Tissue antioxidant enzyme activities and lipid profile of experimental rats fed with *Waltheria indica* leaf diets

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Abstract:
Hypercholesterolemia is a specific genetic condition characterized by excessively increased plasma cholesterol concentrations. Although treatments through statins have recorded exponential success over time, regrettably, several patients have yet to attain the minimum levels of low-density lipoprotein-cholesterol (LDL-Ch), evidenced by the high cardiovascular outcomes after treatment. Hence, this research aimed at evaluating the effect of *Waltheria indica* leaf diets on the lipid profiles and antioxidant activities in male albino rats weighing 40-45 g, which were grouped into three: Group A (basal group without cholesterol), Group B control (diet + 1% cholesterol) and Group C test diet (1% cholesterol + 10% *Waltheria indica* leaf sample) and fed for 21 days and weighed before sacrifice. After the last day of feeding, the Serum and liver were excised, and lipid profile and antioxidant enzyme activities were analyzed using standard methods. Serum total cholesterol (mg/dl) decreased significantly ($p<0.05$) from 83.88 ± 2.74 in control rats (group B) to 23.14 ± 1.05 in group C rats. Also, serum high-density lipoprotein (mg/dl) increased significantly ($p<0.05$) from 17.09 ± 0.71 in control rats to 33.25 ± 4.01 in group C rats. The serum malondialdehyde level decreased markedly from 8.68 ± 0.07 in group B rats to 5.74 ± 0.40 in group C rats. The activity of Serum Catalase significantly increased from 2.29 ± 0.15 in group B rats to 3.75 ± 0.13 in group C rats. This study demonstrates the lipid-lowering potential of *Waltheria indica* leaf diets and its protective effects against oxidative stress, which holds promise in managing and treating hypercholesterolemia.

Keywords: **Antioxidant, Hypercholesterolemia, Lipid profile, Total Cholesterol, *Waltheria indica*.**

1. Introduction

Hypercholesterolemia is a hereditary condition marked by elevated plasma cholesterol levels due to dysfunctional clearance of low-density lipoprotein (LDL) particles. Cholesterol levels higher than 240 mg/dl are frequently thought to pose twice the cardiovascular risk of those in the recommended range [1]. Cholesterol displays the ability to disrupt and rework the structure and functions of blood vessels due to its accumulation within the walls of the blood vessel. This accumulation can lead to dysfunctional endothelium and consequently result in plaques and lesion formation accompanied by a deteriorated healing process and proper ischemia management [2]. All lipoproteins carry cholesterol, but elevated levels of non-HDL cholesterol, particularly low-density lipoprotein-cholesterol (LDL-Ch), accompanied by simultaneously decreased levels of high-density lipoprotein-cholesterol (HDL-Ch), are markers for myocardial ischemia [3]. Also, increased triacylglycerol concentrations are considered detrimental as this is well correlated with poor cardiovascular outcomes [4]. Fat-mediated oxidative stress and a simultaneous decrease in the activity of antioxidant enzymes responsible for scavenging free radicals and protecting cell integrity have been reported to be associated with elevated fat levels [5]. Hypercholesterolemia increases the cholesterol pool, which can alter the physical properties of the cell membrane [6] and facilitate the leakage of reactive oxygen species (ROS) from the mitochondrial electron transport chain or activate nicotinamide adenosine dinucleotide phosphate (NADPH) oxidase [7]. These reactive radicals induce lipid peroxidation in the cell membrane, forming lipid peroxides and other free radicals.

More recently, phytomedicine and herbal medicine have gained maximum popularity due to their benefit in ameliorating health conditions and reducing toxicity [8]. Although therapeutic approaches to the regulation of cholesterol have greatly mitigated atherosclerotic events and associated cardiovascular disease [9], nonetheless, adverse effects associated with therapeutic drugs, such as liver damage, have been reported, and this proves to be a great concern [10]. Hence, the search for novel natural therapies for the control of cholesterol levels is necessary. The current research, therefore, sought to evaluate the cholesterol-lowering and antioxidant potentials of *Waltheria indica* (*W. Inidica*) leaf diets in experimental rats.

Waltheria indica has been recognized as a medicinal plant, also called sleepy morning, with a pan-tropical distribution [11]. They can be found throughout the tropics and warmer subtropics. It boasts of small, bright yellow fragrant flowers, and many plant parts are hairy. Phytochemical evaluation of *Waltheria indica* root, stem and leaf extracts indicated the presence of bioactive chemicals such as anthraquinones, saponins, tannins/phenols, flavonoids, alkaloids, and cardiac glycosides at varied concentrations.

The three evaluated plant parts exhibited comparatively higher concentrations of saponins and anthraquinones than other phytochemicals. Although cardiac glycosides and tannins were evident in the root, their concentrations were more pronounced in the leaf and root extracts [12]. Traditionally, *W. Indica* has

been used as a therapeutic agent in some parts of Africa, Hawaii and South America in the management of inflammatory reactions, seizures, and dysentery, to mention a few [13]. Furthermore, it has been employed in the treatment of cataracts due to its protection of the lens against naphthalene damage due to its free radical scavenging potential [14]. In Nigeria, various parts of *Waltheria indica* are commonly used to ameliorate varieties of infections in humans [15]. The plant extracts have been used as an ameliorative agent in combating bacterial infections [16]. Following its relevance in phytomedicine and its medicinal influence, this research was carried out to evaluate the effects of diet formulation containing *Waltheria indica* leaves on the lipid profile and tissue antioxidant enzyme activity of male albino rats.

II. Material and Method

Reagent and Equipment

The reagents used, including sodium carbonate, hydrogen peroxide, potassium phosphate buffer, ammonium molybdate, chloroform, and isopropanol during the experiment were analytical grade and obtained from the Department of Biochemistry, Faculty of Medicine, Ekiti State University, Nigeria. The apparatus used includes a conical flask, fennel, beaker, test tube, filter paper, warming blender, measuring cylinder, weighing balance, oven petri dish porcelain dish, desecrator, muffle furnace, poplin cloth, Kjeldahl flask and sixtieth extraction. They were all present in the same location.

Sample Preparation

Freshly harvested *Waltheria indica* leaves were harvested from a private farm in Itele, Ogun State, Nigeria, and identified at the Plant Science and Biotechnology Department, Ekiti State University, Ado-Ekiti. The leaves were cut into small pieces and air-dried for approximately two weeks. After drying, they were ground into a fine powder using a Lexus blender in the laboratory and stored in an airtight container.

Proximate Analysis

The proximate plant extract analysis followed the procedure described by [17]. The protein, fat, fibre, ash and dry matter were determined using the protocol.

Diet Formulation

The component of the diet used for the study consists of corn starch, skimmed milk, vegetable oil, cholesterol, vitamin-mineral mix and *Waltheria indica* powder in varying ratios per group, as can be seen in Table 1, which contains the diet component for each group with the basal group without cholesterol and *Waltheria indica*, the control group without cholesterol and the group C containing all diet with 1% cholesterol and 19% *Waltheria indica* leaf.

Group A: diet without cholesterol (basal)
Group B: diet + 1% cholesterol (control)
Group C: diet + 1% cholesterol + 10% *Waltheria indica* leaf

Experimental Animal

Twenty-four (24) male albino rats, weighing between 40g and 45g, were obtained from the animal house of the College of Medicine, Ekiti State University, Ado-Ekiti, Nigeria, and ethical approval was obtained. After a 5-day acclimation, the rats were weighed and randomly assigned into three groups of eight (8) rats each, where they were fed formulated diets for 21 days with the prepared feed consisting of the *Waltheria indica* Plant, vitamin and mineral, corn starch, skimmed milk, vegetable oil, cholesterol in varying proportion depending on the groups. The weight was monitored to examine growth. The animals were fasted overnight prior to the commencement of the experiments.

Preparation of Serum and Tissue Homogenate

Using chloroform, 24 rats were numbed and subsequently sacrificed via an incision of the jugular vein after fourteen days of feeding and administration. Blood samples were collected into EDTA and plain vials for serum and hematological analyses. Blood samples designated for serum were allowed to coagulate at room temperature for 30 minutes before being centrifuged at 3000xg for 15 minutes. Following centrifugation, the clot settled at the bottom, and the serum (supernatant) was carefully collected using a Pasteur pipette. The serum was then correctly labelled and stored in a freezer at -50°C until required for further analysis.

The animals were sacrificed, followed by liver isolation. The liver was sterilized, weighed and promptly kept under 0.25 M (1:5 w/v) solution of sucrose in ice-cold conditions. The liver was homogenized using a D160 homogenizer with subsequent storage under low temperatures for further analysis.

Biochemical Assays

Lipid peroxidation measured through malondialdehyde was determined in the serum and liver using standard diagnostic kits according to the method of [18]. Also, high-density lipoprotein (HDL), total cholesterol (T-Ch), triglycerides, very low-density lipoprotein (VLDL) and low-density lipoprotein LDL levels were estimated in the liver and serum using Randox lab kits. Friedewald`s equation was employed to estimate the Atherogenic index of the lipid profiles.

Catalase and Superoxide dismutase activities in serum and liver were also determined according to [19] and [20] methods, respectively. Methods by [21] were adopted to determine the activities of alkaline phosphatase (ALP), Aspartate transaminase (AST) and alanine transaminase (ALT).

Statistical Analysis

The data collected from the study were analyzed using a one-way analysis of variance (ANOVA), followed by Duncan's multiple range test to compare the experimental groups. Results are presented as mean ± standard deviation, with statistical significance at $p<0.05$.

III. RESULTS AND DISCUSSIONS

PROXIMATE ANALYSIS

Table 2 shows the proximate composition of Waltheria indica. The analysis shows that carbohydrate composition has the highest percentage with 41.01 ± 0.105, and crude lipid has the lowest composition with 2.31 ± 0.105. The results are consistent with that of Basiru et al. [22], who observed a similar proximate composition for Waltheria indica.

The high moisture observed in the plant sample proves it is prone to deterioration. The quantity of crude fat extracted revealed that the plant is more appealing because dietary fats promote food palatability by absorbing and retaining tastes. Fat contents are moderate and can be used for storage and transport forms of metabolic fuel.

Table 1: Diet formulation for control and experimental diets (g/kg)

DIET COMPONENTS (g/kg)	A	B	C
Corn Starch	537.50	527.50	427.50
Skimmed milk	312.50	312.50	312.50
Vegetable Oil	100	100	100
Cholesterol	-	10	10
*Vitamin-Mineral mix	50	50	50
Waltheria indica leaf	-	-	100

**Vitamin-Mineral mix (per 2.5kg): Folic acid 500 mg, Vit.B_{12} 10 mg, Vit.B_6 2,000 mg, Panthothenic acid 5,500 mg, Niacin 15,000, Vit.B_2 2,500 mg, Vit.B_1 2000 mg/2.5kg, Vit.K_3 1,550 mg, Vit.E 7,000 mg, Vit.D_3 1,500,000 IU, Vit.A 8,000,000 IU, Biotin H_2 250 IU, Cobalt 200 IU, Copper 3,000 IU, Iodine 1,000 IU, Iron 21,000 IU, Manganese 40,000 IU, Selenium 200 mg, Zinc 30,000 mg, Choline Chloride 175,000 mg, antioxidant 1,300 mg.*

Group A: diet without cholesterol (basal)

Group B: diet + 1% cholesterol (control)

Group C: diet + 1% cholesterol + 10% *Waltheria indica* leaf

Table 2: Proximate composition of *Waltheria indica*

Parameters	**Composition (%)**
Moisture content	**15.53** ± 0.03
Total ash	13.43 ± 0.03
Crude lipid	2.31 ± 0.015
Crude fibre	9.39 ± 0.035
Crude protein	18.34 ± 0.115
Carbohydrates	41.01 ± 0.105

Values are presented as the mean of duplicates expressed as mean ± SD.

Table 3: Effects of *Waltheria indica* leaf diet on the liver lipid profile of experimental rats

Lipid profile (µ/mg protein)	Groups		
	A	B	C
T-Ch	17.50 ± 0.59^a	83.88 ± 2.74^b	23.14 ± 1.05^a
HDL-Ch	35.94 ± 0.22^b	17.09 ± 0.71^a	33.25 ± 4.01^b
LDL-Ch	46.28 ± 1.82^a	85.33 ± 10.70^b	42.38 ± 1.11^a
VLDL-Ch	52.33 ± 1.19^a	135.32 ± 5.82^c	70.82 ± 0.64^b
TG	36.23 ± 12.08^a	36.74 ± 2.76^a	37.95 ± 11.70^a
HDL/T-Ch ratio	2.05 ± 0.41^b	0.20 ± 1.73^a	1.44 ± 2.53^b
LDL/HDL ratio	1.29 ± 1.02^a	4.99 ± 5.71^b	1.27 ± 2.56^a
Atherogenic index	0.00 ± 0.00^a	0.33 ± 0.59^b	0.06 ± 0.47^a

Values are presented as mean ± SD. Values with different superscripts are significantly different at ($p<0.05$).

T-Ch, Total Cholesterol; HDL-Ch, LDL-Ch, VLDL-Ch, high-, low- and very low-density lipoproteins, respectively; TG, Triglyceride; HDL/T-Ch, high-density lipoprotein-total cholesterol ratio; LDL/HDL, Low-density lipoprotein-high density lipoprotein ratio.

Table 4: Effects of *Waltheria indica* leaf diet on the liver lipid profile of experimental rats

Lipid profile (µ/mg protein)	Groups		
	A	B	C
T-Ch	16.95 ± 2.85^a	45.53 ± 2.11^c	27.39 ± 1.80^b
HDL-Ch	5.78 ± 2.22^b	2.53 ± 1.58^a	4.91 ± 1.01^b
LDL-Ch	25.86 ± 7.43^a	49.12 ± 2.26^b	28.15 ± 0.42^a
VLDL-Ch	3.38 ± 3.41^a	13.73 ± 1.62^c	9.90 ± 2.19^b
TG	25.78 ± 4.59^a	26.31 ± 5.23^a	25.45 ± 7.39^a
HDL/T-Ch ratio	0.34 ± 2.54^a	0.06 ± 1.85^a	0.18 ± 1.41^a
LDL/HDL ratio	4.47 ± 4.83^a	19.42 ± 1.92^b	5.73 ± 0.72^a
Atherogenic index	0.65 ± 0.32^a	1.02 ± 0.52^b	0.72 ± 0.86^a

Values are presented as mean ± SD. Values with different superscripts are significantly different at ($p<0.05$).

T-Ch, Total Cholesterol; HDL-Ch, LDL-Ch, VLDL-Ch, high-, low- and very low-density lipoproteins, respectively; TG, Triglyceride; HDL/T-Ch, high-density lipoprotein-total cholesterol ratio; LDL/HDL, Low-density lipoprotein-high density lipoprotein ratio.

Table 5: Antioxidant enzyme activity of Experimental rat fed with *Waltheria indica* leaf diet

Entities		Units	Groups		
			A	B	C
MDA	Serum MDA	(nmol/ml)	2.86±0.45^a	8.68±0.70^c	5.74±0.40^b
	Liver MDA	(µg/ml)	1.62±0.17^a	2.59±1.30^b	1.60±0.29^a
CAT	Serum CAT	(U/I)	5.18±0.04^c	2.29±0.15^a	3.75±0.13^b
	Liver MDA	(µ/mg protein)	5.90±0.09^c	3.11±0.10^a	4.31±0.09^b
SOD	Serum SOD	(U/I)	3.67±0.17^b	1.11±0.07^a	1.26±0.15^a
	Liver SOD	(µ/mg protein)	2.93±0.08^b	0.91±0.03^a	2.08±0.03^b
GSH	Serum GSH	(U/I)	249.55± 0.64^c	289.23±11.48^a	421.77±11.36^b
	Liver GSH	(µ/mg protein)	336.82±55.49^c	271.37±0.64^a	308.19± 46.28^b
GR	Serum GR	(U/I)	13.7 ± 1.63^c	17.89 ± 1.90^a	22.81± 2.60^b
	Liver GR	(µ/mg protein)	42.88±2.71^c	38.38±0.47^a	19.46± 2.08^b
GPx	Serum GPx	(U/I)	4.05 ± 0.16^c	1.85 ± 0.24^a	6.52 ± 1.45^b
	Liver GPx	(µ/mg protein)	12.49±1.90^c	10.35 ± 1.58^a	5.35 ± 1.20^b

Values are presented as mean ±SD. Values with different superscripts are significantly different at ($p<0.05$). MDA, Malondialdehyde; CAT; Catalase; SOD, Superoxide dismutase; GSH, Reduced Glutathione; GP, Glutathione Peroxidase and GPx, Glutathione Reductase

Table 6: Effects of *Waltheria indica* Leaf Diet on Liver Enzyme Activity

Entities		Units	Groups		
			A	B	C
ALT	Serum ALT	(U/l)	2.86±0.45[a]	8.68±0.70[c]	5.74±0.40[b]
	Liver ALT	(µ/mg protein)	1.62±0.17[a]	2.59±1.30[b]	1.60±0.29[a]
ALP	Serum ALP	(U/l)	5.18±0.04[c]	2.29±0.15[a]	3.75±0.13[b]
	Liver ALP	(µ/mg protein)	5.90±0.09[c]	3.11±0.10[a]	4.31±0.09[b]
AST	Serum AST	(U/l)	3.67±0.17[b]	1.11±0.07[a]	1.26±0.15[a]
	Liver AST	(µ/mg protein)	2.93±0.08[b]	0.91±0.03[a]	2.08±0.03[b]

Values are presented as mean ±SD. Values with different superscripts are significantly different at ($p<0.05$).
ALT, Alanine-amino transferase; ALP, Alkaline phosphate; AST, Aspartate-amino transferase

Lipid Profile in the Serum

Table 3 shows the effects of 10% *Waltheria indica* leaf diets on the serum lipid profile of experimental animals. Serum Total Cholesterol (TC) (mg/dl) was significantly elevated ($p<0.05$) from 17.50 ± 0.59 in group A (basal group) to 83.88 ± 2.74 in group B (control rats fed cholesterol diets). Reference [23] observed similar results in elevated serum TC of rats fed 0.3 % cholesterol diets. Also, it was observed that serum TC (mg/dl) reduced significantly from 83.88 ± 2.74 in control rats to 23.14 ± 1.05 in group C rats fed cholesterol and *Waltheria indica* leaf diets. In a study [24], a significant reduction in TC lowers the risk of coronary heart disease by 2 %. The ability of *Waltheria indica* leaf diets to lower serum TC concentration shows its potential in managing hypercholesterolemia. This impact could be attributed to the availability of phytochemicals, particularly saponins, which have been demonstrated to reduce serum cholesterol levels in animal studies [25]. Reference [26] found about 0.46% of saponins in *Waltheria indica* leaves.

Serum high-density lipoprotein cholesterol (mg/dl) decreased significantly from 35.94 ± 0.22 in the basal group to 17.09 ± 0.71 in the control. This is similar to the report of [21], who showed a significant reduction in HDL-Ch in rats fed a 1% cholesterol diet. However, serum HDL-Ch (mg/dl) increased significantly ($p<0.05$) from 17.09 ± 0.71 in basal rats to 33.25 ± 4.01 in group C rats fed *Waltheria indica* leaf diets. The increase in high-density lipoprotein cholesterol (HDL-Ch) may be attributed to the ability of the leaves to accelerate the breakdown of free radicals generated during cholesterol administration [27]. No significant difference ($p>0.05$) in serum HDL-Ch levels between the basal rats and those fed the test diets. The elevated serum HDL-Ch suggests increased cholesterol transport from peripheral tissues to the liver for metabolism and clearance. Feeding *Waltheria indica* leaves significantly boosted HDL-Ch levels in rats on a high-cholesterol diet, indicating that the leaves may offer valuable health benefits in managing cholesterol-related conditions.

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Serum LDL-cholesterol (mg/dl) significantly increased from 46.28 ± 1.82 in basal rats' group to 85.33 ± 10.70 in group B (cholesterol diet-fed rats). This finding is consistent with that of [28], who found that rats given 1% cholesterol diets are more likely to have high LDL-C levels. However, in group C rats fed test diets, serum LDL-C (mg/dl) decreased significantly ($p<0.05$) to 42.38 ± 1.11. The reduction in LDL-C observed in this study could be ascribed to cholesterol-induced up-regulation of LDL receptors [29]. Saponins have been shown to preferentially lower serum LDL-C in rats and humans [30].
Serum VLDL cholesterol levels (mg/dl) increased considerably from 52.33 ± 1.19 in group A (basal) to 135.32 ± 5.82 in group B (control rats fed a cholesterol diet). However, there was an obvious drop to 70.82 ± 0.64 in group C rats fed the test diet. Feeding *Waltheria indica* leaf diets to rats with elevated blood cholesterol may have improved VLDL-C fractional turnover [23]. Thus, reduced serum VLDL-C levels may be linked to enhanced lipoprotein lipase activity in peripheral vascular endothelial cells and reduced hepatic release, due to increased cholesterol elimination through stool [28].
Serum Triglyceride levels were not substantially different ($p>0.05$) across dietary groups. High blood triglyceride levels exceeding 500 mg/dL have been associated with atherosclerosis and stroke in humans [31]. This could be attributed to decreased triglyceride clearance due to decreased lipoprotein lipase activity [32]. Furthermore, we found an elevation in the ratio of HDL-Ch to TC from 0.20 ± 1.73 in control rats to 1.44 ± 2.53 in the test diet group.

The serum LDL to HDL-cholesterol ratio statistically reduced from 4.99 ± 5.71 in control rats to 1.27 ± 2.56 in the test diet group. This decrease may be due to the reduction in the concentration of LDL-C. This is beneficial and has positive health implications. However, the ratio did not statistically differ in basal rats and rats fed test diets. The serum atherogenic index of the test diet group reduced significantly ($p<0.05$) from 0.33 ± 0.59 in the control to 0.06 ± 0.47 in rats fed the test diet. A decrease in atherogenic lipids shows that the plant possesses lipid-lowering properties. Lowering atherogenic lipids has been shown to lessen the risk of heart attack, atherosclerosis and other related disorders [33]. However, no substantial difference in the atherogenic index was not significantly different in basal rats and rats fed test diets. This result, therefore, shows that consumption of 10% *Waltheria indica* as part of the diet will enhance a reduced risk of developing atherosclerosis.

Lipid Profile in the Liver

Table 4 displays the impact of 10% *Waltheria indica* leaf meals on the hepatic lipid profile of experimental rats. Cholesterol supplementation in rats has been demonstrated to improve hepatic lipid metabolism and triglyceride concentrations [34]. Total liver cholesterol (µg/mg protein) increased significantly from 16.95 ± 2.85 in basal diet-fed rats to 45.53 ± 2.11 in control. This result agrees with the findings of [28], who found a significant increase in liver TC concentration in rats fed 1% cholesterol diets. However, there was a significant decrease ($p<0.05$) in total TC (µg/mg protein) from 45.53 ± 2.11 in control rats to 27.39 ± 1.80 in the test diet-fed group. *Waltheria indica* leaf may decrease cholesterol by increasing the activity of 7α-hydroxylase, which catalyzes the breakdown of

cholesterol and bile acids and eliminates them through the feces [28]. Increased cholesterol elimination through bile may be linked to the reduction in total hepatic cholesterol.

Liver HDL-Ch (µg/mg protein) decreased significantly ($p<0.05$) from 5.78 ± 2.22 in the basal group to 2.53 ± 1.58 in the control. However, there was a significant increase ($p<0.05$) in liver HDL-Ch (µg/mg protein) from 2.53 ± 1.58 in control rats to 4.91 ± 1.01 in the test diet group.

Liver LDL-C (µg/mg protein) increased significantly ($p<0.05$) from 25.86 ± 7.43 in basal diet-fed rats to 49.12 ± 2.26 in group fed with cholesterol diet (CD). However, we recorded a gradual reduction in LDL-C (µg/mg protein) from 49.12 ± 2.26 in control rats to 28.15 ± 0.42 in group C-fed test diets. These findings are similar to those of [35], who showed a substantial decrease in LDL-C in rats fed 1% cholesterol. This reduction following the administration of *Waltheria indica* leaf meals is consistent with a previous study that found enhanced clearance of oxidized LDL-C [36]. LDL-C levels were not substantially distinct ($p>0.05$) between basal rats and rats fed test diets.

Liver VLDL-C (µg/mg protein) significantly increased ($p<0.05$) from 3.38 ± 3.41 in basal diet-fed rats to 13.73 ± 1.62 in control. However, the concentration decreased significantly ($p<0.05$) from 13.73 ± 1.62 in control rats to 9.90 ± 2.19 in test diet-fed rats. The reduction may be due to diminished VLDL-C production and uptake by the liver [37]. There were no significant variations in liver triglyceride levels across dietary groups. Rats given test diets had a significantly reduced hepatic LDL cholesterol to HDL cholesterol ratio compared to control rats on a cholesterol diet (19.42 ± 1.92). This increase might be related to higher HDL-Ch levels. There was no significant difference in hepatic LDL-C to HDL-Ch ratios between rats fed basal and test diets. The liver atherogenic index was significantly lower (0.72 ± 0.86) in group C rats fed cholesterol and test diets compared to group B rats (1.02 ± 0.52).

The atherogenic index was predicted to be greatly lowered due to a non-significant change in triglyceride concentrations and increased HDL cholesterol in the livers of group C rats. However, there was no statistical difference in the atherogenic index between basal rats and rats fed test diets.

Antioxidant Enzyme activity

Table 5 shows the hepatic and serum activities of antioxidant enzymes exposed to test diets and basal meals. Serum malondialdehyde (nmol/ml) was statistically different as it increased from 2.86 ± 0.45 in basal diet-fed rats to 8.68 ± 0.70 in control (cholesterol-fed rats). Erythrocytes are a specialized location for generating reactive species [38], making them particularly susceptible to the harmful effects of reactive oxygen species, which explain the increased hemolysis of red blood cells. An increase in serum malondialdehyde of animals fed cholesterol diets is consistent with another experimental model, which also showed that increased lipid peroxidation may be associated with high serum cholesterol [39]. However, serum malondialdehyde (nmol/ml) decreased significantly ($p<0.05$) to 5.74 ± 0.40 in rats fed test diets. The decrease in malondialdehyde concentration shows the reduction in lipid peroxidation due to the inclusion of *Waltheria indica* leaf in the diet. This suggests the lipid-lowering potential of *Waltheria indica* leaves. [40] also reported a significant reduction in lipid peroxidation in rats fed 2% cholesterol and methanolic extracts of *T. violacea*. This action may be attributed to phytochemicals, including phenols, whose presence has been reported in *Waltheria indica* leaves in a study [12].

Liver malondialdehyde (µg/ml) was significantly elevated from 1.62 ± 0.17 in the basal group to 2.59 ± 1.30 in the control. This suggests increased oxidative stress in high-cholesterol animals [41]. Liver malondialdehyde (µg/ml), however, decreased significantly ($p<0.05$) from 2.59 ± 1.30 in group B (cholesterol-fed rats) to 1.60 ± 0.29 in rats fed cholesterol and test diets. The reduction in lipid peroxidation product formed after treatment is also seen in another study by [42], who showed a significant decrease in lipid peroxidation product in rats fed 2% cholesterol with parsley and carob. This shows that *Waltheria indica* leaves may offer protective effects against cholesterol-induced free radical injury, thereby inhibiting oxidative stress in hypercholesterolemia.

Serum catalase activity (U/I) showed a substantial decrease from 5.18 ± 0.04 in the basal group to 2.29 ± 0.15 in control rats. Reference [42] observed a similar decline in catalase activity in rats fed a 2% cholesterol diet. This reduction in catalase activity may suggest that the accumulation of reactive oxygen species (ROS) increases with high cholesterol intake, leading to elevated oxidative stress. This, in turn, contributes to hypercholesterolemia and related cardiovascular issues [43]. However, serum catalase activity significantly increased in rats fed test diets to 3.75 ± 0.13. This rise in catalase activity can be linked to phytochemicals, such as flavonoids, found in *Waltheria indica* leaves at a concentration of about 0.02% [12]. In the liver, catalase activity (µ/mg protein) decreased significantly ($p<0.05$), dropping from 5.90 ± 0.09 in basal rats to 3.11 ± 0.10 in control rats. This decline could be attributed to the buildup of ROS, as liver catalase activity was suppressed by the production of malondialdehyde, leading to oxidative stress in rats fed high-cholesterol diets. Nevertheless, liver catalase activity (µ/mg protein) significantly increased ($p<0.05$) to 4.31 ± 0.09 in rats fed test diets. These findings align with those of [14], who also reported a significant increase in serum catalase activity in rats administered *Waltheria indica* leaf diets.

The serum superoxide dismutase activity (U/I) decreased significantly ($p<0.05$) from 3.67 ± 0.17 in the basal group to 1.11 ± 0.07 in the control (cholesterol-fed rats). A decrease in serum superoxide dismutase activity may be due to enhanced production of superoxide during high blood cholesterol, resulting in consumption of the available enzyme, thereby making it less available. The decrease in superoxide dismutase activity agrees with a previous report of decreased activity of antioxidant enzymes during hypercholesterolemia [4]. However, superoxide dismutase (U/I) serum activity did not significantly differ in control and rats fed cholesterol and test diets. Increased serum superoxide dismutase activity characterizes reduced hemolysis of red blood cells due to a reduction in oxidative stress that initially occurred due to hypercholesterolemia [38]. Liver superoxide dismutase activity (µ/mg protein) decreased significantly ($p<0.05$) from 2.93 ± 0.08 in the basal group to 0.91 ± 0.03 in control rats fed cholesterol diet. The reduction may be due to increased

oxidative stress caused by high-cholesterol diets. There was a relatively marked increase ($p<0.05$) in liver superoxide dismutase activity (μ/mg protein) from 0.91 ± 0.03 in control rats to 2.08 ± 0.03 in test diet rats. Increased liver superoxide dismutase activity means that the oxidative stress induced by hypercholesterolemia was almost completely eliminated by administering *Waltheria indica* leaf diets [44].

The results of the effect of Glutathione reductase (GR), Reduced Glutathione (GSH) and Glutathione peroxidase (GPx) were also reported in Table 5. The result shows an increase in the GSH in both liver and serum in experimental rats exposed to *Waltheria indica* relative to the control group with cholesterol (421.77± 11.36 vs 289.23 ± 11.48 and 308.19 ± 46.28 vs 421.77± 11.36 respectively). Also, an increase was observed in both GR and GPx of the serum, while a decrease was observed in both GR and GPx of the liver. The decrease in Reduced Glutathione was probably a consequence of *Waltheria indica's* ability to strengthen the liver's defensive functionality in combating free radicals, similar to the report of [45] on the antioxidant status of *Ulva pertusa* leaf. A statistically significant increase was observed in the activity of Glutathione Peroxidase in Group C containing cholesterol and 10% *Waltheria indica* (6.52±1.45) when compared with Group B containing only 1% cholesterol (1.85±0.24) $p<0.05$. This increase is due to the high cholesterol found in the diet, and this is not different when compared with the observations of [46], who discovered an increase in Glutathione Peroxidase in fat-fed rats in his study. There was a significant decrease in Group C containing cholesterol and 10% *Waltheria indica* (19.46±2.08) when compared with Group B containing 1% cholesterol (38.38±0.47) $p<0.05$. Glutathione peroxidase decreased in Group C rats whose diets contained cholesterol and 10% *Waltheria indica* (5.35±1.20) when compared to Group B fed with 1% cholesterol (10.35±1.58) $p<0.05$. GPx is actively involved in the clearance of lipid peroxide; a decrease in its activity would lower the protective capabilities of the liver against lipid peroxidation.

Liver Enzyme Activity

Table 6 presents the serum and liver activities of aspartate aminotransferase (AST), alkaline phosphatase (ALP), and alanine aminotransferase (ALT), which significantly increased in the cholesterol-fed group compared to the basal group without cholesterol, consistent with the findings of [47]. However, the experimental group treated with the plant extract exhibited a significant reduction in these enzyme activities. These results highlight the plant's potential to lower cholesterol levels in experimental animals, as AST, ALT, and ALP are specific biochemical markers used in diagnosing liver dysfunction [48].

Hypocholesterolemia activity

The present study reveals that consumption of the *Waltheria indica* leaf diet showed ameliorative effects on the lipid profiles of experimental rats. Hypercholesterolemia is characterized by excessively high plasma cholesterol levels, as evidenced by increased plasma low-density lipoprotein cholesterol [49]. Reference [50] suggests that rats are naturally resistant to hypercholesterolemia and atherosclerosis, with a strong ability to regulate plasma cholesterol levels. We supplemented their diet with 1 % cholesterol to induce hypercholesterolemia in experimental rats. Elevated lipid peroxidation and the subsequent production of malondialdehyde serve as markers of high cholesterol, promoting the generation of reactive oxygen species during mitochondrial oxidative phosphorylation and the electron transport chain. However, consequent to negative cardiovascular events associated with cholesterol-lowering drugs like fibrates, it is a thing of interest to evaluate certain natural products which may possess specific lipid-lowering potentials in mitigating the risk of onset and progression of cardiovascular diseases, a specific indicator of both atherosclerosis and coronary artery disease [51].

IV. Conclusion

Conclusively, the current study shows that the diet formulation of *Waltheria indica* leaf diets in high-cholesterol rats significantly regulated lipid profile, promoted antioxidant enzyme activities and ameliorated lipid peroxidation. This reveals the possible potential of *Waltheria indica* leaf as a supplement in ameliorating high cholesterol concentration, which will be useful in managing and treating hypercholesterolemia-related disorders. However, the toxicity of the plant extracts on the animal model needs to be further studied.

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