Original Research Article
Comparative studies on the effect of quercetin and *Emilia sonchifolia* leaf extract on lipid profile of cadmium induced male wistar rats.

ABSTRACT

Aims: The effects of quercetin and *Emilia sonchifolia* on the lipid profile in Cadmium chloride induced oxidative stress were investigated in this study

Methodology: Twenty-four (24) male albino Wistar rats aged 5-6 weeks old and weighing 100-170g were divided into four groups of six rats each; consisting of normal control group, $CdCl_2$ untreated group, QE treated group and ES treated group. In the initial phase of the experiment called the induction phase, Group 1 being the NC group received distilled water orally and the rest of the experimental groups were orally administered $CdCl_2$ at a dose level of 5mg/kg b.w/day for 21 days. In the next phase of the experiment called the treatment phase Group 1 and 2 being the NC and $CdCl_2$ untreated groups respectively, were orally administered 0.07ml of 2% tween80. Group 3 being QE treated group was administered quercetin at a dose level of 20mg/kg b.w/day + 0.07ml of 2% tween80. Group 4 being ES treated group was administered *Emilia sonchifolia* at a dose level of 575.8mg/kg b.w/day + 0.07ml of 2% tween80 for 28 days. After the treatment period of 28 days, the animals were fasted overnight, anesthetized and sacrificed. Whole blood was collected for biochemical assay.

Results: Results showed that cadmium decreased body weight and significantly increased serum levels of TG, TC, LDL-C and VLDL-C across the treatment groups.

Conclusion: Marked positive response were observed following treatment with quercetin and *Emilia sonchifolia* in all treated groups thus suggesting the hypolipidemic activities of quercetin and *Emilia sonchifolia* in cadmium induced oxidative stress.

Keywords: Cadmium chloride; Emilia sonchifolia; lipid profile; oxidative stress; Quercetin.

1. INTRODUCTION

Heavy metals exposure and resulting disease conditions have become a global issue and a matter of serious concern. In recent times, research has focused on the elucidation of possible mechanism of toxicity of various environmental and occupational heavy metals, how they affect health and developing a safe and cheap therapeutic approach to ameliorating disease conditions resulting from exposure. Heavy metals through the generation of reactive oxygen species has been known to generally affect man's health. Normally these radicals are produced in such an amount that it overwhelms the body natural defense system and thus cause damage to important body tissues.

Cadmium is a heavy metal and has been identified as potentially toxic [1]. It is among the top 10 most toxic compounds that adversely affect human health [2]. It naturally occurs in the environment at concentration that poses no risk to man; however, the involvement of man and his activities has greatly increased these concentrations. Cadmium is used in industries for protective plating of steel, rubber processing, galvanizing, electrode materials in nickel cadmium batteries, pigment in glass and plastic, production of pesticides and as stabilizer for poly vinyl chloride (PVC) products [3]. Cadmium, through these activities can over time be

detected in food, air, water [4, 5] and can be easily transported from soil to plants and bioaccumulates in the food chain [4]. Man's exposure to cadmium may be through; the daily ingestion of cadmium contaminated food, involvement in heavy metal industrial activities, smocking of tobacco and consumption of tobacco products [4]. WHO reports [4] showed that food may make up a major environmental source of cadmium exposure especially for the population that are not involved in the consumption of tobacco products. However, in this same report it was established that some crops can overtime store high concentration of cadmium if cultivated in an environment that is contaminated with cadmium. The human body has a natural anti-oxidant protection system which detoxifies/eliminates free radical and it metabolites when introduced into the body, but a prolong exposure to toxic compounds and heavy metals including cadmium can result in the generation of free radicals and when the free radical load overwhelms the protective mechanism of the anti-oxidants it can result to oxidative stress and cellular injuries [6].

Plant derived antioxidants have recently gained attention in research and are been routinely employed in the fight against toxicants. Quercetin is a flavonoid that possesses high antioxidant properties; this flavonoid can prevent cellular oxidative damage by scavenging/mopping up reactive oxygen species thereby preventing the process of cellular lipid peroxidation. Several documented research articles have demonstrated the protective potential of dietary quercetin against cellular oxidative damage which is initiated by heavy metals or ultraviolet radiation on experimental animals liver, renal system and sperm. The antioxidant activity of quercetin could be due to its ability in chelating transition metal ions.

The use of plants in ethno-botanical medicine can be attributed to the presence of bioactive compounds that are responsible for the mopping up of free radicals and their toxic metabolites thus preventing subsequent cellular oxidative stress. *Emilia sonchifolia* is a bushy annual herb that has found wide applications in traditional medicine in most developing part of the world. It is reported to be rich in Phytochemicals and possess potent antioxidant properties [7]. In Nigeria, *Emilia sonchifolia* has found wide applications in ethnomedicine which includes the treatment of several ailments viz inflammation, diabetes, cancer, fever, cataract, convulsion, malaria etc. [7-9]. Phytochemical studies of the aerials parts *Emilia sonchifolia* have indicated the presence of bio-active compounds like alkaloids which have been identified as senkirkine and doronine, flavonoids (i.e quercetin) and terpenes [10] that may be responsible for the plant antioxidant activity. This present study seeks compare the effect of quercetin and Emilia sonchifolia leaf extract on lipid profile of cadmium induced male wistar rats.

2. MATERIAL AND METHODS

2.1 Collection of plant specimen and preparation

Fresh leaves of *Emilia sonchifolia* were harvested in July 2017, from University of Calabar Staff quarters. The leaves were later identified at the Herbarium with voucher specimen (BOT/ES/1/2017), in the Department of Botany, University of Calabar, Cross River State, Nigeria. The collected fresh leaves were separated from stalk, rinsed thoroughly with tap water to remove debris and thereafter shade dried under room temperature for nine days. The dried leaves of plant sample (5kg) were then pulverized into powder using mechanical food blender (corona). The powder obtained was then soaked in ethanol and allowed for 48 hours before filtration. The homogenate was filtered using cheesecloth and filter paper. The filtrate obtained was then exposed to mild heat of $40^{o}C$ in water bath to obtain a semi-solid extract [11]. The extract was then stored in a refrigerator at 4 ^{0}C until when needed for administration to experimental animals.

2.2 Experimental animal and protocol

Twenty-Eight male albino wistar rats aged 6-8 weeks and weighing 120- 170g, were obtained from the University of Calabar animal house in Nigeria. The rats were kept in standard well ventilated cages under room temperature and 12 hours light/dark cycle and maintained following the guidelines for animal care as contained in the animal ethics handbook of the Faculty and Ethical Committee on the experimental use of animals for research purposes.

Table 1. Grouping and treatment schedule for the various experimental animals

Groups	No. of animal	Induction Phase	Treatment Phase (28 days)
A	7	Distilled water	0.07ml of 2% twin80 + Distilled water
B	7	$CdCl_2$+Distilled Water	0.07ml of 2% twin80 + Distilled water
C	7	$CdCl_2$+Distilled water	QE (20mg/kg b.w/day) + 0.07ml of 2% twin80 + Distilled water
D	7	$CdCl_2$+Distilled water	ES (575.8mg/kg b.w/day) + 0.07ml of 2% twin80 + Distilled water

2.3 Experimental design

The experimental design was carried out as illustrated by Nna *et al.* [12] (modified). Rat models were randomly allocated to four experimental groups namely: A, B, C, and D with each group having seven rats each. A: Normal, B: $CdCl_2$ untreated, C: $CdCl_2$ + QE (QE treated), D: $CdCl_2$ + ES (ES treated) Rats in group A were orally administered distilled water for the first 21 days of the experiment and then given 0.07mls of tween80 dissolved in 3.43mls of distilled water throughout the remaining 28 days of the experiment. Rats in group B were treated with $CdCl_2$ at 5mg/kg b.wt./day for the first 21 days of the experiment (induction phase) and given 0.07 ml of tween80 disolved in 3.43 mls of water throughout the remaining 28 days of the research period. Rats in group C were pretreated with $CdCl_2$ dissolved in distilled water at 5mg/kg b.wt. /day for 21 days before the administration of quercetin at 20mg/kg b.wt/day for another 28 days. Rats in group D were also pretreated with $Cdcl_2$ at 5mg/kg b.wt./day for 21 days before the administration of *Emilia sonchifolia* extract at 575.8mg/kg b.w./day for another of 28 days. QE and ES were dissolved in 0.07ml of 2% tween80 before the supplementation of water to make up the daily required quantity of extract (3.5mls/day) for administration. The experimental design used during the study can be summarized in Table 1.

2.4 Assessment of body weight changes

To get the body weight change of each experimental animal, its initial body weight was subtracted from the final body.

2.5 Determination of serum lipid profile

Fresh serum collected was utilized in the estimation of TC, TG, HDL-c, LDL-c and VLDL-c using Randox kits assay procedures.

2.6 Statistical analysis

The data obtained were expressed as mean ±SEM. The results gotten after laboratory findings were then subjected to statistical analysis for significance by one way ANOVA using SPSS statistical package and post Hoc Test (LSD) between groups. The value gotten were considered statistically significant at $p < 0.05$ level.

3. RESULTS AND DISCUSSION

The results of the effects of quercetin (QE) and *emilia sonchifolia* (ES) leaf extract on body weight change (table 2), lipid profile (FIG 1-6) of cadmium induced oxidative stressed rats are presented here.

3.1 Effect of CdCl$_2$, *Emilia sonchifolia* and quercetin on body weight changes

CdCl$_2$ untreated group had a significantly ($p < 0.05$) decline in body weight when compared to the control group which had a higher body weight. In the course of treatment with QE and ES, body weight changes were increased in the treated groups but these increase were not significant ($p < 0.05$) when compared to the CdCl$_2$ untreated animals.

3.2 Effect of CdCl$_2$, *Emilia sonchifolia* and quercetin on serum lipid parameters and atherogenic index of plasma (AIP)

Total cholesterol (TC) significantly ($p<0.05$) increased in CdCl$_2$ untreated group when compared with normal control group (NC). The result revealed a general decrease in the serum levels of TC in QE and ES treatment groups compared to CdCl$_2$ untreated group.
Triacylglycerol significantly ($p<0.05$) increased in CdCl$_2$ untreated group when compared with the NC. The results obtained also showed that serum levels of TG were significantly ($p<0.05$) reduced in QE and ES treated groups when compared with the CdCl$_2$ untreated group. It was also observed that TG levels in the ES treated group were also significantly ($p<0.05$) reduced when compared with QE Treated group.
High density lipoprotein (HDL-c) was significantly ($p<0.05$) reduced in the CdCl$_2$ untreated animals compared to NC. HDL-c levels were markedly ($p<0.05$) elevated in the QE and ES groups when compared with the CdCl$_2$ untreated group. No marked ($p<0.05$) difference was observed in HDL-c levels of QE and ES groups.
Low density lipoprotein (LDL-c) was significantly ($p<0.05$) elevated in the CdCl$_2$ untreated group when compared with the NC. The results obtained also showed that levels of LDL-c decreased significantly ($p<0.05$) in the QE qnd ES treatment groups when compared with the CdCl$_2$ untreated group.
Very low density lipoprotein (VLDL-c) levels were significantly ($p<0.05$) elevated in CdCl$_2$ untreated group compared with the NC. Results also revealed levels of VLDL-c were decreased significantly ($p<0.05$) in the QE and ES groups compared with CdCl$_2$ untreated group. A significant difference was also observed in the ES group when compared with QE.
Although there was elevation in serum TC, TG, LDL-c and VLDL-c concentrations and a decline in serum concentration of HDL-c of the CdCl$_2$ untreated group when compared to the NC, there was no risk of cardiovascular diseases as shown by the artherogenic index of plasma (AIP) analysis.

TABLE 2. **Effect of cadmium, *Emilia sonchifolia* and quercetin on weight change of the experimental animal models**

Groups	Body Weight Change (g)
CdCl$_2$ untreated	47.34±3.44
ES Treated	55.10±4.10
QE Treated	52.32±5.24
Normal control	67.96±4.67[*]

Values expressed as Mean ± SEM and n = 5
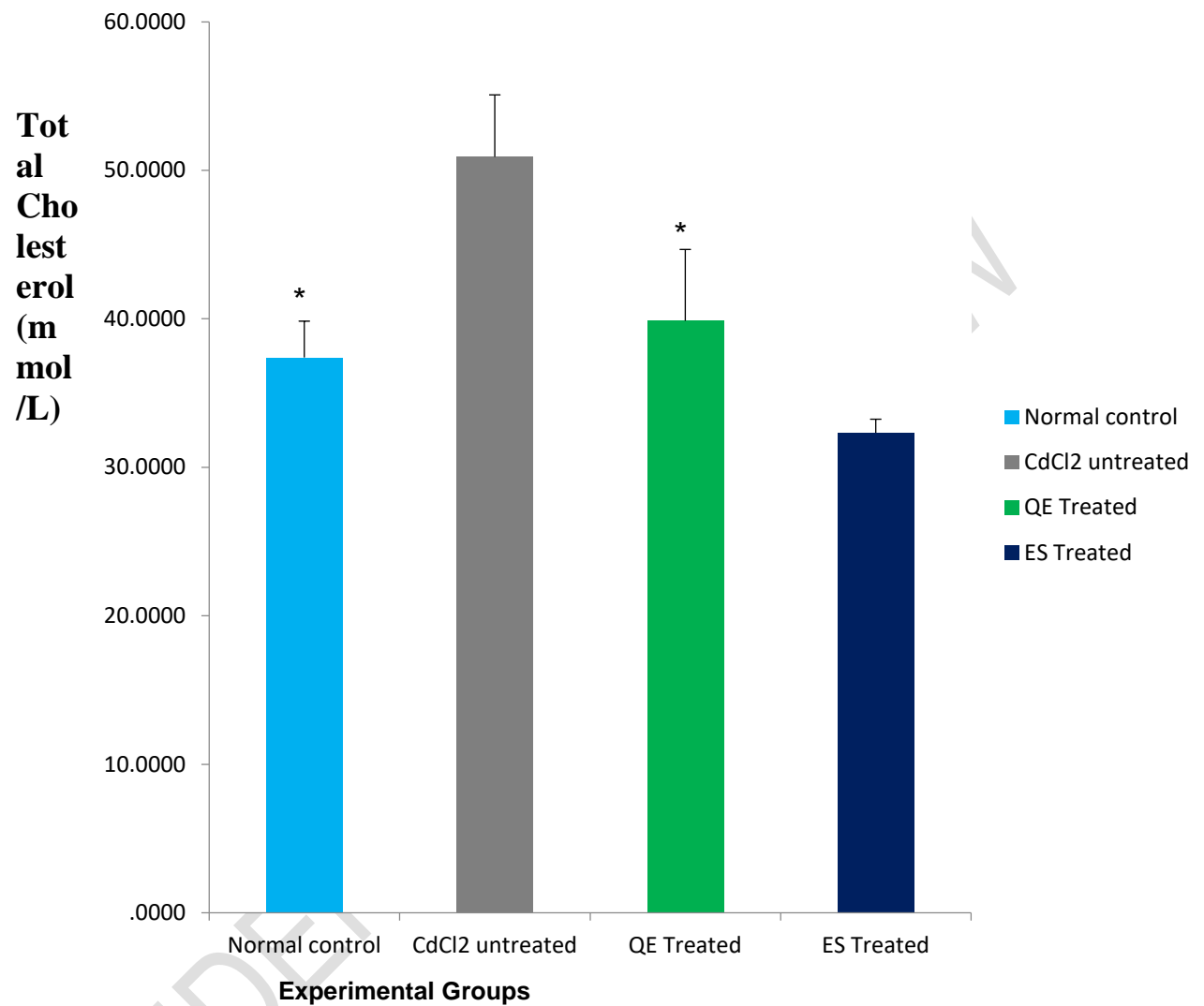[*] = P = .05 vs CdCl$_2$ untreated group

Fig. 1. Total cholesterol concentration of the various groups of experimental   animals
Values expressed as mean ±SEM and n = 5
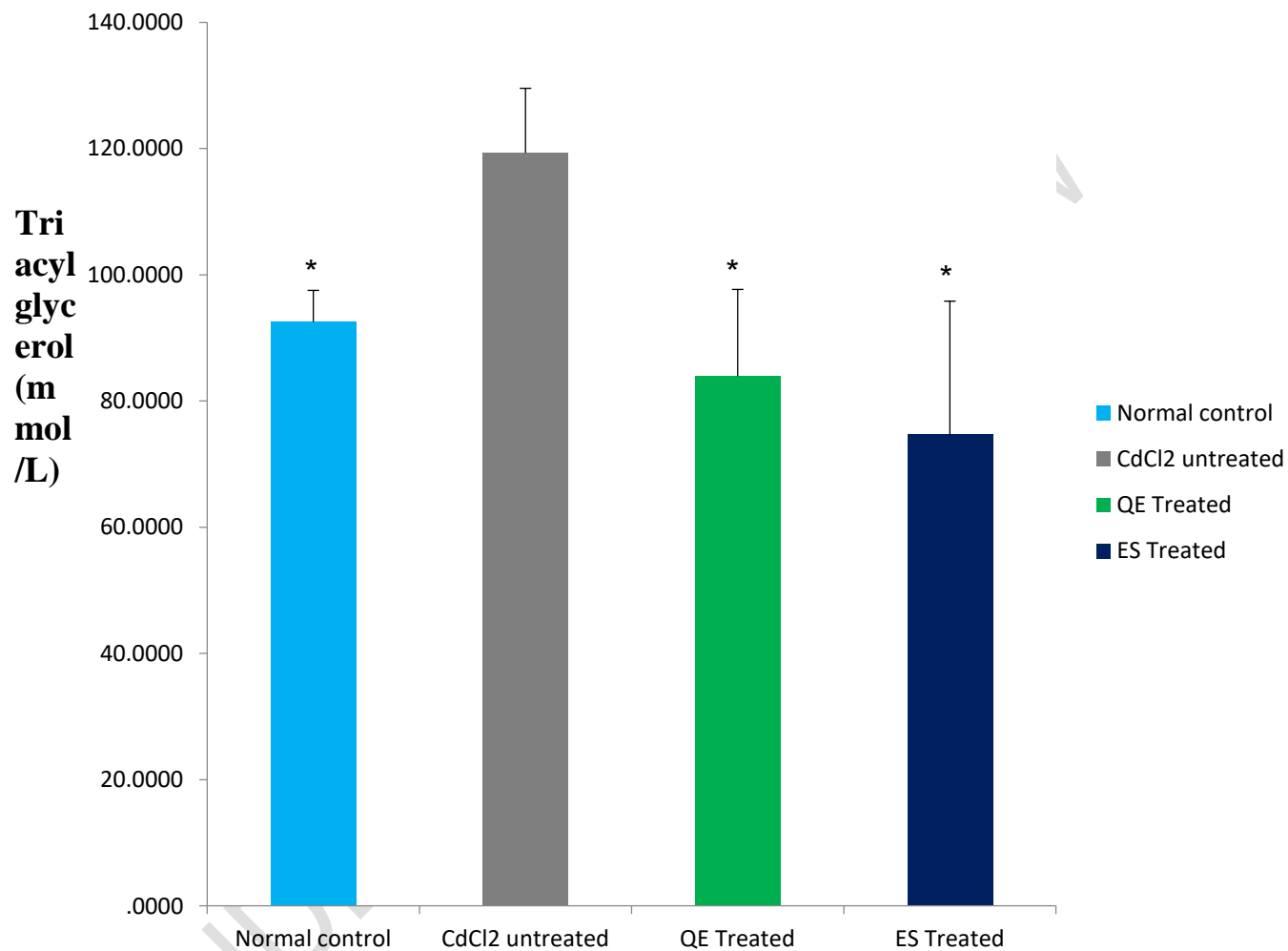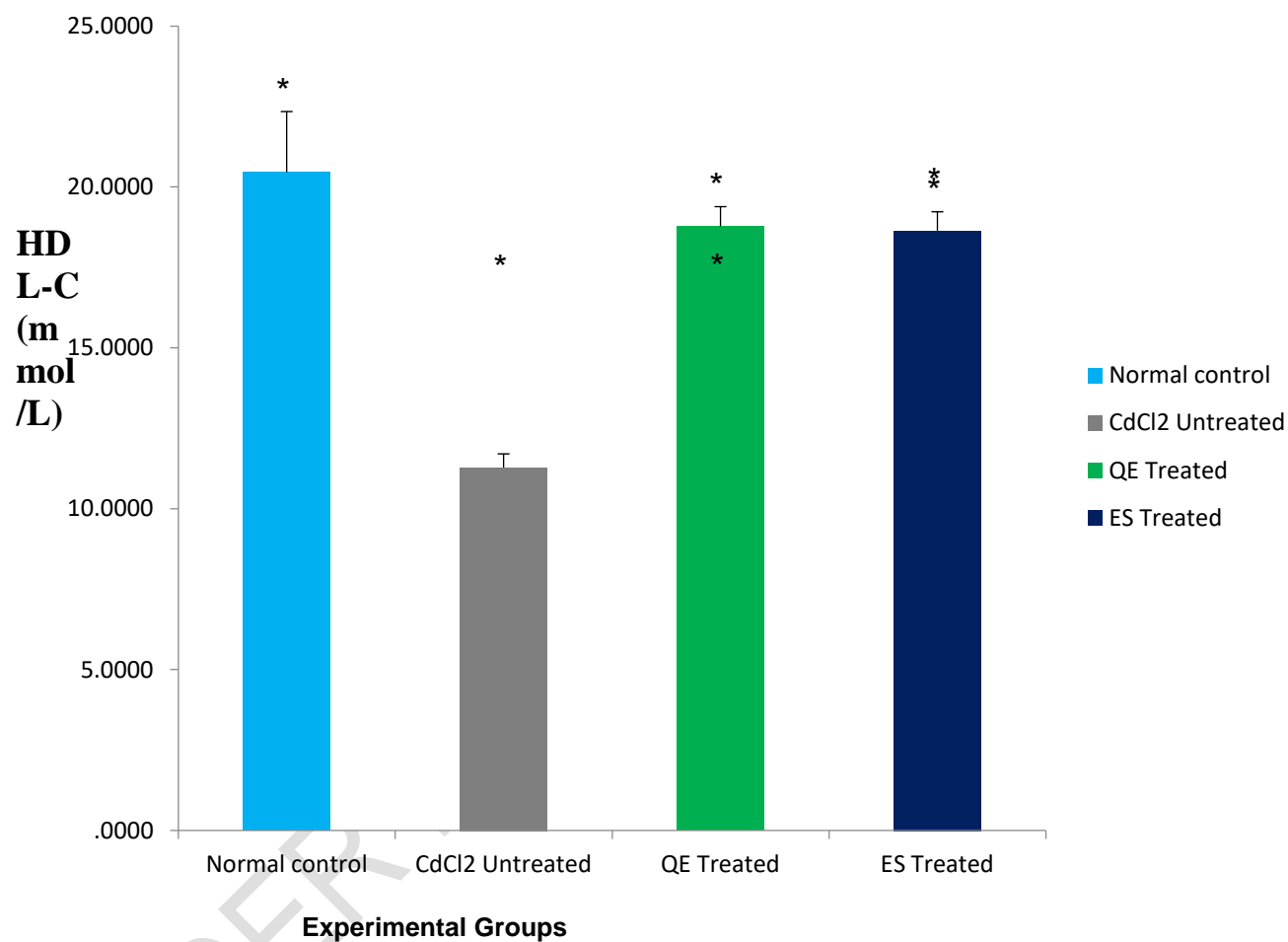** = p = .05 vs CdCl$_2$ untreated group*

Fig. 2. Triacylglycerol concentration of the various groups of experimental   animals
Values expressed as mean ±SEM and n = 5
* = p = .05 vs CdCl$_2$ untreated group

Fig. 3. T HDL-c concentration of the various groups of experimental animals
Values expressed as mean ±SEM and n = 5
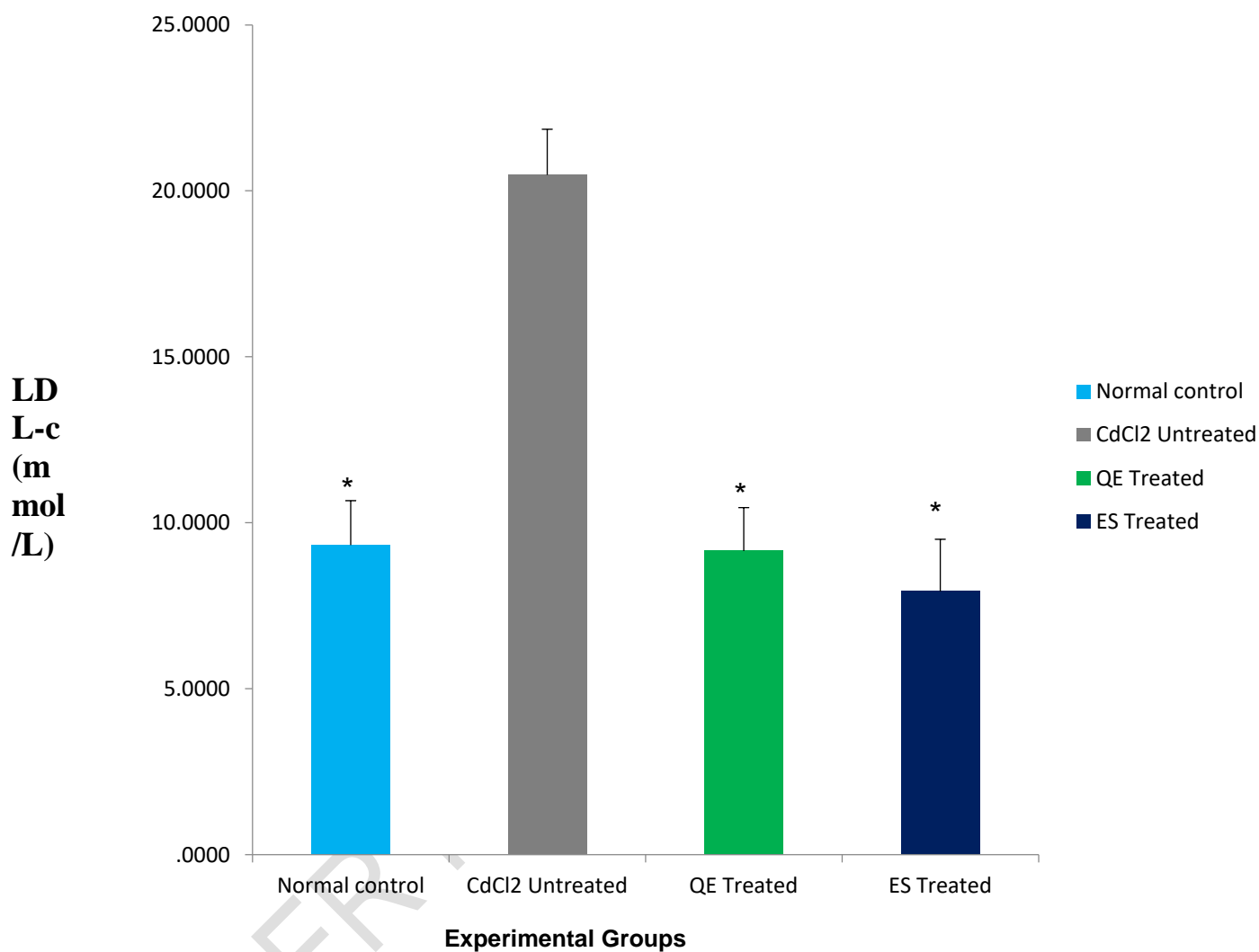*$*$ = p = .05 vs CdCl$_2$ untreated group*

Fig. 4. LDL-c concentration of the various groups of experimental animals
Values expressed as mean ±SEM and n = 5
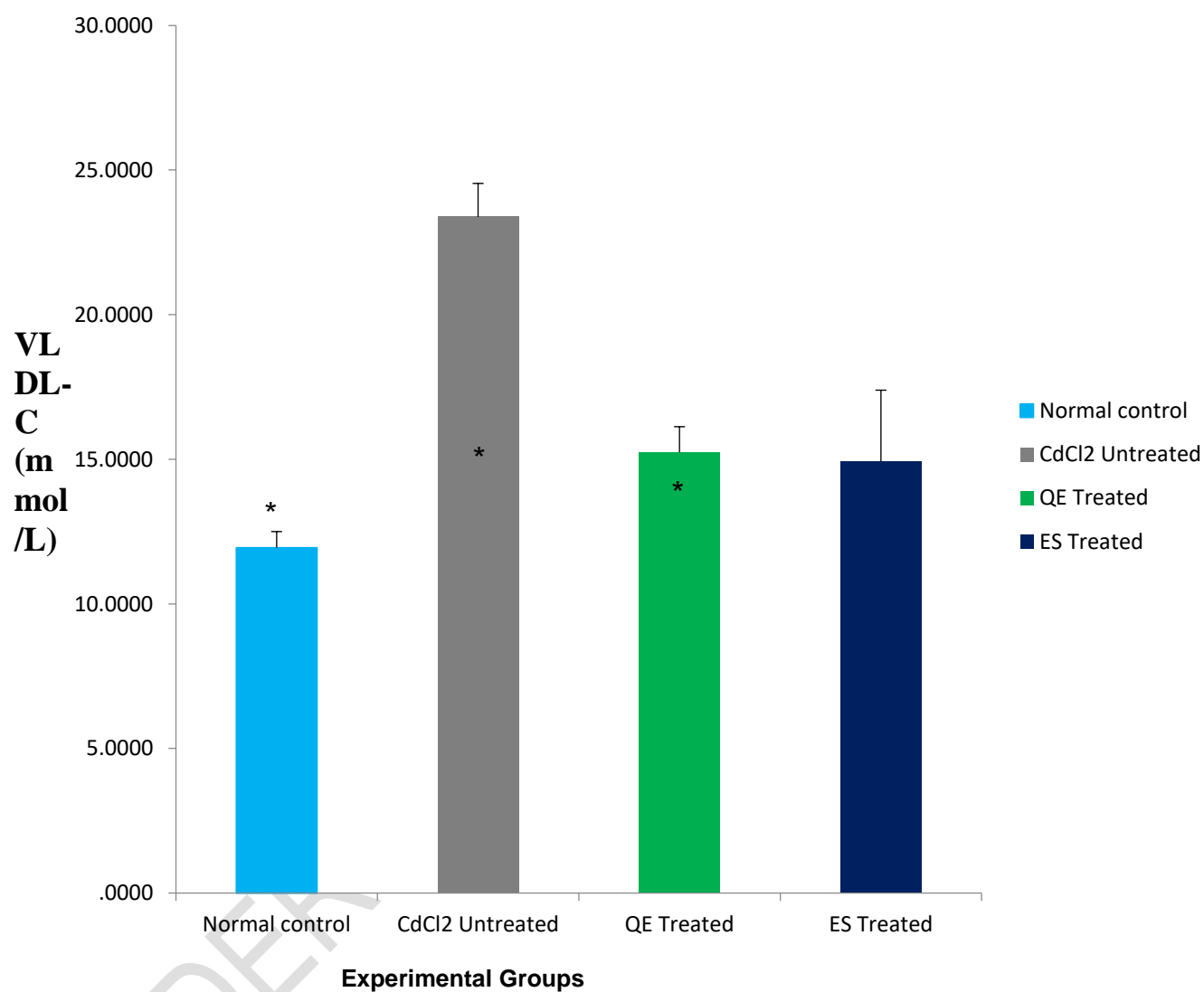** = p = .05 vs CdCl$_2$ untreated group*

Fig. 5. VLDL-c concentration of the various groups of experimental animals
  Values expressed as mean ±SEM and n = 5
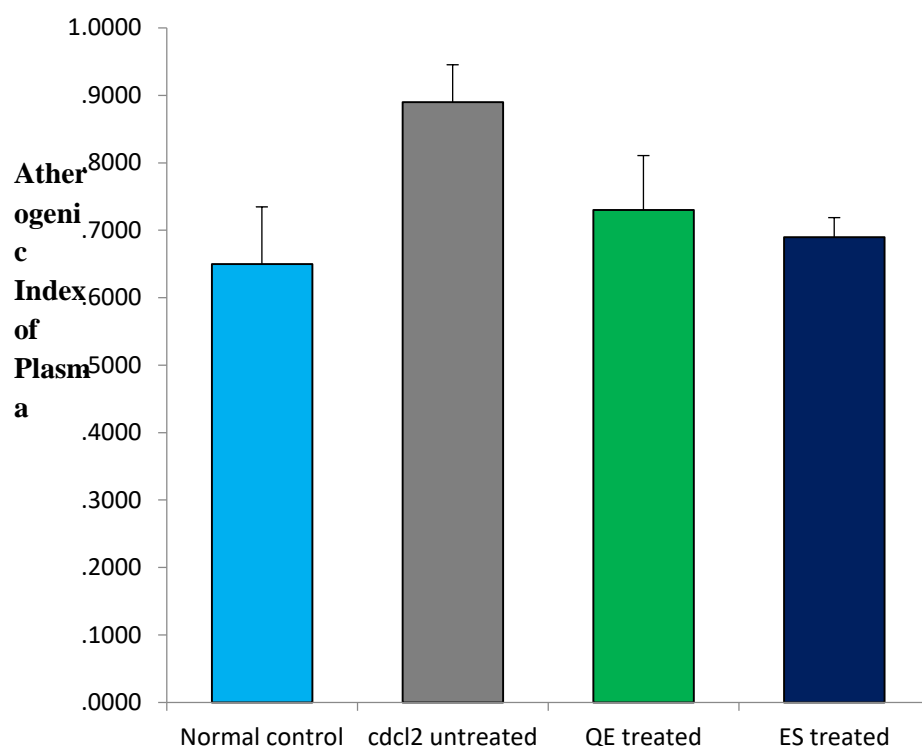** = p = .05 vs CdCl$_2$ untreated group*

Fig. 6. Effect of the various treatments on atherogenic index of plasma

In this study, we investigated the short term effect of cadmium exposure on the lipid profile of cadmium induced male wistar rats. Additionally, since quercetin and *Emilia sonchifolia* have both been proven to confer protection against oxidants and have been tagged "strong antioxidants" we also investigated the capacities of these antioxidants in combatting deleterious effects associated with cadmium exposure.

The results of the study showed that exposure to cadmium at a dose level of 5mg/kg bw/day significantly reduced body weights of the exposed experimental animals while quercetin and *Emilia sonchifolia* were both potent in combating cadmium induced wasting (table 2). The significant reduction in body weight may be attributed to the necrotic and degenerative

capacities of cadmium [13]. And the protective capacities of quercetin and *Emilia sonchifolia* is attributed to the presence of phytochemicals and consequently antioxidants properties which might be responsible for their abilities to protect the body against cadmium induced organ and tissue wasting thereby bringing back body weight gain to nearly normal.

Cd exposure resulted in higher levels of TC, TG, LDL-c and VLDL-c in exposed rat models compared with non-exposed rat models. Whereas Cd administration markedly decreased serum HDL-c levels compared to the non-exposed animal models. These findings can be linked to the excessive generation of free radicals that are involved in eliciting oxidative stress and subsequently many pathophysiological conditions and disease development [13].

4. CONCLUSION

We can say that quercetin and *Emilia sonchifolia* both had beneficial effects on lipid profile in cadmium induced toxicity, and this is attributable to their various antioxidant and antiapoptotic properties.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee
COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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