

# Effect of Methanolic Leaf Extract of *Talinum triangulare* (Jacq.) Willd. on Biochemical Parameters in Diet induced Dyslipidemia Wistar Rats

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## ABSTRACT

**Objective:** To investigate the effect of methanolic leaf extract of *Talinum triangulare* on hematological parameters, enzymatic and non-enzymatic antioxidant status, and serum lipid in Wistar rats fed standard laboratory, or 2% cholesterol-enrich diet. **Material and Methods:** Wistar rats (180-210g) divided into six groups of six animals (males) each were fed 2% cholesterol-enriched diet and orally treated with 0.9% saline or extract of *Talinum triangulare* (250, 500, and 1000 mg/kg per body weight) daily for eight weeks. Lipid profile, lipid peroxidation (MDA), hematological parameters, and their functional indices and serum antioxidant enzymes (catalase, glutathione -S-transferase, and superoxide dismutase) activities and glutathione status were assessed in normal and diet-induced hypercholesterolemic extract treated rats and compared with the rats treated with 100 mg/kg per bwt standard drug gemfibrozil. **Results:** A significant ( $P < 0.05$ ) increase in lipid profile (total glyceride, total cholesterol, low-density lipoprotein, and very low-density lipoprotein), MDA and reduction ( $P < 0.05$ ) in enzymatic and nonenzymatic antioxidant status coupled with alterations in hematological parameters was observed in the serum of hypercholesterolemic rats when compared with animals on a normal diet. Coadministration of methanolic leaf extracts of *Talinum triangulare* or gemfibrozil significantly ( $P < 0.05$ ) restored the elevated serum lipid profile, MDA, and the deranged hematological parameters to near normal. The extract also protected against hypercholesterolemic-induced diminished enzymatic and nonenzymatic antioxidant status. The activities of the plant extract are dose (250, 500, and 1000 mg/kg) dependent and it compared favorably with the standard drug gemfibrozil. **Conclusion:** The present study suggested that the extract of *Talinum triangulare* might protect against hypercholesterolemic-induced altered lipid profiles, oxidative stress, and also improve the status of antioxidant defense system and hematopoiesis.

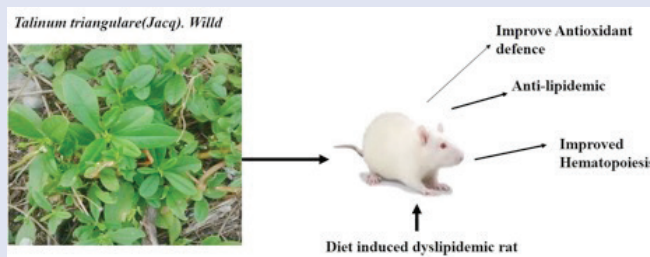
**Key words:** antioxidant, anti-lipidemia, antioxidant enzymes, hematopoiesis, *Talinum triangulare*

## SUMMARY

- Elevated lipid profile (total glyceride, total cholesterol, low-density lipoprotein, and very low-density lipoprotein), lipid peroxidation (MDA), and reduced enzymatic and nonenzymatic antioxidant status coupled with alterations in

hematological parameters was observed in the serum of hypercholesterolemic rats when compared with animals on a normal diet

- Coadministration of methanolic leaf extracts of *Talinum triangulare* significantly ( $P < 0.05$ ) restored the elevated serum lipid profile, MDA, and the deranged hematological parameters to near normal.
- The extract also protected against hypercholesterolemic-induced diminished enzymatic and nonenzymatic antioxidant status.
- The activities of the plant extract was dose-dependent and it compared favorably with the standard drug gemfibrozil.



**Abbreviation used:** Lipid peroxidation (MDA), catalase (CAT), glutathione-S-transferase (GST), superoxide dismutase (SOD), glutathione (GSH), Thrombocytes indices (PLT), Red blood cell (RBC), Packed cell volume (PVC), Mean corpuscular hemoglobin (MCH), Mean corpuscular hemoglobin concentration (MCHC), Total glyceride (TG), Very low density lipoprotein (VLDL), Total cholesterol (TC), Low density lipoprotein (LDL), High density lipoprotein (HDL) and 3-Hydroxy-3-methylglutaryl-CoA reductase (HMG-CoA).

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## INTRODUCTION

The prevalence of cardiovascular diseases (CVD) is assuming an alarming rate worldwide. It was reported that by 2020 CVD it will account for, approximately, 40% of all global deaths.<sup>[1]</sup> Recent studies have revealed that dyslipidemia,<sup>[2,3]</sup> inflammation,<sup>[4]</sup> and increased free radicals/reactive oxygen species generation<sup>[2]</sup> play a critical role in the etiopathogenesis and progression<sup>[5]</sup> of cardiovascular diseases.<sup>[6]</sup> Hyperlipidemia or dyslipidemia is caused by derangement in lipid biosynthesis or catabolism<sup>[7]</sup> and is characterized by elevated blood low-density lipoprotein (LDL), very low-density lipoprotein (VLDL), triglycerides, and decrease high density lipoprotein (HDL) cholesterol

level.<sup>[2]</sup> Excessive lipids (Hypercholesterolemia or hypertriglyceridemia)

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within the arterial wall are recurring decimals in the development of cardiovascular diseases such as atherosclerosis.<sup>[8]</sup> One possible mechanism by which hyperlipidemia contributed to the development of cardiovascular diseases involved the generation of free radicals [malondialdehyde (MDA) and conjugated dienes] and decrease antioxidant enzyme (superoxide dismutase, catalase and glutathione peroxidase) activities and nonenzymatic antioxidants (vitamin C and E) status.<sup>[9]</sup> It is an established fact that most of the orthodox drugs (statins and fibrates) commonly employed in the treatment of cardiovascular diseases are tailored to correct the deranged blood lipid profile, whereas some of them act by inhibiting the biosynthesis of cholesterol or enhancing the clearance of triglyceride. However, due to the toxicity and high cost of procurement,<sup>[10]</sup> attention of the world has shifted to the use of medicinal plants. One of the plants commonly use in Ogbomoso for the treatment of cardiovascular-related diseases is *Talinum triangulare* (Jacq). Willd.

*Talinum triangulare* (water leaf) a vegetable, usually consumed in Nigeria is an herbaceous, perennial, coalescent, and a glabrous plant.<sup>[11]</sup> It is widely distributed among the Southern ecological zones and its leaf is used as a softener of other vegetable species in vegetable soup.<sup>[12,13]</sup> The alcoholic root extract is traditionally used to treat hypertension in Ogbomoso Southwest region of Nigeria. "Gbure" as it's locally called is employed in the management of cardiovascular diseases like stroke and obesity.<sup>[14]</sup> In spite, of the reported local use of the *Talinum triangulare* in the management of cardiovascular diseases, there is still a paucity of information on its antilipidemic, antioxidant potential, and effect on blood parameters. Therefore, the present study was designed to investigate the effect of leaf extract *Talinum triangulare* (Jacq). Willd. on lipid profile, oxidative stress and hematological indices in animal model.

## MATERIALS AND METHODS

### Sample collection, preparation, and extraction

The leaf of *Talinum triangulare* (figure 1) was collected from Ogbomoso, Oyo State, Nigeria and were identified by a Taxonomist Dr A.T.J, Ogunkunle, of the Department of Pure and Applied Biology of Ladoko Akintola, University of Technology Ogbomoso, Oyo State, Nigeria and a voucher specimen was deposited (DSO 075) at the University Herbarium. Leave preparation was done according to the method described by<sup>[13]</sup> with slight modification. Briefly, the leaves were destalked, washed, and oven dried at 30°C with continuous turning for 3 days to avert fungal growth. The leaves were later grounded into fine powder using an electric blender (Waring Products Division, Torrington, USA) and kept in well labeled airtight containers and stored in the refrigerator. Two hundred grams (200 g) of coarse powder was extracted by using methanol in Soxhlets apparatus. The extract was concentrated to a semisolid alcohol-free material and final extract yield was 16.99% [based on dry material, Dry plant sample (200)/dry extract (33.90) x 100 = 16.99%]. The extract was kept in the refrigerator until when needed.

### Animals

Male Wistar albino rats (180–210) g were used for the present study. They were maintained at standard laboratory conditions (temperature 22 ± 1°C: photoperiod: 12 h light and dark cycle each throughout the experimental period; humidity: 45–50%) and fed with standard commercial rats pellet diet (Alanko feeds, Mokola, Ibadan) water ad libitum. The experiment was carried out after its approval by the Faculty of Basic Sciences, Animal Ethics Committee, Ladoko Akintola University of Technology, Ogbomoso, Oyo State, Nigeria, in accordance with the "Principles of Laboratory Animal Care" from NIH Publication No.85–23.

### Cholesterol supplemented diet

Hypercholesterolemia was induced according to the method described by Onody *et al.*<sup>[15]</sup> and Olorunnisola *et al.*<sup>[16]</sup> Briefly, cholesterol (2% w/w) powder was thoroughly mixed with crushed pellet diet and reconstituted with water and allowed to dry properly to prevent microbial contamination.

### Experimental design

Thirty-six male rats were divided into six groups (six animals each) after 2 weeks of acclimatization.

**Group 1:** animals were fed with a standard diet and was given 0.9% saline once daily for 8 weeks with the aid of oropharyngeal cannula.

**Groups 2:** animals served as hypercholesterolemic (fed with 2% w/w pure cholesterol enriched diet) negative control.<sup>[17]</sup>

The animals in group 3, 4 and 5 were fed with 2% w/w pure cholesterol enriched diet supplemented orally with 1 ml of the extract corresponding to 250, 500, and 1000 mg/kg per bwt (LD<sub>50</sub>>2500.<sup>[18]</sup>), respectively, once daily for 8 weeks.

Group 6 were fed with 2% w/w pure cholesterol enriched diet and supplemented orally with 1 ml of gemfibrozil (100 mg/kg per bwt) once daily for 8 weeks.

### Biochemical assay

All the animals from each group were euthanized using ether anesthesia 24 h after 8 weeks of daily doses of the methanolic extract and the standard drug. Blood samples obtained by cardiac puncture were rapidly centrifuged at 3,000 rpm for 15 mins at 4°C. The serum obtained was stored at -20°C before analysis. Serum total cholesterol, triglyceride, LDL, HDL, MDA concentrations were determined using commercial kits (Sigma, USA) and analysis were carried out according to the instructions of the manufacturer.

### Evaluation of antioxidant enzymes activities

#### Determination of catalase activity

Catalase was assayed according to the method described by Cohen and Dembiec.<sup>[19]</sup> Briefly, 1.8 ml of 30 mmol/l H<sub>2</sub>O<sub>2</sub> was added to 2 ml (200 µl) of serum or serum and extract sample, Reagent buffer was used as the blank and their absorbance readings were taken at 240 nm, at 60 s intervals for 5 min.

#### Determination of superoxide dismutase activity

Superoxide dismutase (SOD) enzyme activity was determined according to the method described by Sun and Zigman.<sup>[20]</sup> The SOD enzyme assay determines the difference between superoxide anion decomposition and production that is, its ability to inhibit the autoxidation of epinephrine. The assay was performed in 3.0 ml of 50 mmol/l Na<sub>2</sub>CO<sub>3</sub> buffer (in two different test tubes) to which 0.02 ml of each serum sample was added. As 0.03 ml of the epinephrine stock solution was then added to the above before taking absorbance readings at 480 nm for 3–5 min.

#### Determination of Serum glutathione-S-transferase activity

The activity of glutathione-S-transferase (GST) was determined according to the method of Habig *et al.*<sup>[21]</sup> 0.1 ml of CDNB=1-Chloro 2, 4- 2,4-Dinitrochlorobenzene solution was pipetted into a conical flask before adding 1 ml of phosphate buffer and 1.7 ml of distilled water. Next, the mixture was incubated at 37°C for 5 min. After the incubation, 0.1 ml of the serum sample and also 0.1 ml of glutathione (GSH) solution were added. A blank of serum was prepared for control.

### Determination of reduced glutathione activity

Reduced GSH level was determined by the method of Ellman modified by Jollow *et al.*<sup>[22]</sup> 0.5 ml 10% sulphosalicylic acid was added to mixture of 0.4 ml homogenate and 0.6 ml of distilled water as protein precipitant. As 0.5 ml of supernatant was mixed with the reaction mixture of 4.5 ml of 0.5 mol/l Tris-buffer and 0.5 ml of 10 mmol/l DTNB= 5,5'-dithio-bis-[2-nitrobenzoic acid and the absorbance was measured immediately at 412 nm. The GSH contents were calculated using GSH as standard and expressed as mmol/l/g tissue.

### Estimation of lipid peroxidation

The thiobarbituric acid (TBARS) assay method<sup>[23]</sup> was used for the lipid peroxidation analysis. It is used to measure total free radical damage in a biological system; wherein malondialdehyde, the end product of lipid peroxidation serves as a convenient index and can be measured spectrophotometrically at 535 nm to assay for the extent of lipid peroxidation in a sample. Briefly, 1 ml of serum sample was added to 2 ml of TCA-Trichloroacetic acid, TBA-HCl= Thiobarbituric acid and Hydrochloric acid reagent (1 : 1 : 1) in a test tube and mixed thoroughly. The mixture was then heated for 15 min in a boiling water bath and cooled. After cooling, the flocculent precipitate was removed by centrifugation at 1000 g for 10 min. The absorbance of the sample was then determined at 535 nm against a blank.

### Determination of hematological parameters

Hematological parameters such as red blood cells (RBC), hemoglobin, packed cell volume (PCV), White blood cell (WBC), neutrophils, monocytes, lymphocytes, eosinophils, basophils, platelet, and its related indices [mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC)] following manufacturer's instruction were analyzed using Horiba ABX 80 Diagnostics (ABX penetrates Montpellier, France).

### Statistical analysis

Data were expressed as (mean  $\pm$  SD) of five replicates and were subjected to one way analysis of variance. Means were separated by the Duncan multiple test using SAS = Statistical Analysis System). Values were considered statistically significant at *P* value less than 0.05.

## RESULTS

### Effect of *Talinum triangulare* leaves extract on lipid profile in serum of normal and hypercholesterolemic rats

Table 1 show the effect of high cholesterol diet on total cholesterol, triglycerides, LDL, VLDL, and highdensity lipoprotein cholesterol HDL in the serum of normal and hypercholesterolemic rats. A significant (*P* < 0.05) increase in TC, TG, LDL, VLDL, and decrease (*P* < 0.05) HDLc concentration was observed in rats fed with high cholesterol diet (group 2) when compared with animals fed with a standard diet (group

1). Coadministration of methanolic extract of *Talinum triangulare* at 250, 500 and, 1000 mg/kg per bwt and the standard drug (group 3, 4, 5, and 6, respectively) in rats fed with high cholesterol diet significantly (*P* < 0.05) restored the abnormal lipid profile (group 2) in a dose-dependent manner to near normal levels. The activities of the extract at 500 and 1000 mg/kg per bwt compared favorably with standard drug.

### Effect of graded doses of *Talinum triangulare* leaves extract on antioxidant status in serum of rats fed with high cholesterol diet

[Figure 1] showed the effect of *Talinum triangulare* on the activities of antioxidant enzymes in the serum of normal and diet-induced hypercholesterolemic rats. The results revealed a significant (*P* < 0.05) decrease in the activities of SOD, catalase (CAT), GST, level of GSH, and a significant (*P* < 0.05) increase in the level of TBARS in diet-induced hypercholesterolemic rats (group 2) compared with the normal control (group 1). Coadministration with methanolic extract of *Talinum triangulare* (250, 500, and 1000 mg/kg bwt) and Gemfibrozil (100 mg/kg per bwt) for 8 weeks [Table 2] caused a significant (*P* < 0.05) increase in the activities of SOD, CAT, GST, GSH, and significant decrease (*P* < 0.05) in the level of TBARS in a dose-dependent manner. Although the extract caused an increase in the antioxidant enzyme activities, and reduced lipid peroxidation (TBARS) at all doses considered, the activities of the extract only compared favorably with the standard drug at the highest concentration (1000 mg/kg per bwt).

### Effect of graded doses of *Talinum triangulare* leaves extract on hematological parameters of rats fed with high cholesterol diet

At the end of the period of administration, significant (*P* < 0.05) decrease in RBC, PVC, and neutrophil concentration and significant

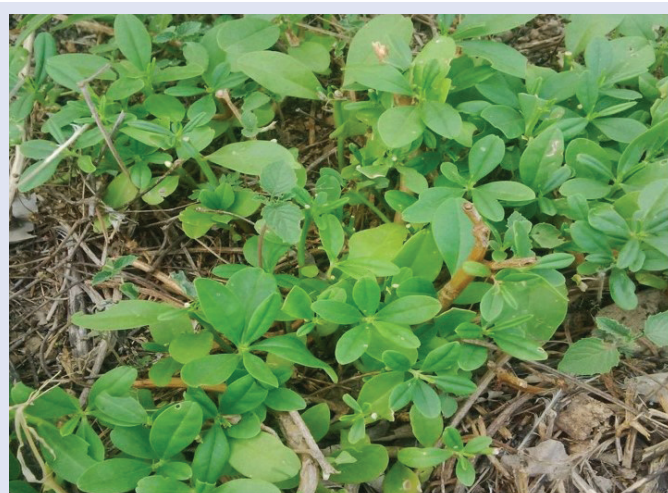


Figure 1: *Talinum triangulare* plant in its natural habitat

Table 1: The effect of graded doses of extract of *Talinum triangulare* on lipid profile in serum of rats on high cholesterol diet.

Sample	Treatment (mg/kg/bw.t).					
	Control	HCD	HCD + 250 TT	HCD + 500 TT	HCD + 1000 TT	HCD + 100 Gb
TC	11.40 $\pm$ 0.25	25.11 $\pm$ 0.40 <sup>a</sup>	19.42 $\pm$ 0.13 <sup>a</sup>	14.33 $\pm$ 0.42 <sup>a</sup>	13.69 $\pm$ 0.22 <sup>a</sup>	12.13 $\pm$ 0.12
TG	4.10 $\pm$ 0.11	11.01 $\pm$ 0.15 <sup>a</sup>	9.08 $\pm$ 0.24 <sup>a</sup>	6.85 $\pm$ 0.31 <sup>a</sup>	5.01 $\pm$ 0.41	4.16 $\pm$ 0.16
LDL-c	3.29 $\pm$ 0.14	10.06 $\pm$ 0.21 <sup>a</sup>	7.04 $\pm$ 0.27 <sup>a</sup>	4.01 $\pm$ 0.22 <sup>a</sup>	4.67 $\pm$ 0.33	4.54 $\pm$ 0.21
HDL-c	4.01 $\pm$ 0.22	2.05 $\pm$ 0.31 <sup>a</sup>	3.30 $\pm$ 0.45 <sup>a</sup>	3.37 $\pm$ 0.50	4.01 $\pm$ 0.30	3.43 $\pm$ 0.15

<sup>a</sup> Test values (mean  $\pm$  SD) carrying superscripts differ from the control across each parameter are significantly different (*p* < 0.05). HCD - high cholesterol, TT-*talinum triangulare*, TC - total cholesterol, TAG - triacylglycerol, LDL-C-low density lipoprotein, HDL-C- density lipoprotein cholesterol. Gb- Gemfibrozil.



( $P < 0.05$ ) increase in Hb, MCV, MCH, WBC, lymphocytes, and platelets counts were observed in rats fed with cholesterol rich diet when compared with control. Oral administration of the methanolic extract of *Talinum triangulare* significantly improved the deranged hematological parameters in dose-dependent manner. The activity of the extract was more pronounced at 500 and 1000 mg/kg per bwt, respectively, and it compared favorably with the standard drug at these concentrations [Table 2].

## DISCUSSION

The development of cardiovascular diseases has been attributed to hyperlipidemia-induced oxidative stress.<sup>[15,24]</sup> Hypertriglyceridemia and hypercholesterolemia-induced oxidative modification of LDL, and other macromolecule with a resultant increase in production of lipid peroxidation products.<sup>[8]</sup> In the present study, we found that high-cholesterol diet leads to significant ( $P < 0.05$ ) increase in serum total cholesterol, LDL, VLDL, triglyceride levels, and a significant ( $P < 0.05$ ) reduction in HDL-cholesterol levels when compared with the group of animals on normal diet [Table 1]. This result is consistent with the report of Shivali *et al.*<sup>[25]</sup> and Olorunnisola *et al.*<sup>[16]</sup> The observed abnormal high serum lipid profile may be due to defects in systemic lipid homeostasis, which involved the redistribution of lipoproteins, triacylglycerol for storage, and utilization by peripheral tissues.<sup>[25-28]</sup> Coadministration of the methanolic extract of *Talinum triangulare* produced a significant ( $P < 0.05$ ) dose-dependent reduction in the elevated levels of total cholesterol, triglyceride, LDL, and VLDL and a significant increase in serum level of HDL-C. The observed anti-lipidemia effect of the extract at 1000 mg/kg. per bwt, compared favorably with the standard drug (Gemfibrozil). The likely antilipidemic effect of the plant extract may be due to the stimulatory effects of its sterols or phytochemicals (flavonoids, steroids, saponins, and anthocyanin)<sup>[13]</sup> on HMG-CoA reductase. HMG-CoA= 3-hydroxy-3-methyl-glutaryl-coenzyme A, an hepatic enzyme catalysis the rate limiting step in cholesterolbiosynthesis in the tissues and its activity closely correlateswith cholesterol-genes in the tissues. Other possible mechanism(s) of antilipidemic activity of the plant may be due to its bioactive agents such as flavonoids that stimulate the activation of AMP= Adenosine Monophosphate-activated protein kinase and serum and hepatic lipoprotein lipase, Enzymes, which has been implicated in the control of fatty acid biosynthesis and oxidation

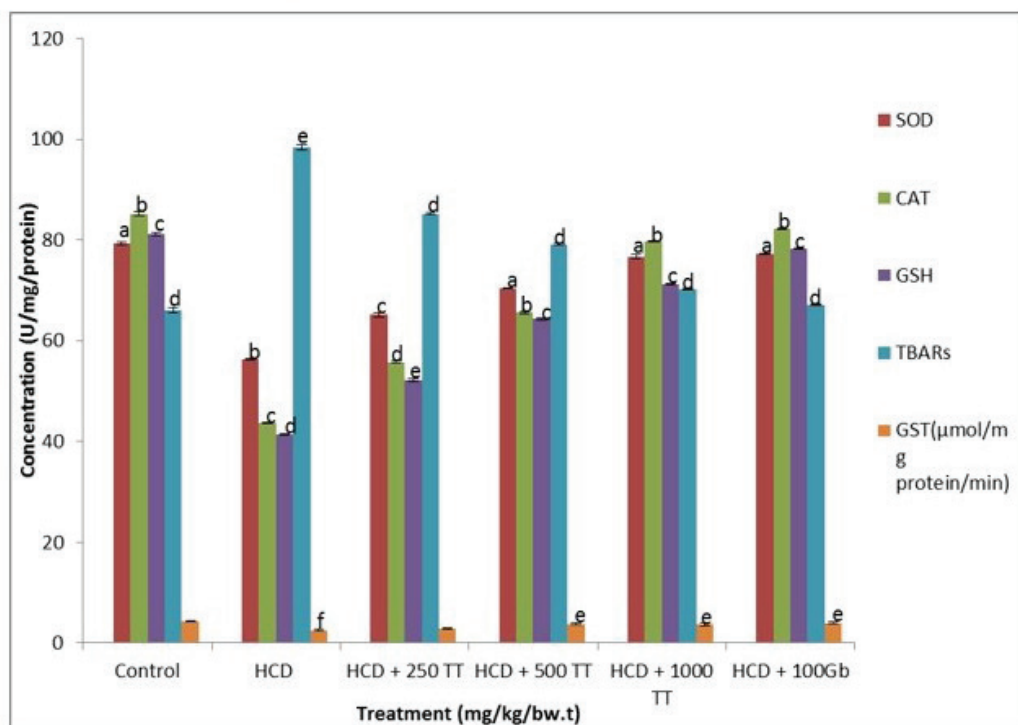
and clearance of both LDL and VLDL from the serum, respectively.<sup>[29]</sup> The above phytochemicals possess antiatherogenic activity,<sup>[30]</sup> also it was reported that plant steroids and triterpenoid reduce the absorption of cholesterol and demonstrate cardio protective ability in experimental hyperlipidemia rats, respectively.<sup>[31,32]</sup> These results suggested that *Talinum triangulare* could be helpful in decreasing hypercholesterolemia-induced diseases such as atherosclerosis and myocardial infarction. The actual mechanism (s) of antilipidemic effect of the plant will be explored in future study [Figure 2].



**Figure 2:** Dried and grounded leaf of *Talinum triangulare*

**Table 2:** The effect of methanolic extract of TT on red blood cells, white blood cells and the differentials in diet induced hyperlipidemia rats ( $n = 5$ , mean  $\pm$  SD).

Parameters	Treatment (mg/kgw bw.t)					
	Control	HCD	HCD + TT 250	HCD+ 500TT	HCD+TT 1000	HCD + Gb 100
RBC (x 10 <sup>6</sup> / $\mu$ L)	7.15 $\pm$ 0.32	5.98 $\pm$ 0.13 <sup>a</sup>	6.01 $\pm$ 0.11 <sup>a</sup>	6.55 $\pm$ 0.20	6.89 $\pm$ 0.23	7.10 $\pm$ 0.15
Hb (g/dl)	13.6 $\pm$ 0.47	8.23 $\pm$ 1.28 <sup>a</sup>	11.20 $\pm$ 0.20	12.50 $\pm$ 0.13	12.99 $\pm$ 0.12	13.01 $\pm$ 0.21
PCV (%)	37 $\pm$ 1.03	30.0 $\pm$ 0.55 <sup>a</sup>	35.0 $\pm$ 1.22	35.6 $\pm$ 0.14	36.5 $\pm$ 0.22	36.9 $\pm$ 0.31
MCV (pg)	92 $\pm$ 0.22	71.1 $\pm$ 0.11 <sup>a</sup>	89.1 $\pm$ 0.21	90.0 $\pm$ 0.33	91.23 $\pm$ 0.44	91.98 $\pm$ 0.41
MCH (pg)	30.2 $\pm$ 0.25	26.1 $\pm$ 0.22 <sup>a</sup>	27.91 $\pm$ 1.23	28.11 $\pm$ 0.43	29.01 $\pm$ 0.54	29.89 $\pm$ 1.01
MCHC (g/dL)	31.2 $\pm$ 0.35	27.2 $\pm$ 1.01 <sup>a</sup>	29.12 $\pm$ 0.24	30.14 $\pm$ 0.12	30.58 $\pm$ 0.31	30.98 $\pm$ 0.31
WBC (x 10 <sup>3</sup> / $\mu$ L)	7.67 $\pm$ 1.21	10.7 $\pm$ 0.10 <sup>a</sup>	9.72 $\pm$ 0.61	9.01 $\pm$ 0.30	8.01 $\pm$ 0.41	7.98 $\pm$ 0.50
Neutrophils (%)	55.2 $\pm$ 1.00	35.1 $\pm$ 1.24 <sup>a</sup>	40.21 $\pm$ 0.31	45.01 $\pm$ 0.18	50.98 $\pm$ 0.05	51.03 $\pm$ 0.40
Monocytes (%)	5.6 $\pm$ 1.02	5.92 $\pm$ 0.14 <sup>a</sup>	5.71 $\pm$ 0.19	5.90 $\pm$ 0.25	5.73 $\pm$ 0.32	5.70 $\pm$ 1.20
Lymphocyte (%)	30.9 $\pm$ 1.45	55.0 $\pm$ 0.15 <sup>a</sup>	47.02 $\pm$ 0.23	40.10 $\pm$ 0.40	34.30 $\pm$ 0.23	32.90 $\pm$ 0.19
Eosinophil (%)	2.0 $\pm$ 0.02	1.92 $\pm$ 0.31 <sup>a</sup>	1.89 $\pm$ 0.41	1.96 $\pm$ 0.51	1.89 $\pm$ 0.23	1.90 $\pm$ 0.33
Basophils (%)	0.26 $\pm$ 0.05	0.32 $\pm$ 0.21 <sup>a</sup>	0.31 $\pm$ 0.17	0.27 $\pm$ 0.23	0.25 $\pm$ 0.50	0.24 $\pm$ 0.31
Platelets (x10 <sup>3</sup> / $\mu$ L))	323.0 $\pm$ 1.12	650.0 $\pm$ 0.22 <sup>a</sup>	420.0 $\pm$ 0.54	390.0 $\pm$ 0.42	351.0 $\pm$ 0.20	340.0 $\pm$ 0.18



**GSH, TBARS, SOD, CAT, GST, HCD and Gb represent glutathione, thiobarbitric acid reacting substance, super oxide dismutase, catalase, glutathione-s-transferase, high cholesterol diet and Gemfibrozil respectively. <sup>a-e</sup> Test values (mean  $\pm$  SD) carrying superscripts differ from the control across each parameter are significantly different ( $p < 0.05$ )**

**Figure 3:** The effect of methanolic extract of *Talinum triangulare* in diet induced changes in antioxidant enzymes, GSH, GST and TBARS in the serum of hypercholesterolemic rats

Increase-free radicals generation and decrease antioxidant enzymes activities have also been reported in diet-induced hypercholesterolemic rats.<sup>[33]</sup> The formation of reactive oxygen species (ROS) is involved in the initiation and progression of various human diseases such as atherosclerosis, diabetes, cancer, and liver diseases.<sup>[34,35]</sup> ROS and lipid peroxidation products (MDA or conjugated dienes) play a critical role in the etiopathogenesis of atherosclerosis.<sup>[36,37]</sup> In the current study, the observed significant ( $P < 0.05$ ) decrease in serum antioxidant enzymes level (SOD, CA) GSH, GST, and the elevated lipid peroxidation product (TBARS) in rats fed with high cholesterol diet (Figure 1) was consistent with the report of Daryoush.<sup>[26]</sup> The decreased antioxidant enzyme activities in hypercholesterolemic rats may be due to the increased oxidative-induced damage occasioned by the high-fat diet.<sup>[38,26]</sup> Coadministration of methanolic extract of *Talinum triangulare* for eight weeks leads to a significant ( $P < 0.05$ ) increase in the activities of the serum antioxidant enzymes (SOD, CAT) GST, GSH, and a significant ( $P < 0.05$ ) reduction in lipid peroxidation product (TBARS) (Figure 1). The ability of *Talinum triangulare* to increase the activities of antioxidant enzymes and protect against hyperlipidemia-induced oxidative injury may be due to its strong free radical scavenging activities or the extract may support the upregulation of the synthesis of the antioxidant. This results suggested that *Talinum triangulare* may contain therapeutic agent(s), which could protect against hyperlipidemia-induced tissue injury and oxidative stress. This could, therefore, explain why *Talinum triangulare*

is employed as a therapeutic agent in the treatment of oxidative-induced stress pathological conditions.<sup>[39]</sup> Blood products and parameters are valuable tools for disease diagnosis, prevention, and treatment.<sup>[40,41]</sup> There appear to be a correlation between hematological parameters and cardiovascular diseases (atherosclerosis and heart diseases).<sup>[42]</sup> Experimental, clinical, and epidemiological evidence revealed that several hemostatic and hemorheological factors such as plasma viscosity, fibrinogen levels, hematocrit, RBC aggregation, and WBC might be involved in pathophysiology of atherosclerosis.<sup>[43,44]</sup> The significant ( $P < 0.05$ ) decrease in the concentration of RBC, PVC, and neutrophil [Table 2] observed in rats fed with cholesterol-rich diet in this study was in agreement with the report of Sodipo *et al.*<sup>[43]</sup> and Mohamed and Sherif.<sup>[45]</sup> They revealed that these parameters were significantly decreased in triton and high-fat diet-induced hyperlipidemic rats and rabbits respectively. However, our findings contradict the report of Choi and Pai<sup>[46]</sup> and Lee *et al.*,<sup>[47]</sup> they reported that there was no significant alteration in erythrocyte indices in participant with or without hyperlipidemia. The reduction in the erythrocytes parameters concentration in this study may be due to hyperlipidemia-induced oxidative stress leading to erythrocyte fragility and hemolysis.<sup>[48]</sup> Mohamed and Sherif,<sup>[45]</sup> corroborated the above statement when they reported that the reduced concentration of hemoglobin in rats fed with high-lipid diet was caused by hyperlipidemia, increased lipid peroxidation, and free radicals. The decreased in hemoglobin and RBC count lead to anemia<sup>[45]</sup> and this was reflected in

the low-PCV count [Table 2] observed in this study. Co-administration of methanolic extract of *Talinum triangulare* resulted in a dose-dependent increase ( $P < 0.05$ ) in RBC, hemoglobin, and PCV concentration [Table 2]. The activities of the extract compared favorably with the standard drug at the highest concentration. The improved values of RBC and hemoglobin suggested that *Talinum triangulare* may possess hematonic potential and it may act as blood enhancer. This observation is consistent with the report of Ezekwe *et al.*<sup>[49]</sup> reported that methanolic leaf extract of *Talinum triangulare* cause a significant increase in RBC concentration in normal rats. The observed hematonic potential of the extract may be due to the presence of flavonoid,<sup>[50]</sup> which has been reported to possess ability to stimulate the formation or secretion of erythropoietin in the bone marrow<sup>[51]</sup> and also improved level of MCH and MCHC.<sup>[52]</sup> The significant ( $P < 0.05$ ) increase in leucocytes (lymphocytes and WBC) and Thrombocytes indices (PLT) concentration observed in this study [Figure 3] agreed with the report of Huang *et al.*<sup>[53]</sup> According to Shurtz-Swirski *et al.*<sup>[54]</sup> and Ros. hypercholesterolemia promote generation of ROS, increased synthesis and release of inflammatory markers, which may enhance activation of WBC, increased platelets counts and promote the formation of platelets-leucocytes aggregates. These chains of biochemical reactions can contribute to the pathophysiological processes leading to increased risk of atherosclerosis and ischemic arterial diseases.<sup>[45]</sup> In addition, the elevated leukocyte count may also suggest low-grade inflammation occasioned by hyperlipidemia.<sup>[50]</sup> However, following the administration of graded doses of methanolic extract *Talinum triangulare* and the standard drug, the level of WBC, lymphocytes, monocytes, and platelets (PLT) were significantly ( $P < 0.05$ ) restored to near normal in dose-dependent manner. The activities of the extract compared favorably with the standard drug at the highest concentrations. The ability of *Talinum triangulare* leaf to positively influence the deranged hematological parameters may be due to its antilipidemic or antioxidant effect or it may contain thrombosis-inducing platelet alleviating factor. Our results, thus, suggested that *Talinum triangulare* extract may protect against elevated PLT-induced vascular disease like microangiopathy and macroangiopathy.

## CONCLUSION

We concluded that methanolic extracts of *Talinum triangulare* may protect against high-fat diet-induced dyslipidemia and oxidative stress through its antioxidant and antilipidemic properties. Further research work is currently going on in our laboratory to isolate and test for antioxidant, antilipidemic, and thrombosis-inducing platelet alleviating bioactive compounds.

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## Conflicts of interest

There are no conflicts of interest.

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