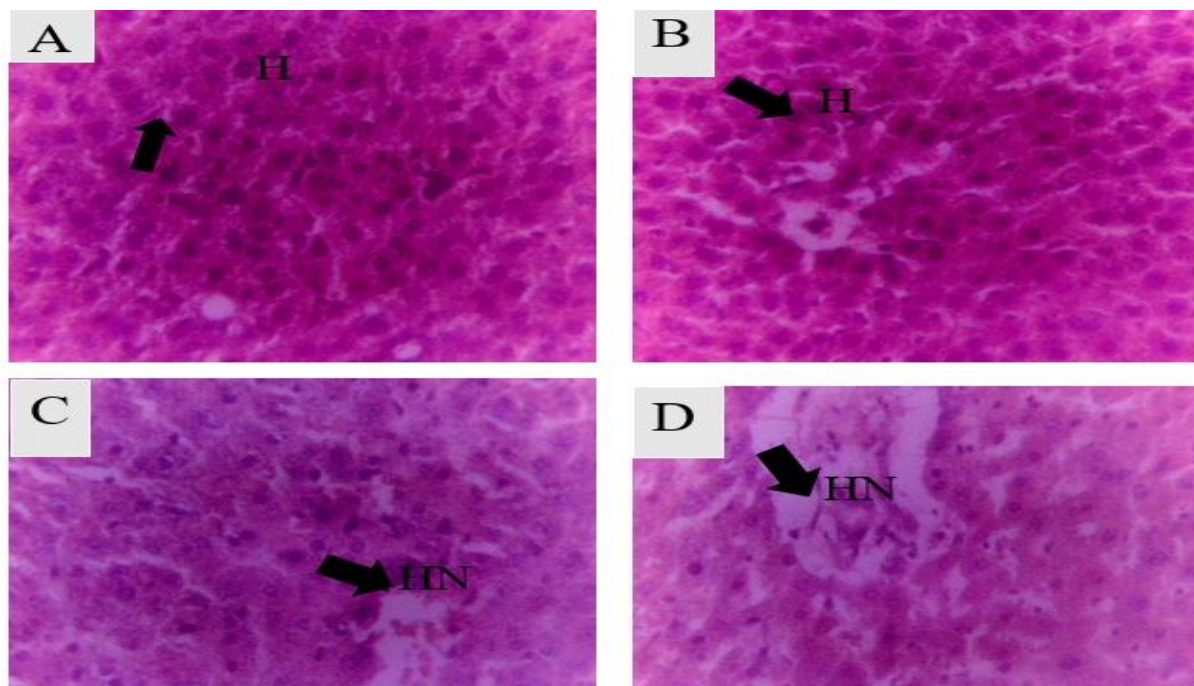
**Figure 2: Photomicrographs of Heart Sections (H & E stained at  $\times 250$  magnification) of Rats Following 28 days oral administrations of Methanol Extract of *Tapinanthus dodoneifolius***

A (Control); B (250 mg/kg); C (500 mg/kg); D (1000 mg/kg) Sections showing normal cardiac muscles (M)



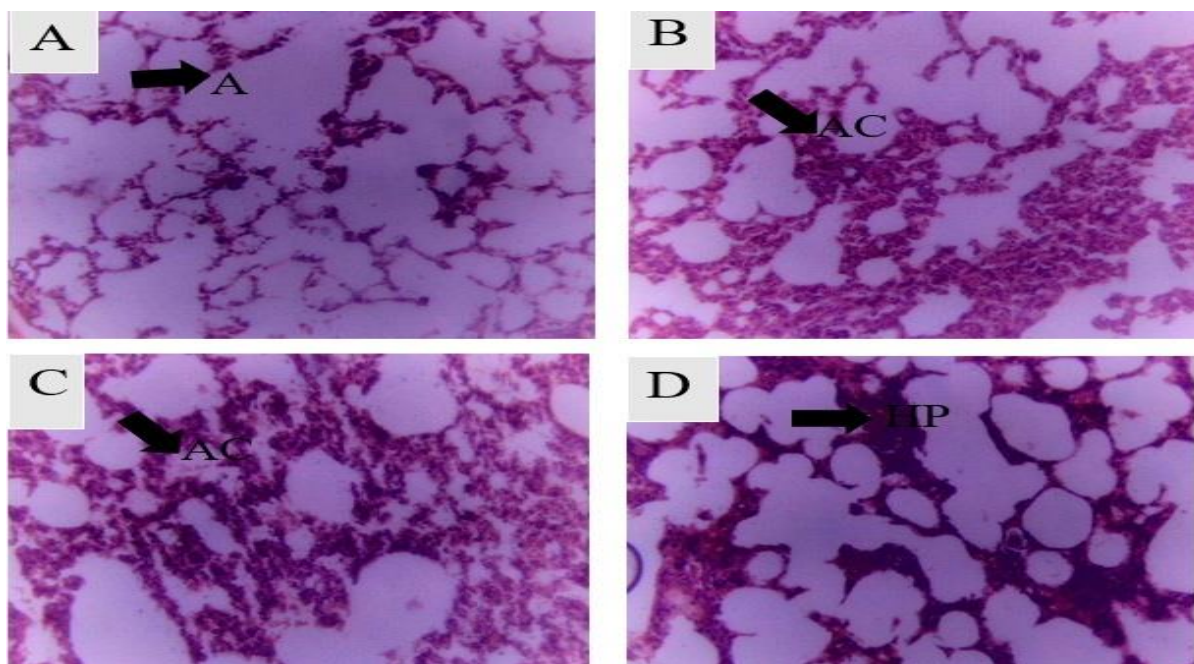
**Figure 3: Photomicrographs of Kidney Sections (H & E stained at  $\times 250$  magnification) of Rats Following 28 days Oral Administrations of Methanol Extract of *Tapinanthus dodoneifolius***

A (Control); B (250 mg/kg); C (500 mg/kg); D (1000 mg/kg) T (Normal kidney tubules and glomerulus); TN (Slight tubular necrosis); LH (Lymphocyte hyperplasia)



**Figure 4: Photomicrographs of Liver Sections (H & E stained at ×250 magnification) of Rats Following 28 days Oral Administrations of Methanol Extract of *Tapinanthus dodoneifolius***

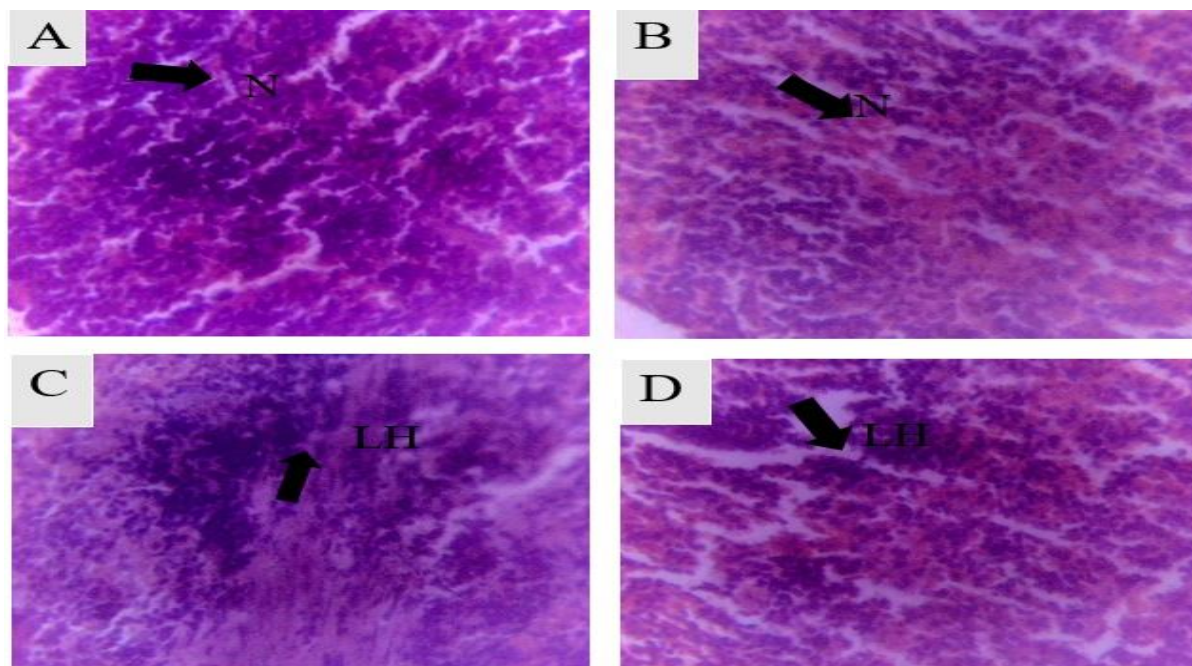
A (Control); B (250 mg/kg); C (500 mg/kg); D (1000 mg/kg) H (Normal hepatocytes); HN (moderate hepatocyte necrosis)



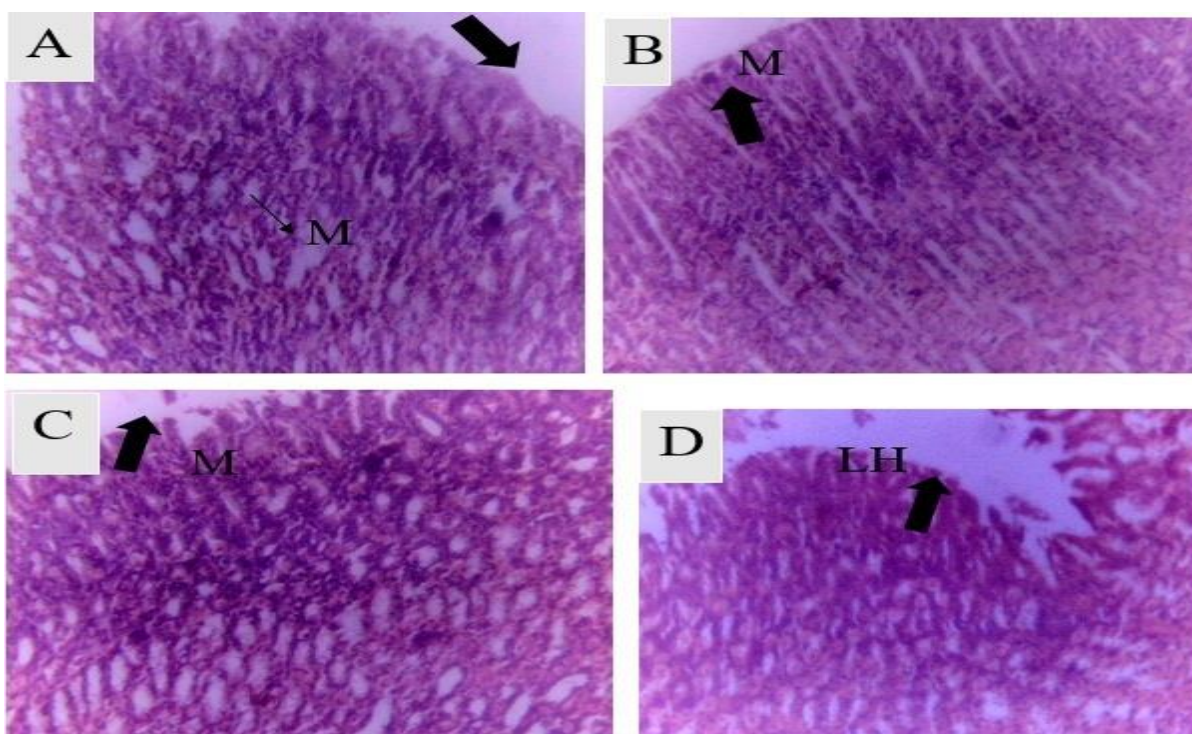
**Figure 5: Photomicrographs of Lung Sections (H & E stained at ×250 magnification) of Rats Following 28 days Oral Administrations of Methanol Extract of *Tapinanthus dodoneifolius***

A (Control); B (250 mg/kg); C (500 mg/kg); D (1000 mg/kg) A (normal lung alveoli); AC (slight alveoli congestion); HP (nuclei hardening and pyknosis)





**Figure 6: Photomicrographs of Spleen Sections (H & E stained at  $\times 250$  magnification) of Rats Following 28 days Oral Administrations of Methanol Extract of *Tapinanthus dodoneifolius***  
 A (Control); B (250 mg/kg); C (500 mg/kg); D (1000 mg/kg) N (normal red and white pulp); LH (Lymphocyte hyperplasia)



**Figure 7: Photomicrographs of Stomach Sections (H & E stained at  $\times 250$  magnification) of Rats Following 28 days Oral Administrations of Methanol Extract of *Tapinanthus dodoneifolius***  
 A (Control); B (250 mg/kg); C (500 mg/kg); D (1000 mg/kg) M (normal mucosa epithelium of the stomach); LH (lymphocyte hyperplasia)

#### 4. DISCUSSION

The use of medicinal plants like *Tapinanthus dodoneifolius* in the treatment of human diseases has enjoyed global acceptability [13]. Comprehensive knowledge about the safety of the plant is yet to be examined despite numerous pharmacological beneficial activities. Therefore, the present study was carried out to ascertain the acute and sub-acute toxicity of *Tapinanthus dodoneifolius* in experimental animals. The methanol whole plant extract of *Tapinanthus dodoneifolius* was found to be non-toxic since there was no sign of toxicity and mortality at 5000 mg/kg. The phytochemical constituents such as alkaloids, cardiac glycosides, saponins, tannins, flavonoids, steroids/triterpenes, terpenoids and carbohydrates present in the extract may be responsible for the observed pharmacological activity [14]. A non-significant increase in body weight (Figure 1) was observed which may be due to the nutritional benefit of the plant extract [15]

Haematological parameters are used to ascertain the level of safety of compounds or extract when exposed to human [16, 17] and has been used to explain haematologically-related functions [18]. Changes in haematological parameters are necessary to risk examination due to their predictive values of toxicity [19]. The non-significant changes in RBC, HCT, Hb, and PLT level by METD (Table 1) show that the plant does not affect erythropoiesis and osmotic fragility of the red blood cells [20]. The decrease in the level of monocytes and granulocytes could suggest that the extract may exert toxic effect on the immune system and cause agranulocytosis at dose of 1000 mg/kg and above. The white blood cells (WBC) are the first line of defence which responds to infectious agents, inflammatory process or tissue injury.

The methanol whole plant extract of *Tapinanthus dodoneifolius* did not show significant changes in AST and ALT enzymes (Table 2), ALP was decreased significantly at dose of 1000 mg/kg suggesting inhibition of the enzyme by the extract.

The serum biochemical assay is used to assess renal and hepatic functions in order to find possible pathological changes due to the extract. Renal and hepatic function analysis is highly useful in the toxicity screening of compounds including medicinal plants as both are important for the excretion and metabolism of drugs by an organism [21]. The liver contains host of enzymes (ALT, AST, ALP) that serve as biochemical markers of liver damages. Once there is a liver damage, these enzymes leak into the serum and show increased activities [22].

The extract at all doses tested could not significantly alter the level of total protein (Table 2). This implies that it is non-toxic to the kidneys and liver. Serum protein and albumin are sensitive indicators of liver function. They are synthesized and metabolized in the liver and serve as a means of assessing hepatocellular injury [23]. Total protein measurement reflects nutritional status and may be used to screen and help diagnose kidney, liver disease and many other conditions. Low total protein levels suggest a liver, kidney disorder or any other condition in which protein is not digested or absorbed properly. High total protein level may be seen with chronic inflammation or liver infections [24].

Measurements of urea, creatinine and uric acid are used to assess renal dysfunction and their normal values indicate absence of renal damage [25]. From the result obtained (Table 3), there was no significant difference among the extract treated groups compared to the control. This suggests that the extract does not seem to possess toxic effect on the kidney. Decrease in organ weight is an expression of toxicity resulting from exposure to toxic substances [26]. The liver; kidney, lungs, stomach, spleen and heart were not adversely affected compared to the control group, throughout the 28 day treatment period (Table 4). Hence, the whole plant extract is nontoxic to the organs in the sub-acute study.

There were no any adverse abnormalities in their gross examinations of the heart, kidney, stomach, spleen, lungs and liver isolated in various group. The histological studies on these organs by the extract did not reveal any pathological changes after treatment compared to the control when administered for 28 days as seen in plate figures 2, 3, 4, 5, 6 and 7.

#### 5. CONCLUSION

The methanol whole plant extract of *T. dodoneifolius* does not seem to possess the toxic effects that could affect its ethnomedicinal use.

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#### CONFLICT OF INTERESTS

There are no conflicts of interest.

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