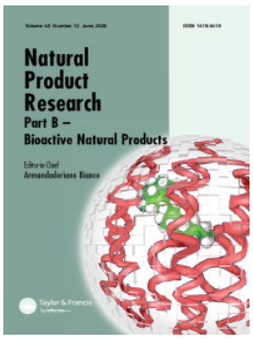

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Ethanolic extract of *Cnidoscolus aconitifolius* leaves alleviates insulin resistance by modulating oxidative stress and inflammation in albino rats exposed to heavy metal mixture

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ABSTRACT

Exposure to heavy metals significantly contributes to insulin resistance, a major factor in type 2 diabetes. This study investigated the antioxidant and therapeutic potential of ethanol leaf extract of *Cnidoscolus aconitifolius* in mitigating heavy metal-induced insulin resistance, oxidative stress and inflammation in albino rats. Thirty rats were divided into five groups: Groups I and II received normal saline and a lead-mercury mixture, respectively, while Groups III, IV and V were treated with the extract (200, 400 and 600 mg/kg) for four weeks after exposure. The extract, rich in catechin, rutin, gallic acid and kaempferol, exhibited strong antioxidant activity. Heavy metal exposure induced hepatic insulin resistance, dyslipidaemia, oxidative stress and inflammation, as shown by elevated HOMA-IR values. Extract treatment reversed these effects in a dose-dependent manner, restoring insulin sensitivity *via* oxidative stress and inflammation modulation. This highlights the potential of *C. aconitifolius* as a nutraceutical for heavy metal-induced metabolic disorders.

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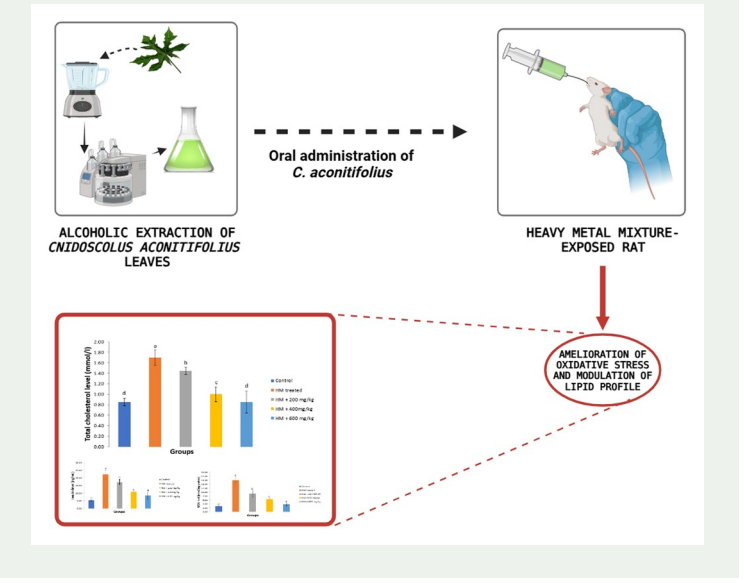

1. Introduction

Insulin resistance (IR), a key risk factor for type 2 diabetes (T2D), manifests as the impaired ability of insulin to regulate glucose metabolism effectively. T2D is a pervasive metabolic disorder that is projected to affect 700 million people globally by 2045 if proactive measures are not implemented (Saeedi et al. 2020). Environmental factors, including mining activities, vehicular emissions, and waste disposal, expose humans to heavy metals, which have been implicated in oxidative stress and subsequent metabolic disturbances (Anetor et al. 2016; Sabir et al. 2019). The weak antioxidative defences of pancreatic beta cells make them particularly vulnerable to oxidative damage, often leading to endocrine dysfunction and exacerbating the pathogenesis of T2D (Newsholme et al. 2016).

Heavy metals such as lead and mercury, classified as metalloestrogens, disrupt endocrine functions and may potentiate diabetes risk by inducing oxidative damage (Choe et al. 2003; Henson and Chedrese 2004). The integral role of oxidative stress in the development of T2D has motivated research into antioxidants as therapeutic agents. Plant-derived natural antioxidants, with their ability to neutralise oxidative stress, hold promise as potential interventions (Valenzuela Soto et al. 2015).

Cnidoscolus aconitifolius, commonly referred to as the spinach tree, is a plant widely consumed as a vegetable and valued for its medicinal properties. Rich in vitamins, minerals, and polyphenolic compounds such as catechin, quercetin, and rutin, this plant exhibits antioxidative, anti-inflammatory, and hypoglycaemic properties (Adeniran et al. 2013). Traditional medicine uses *C. aconitifolius* to manage kidney disorders, inflammation, and hypertension, among other conditions. Previous studies have validated its hypoglycaemic and lipid-modulating effects, suggesting its potential as a therapeutic agent in diabetes management (Loarca-Piña et al. 2010; Achi et al. 2017).

This study explores the therapeutic potential of ethanol leaf extract of *C. aconitifolius* (ELE-CA) in mitigating metabolic and biochemical alterations induced by heavy metal mixtures in Wistar rats. It aims to bridge the gap in knowledge regarding the antioxidant and hypoglycaemic effects of ELE-CA in the context of heavy metal-induced insulin resistance, thereby providing insights into its pharmacological relevance in T2D management.

2. Materials and methods

2.1. Sample collection and preparation

Fresh leaves of Cnidoscolus aconitifolius were harvested from Abakaliki, Nigeria, and authenticated by Prof. Onyekwelu of Ebonyi State University (Herbarium number: EBSU/APB/HB/0314). Ethanol leaf extract (ELE-CA) was prepared using a cold maceration method as described by Orji et al. (2020). The leaves were soaked in ethanol for 72 h, filtered, and concentrated using a rotary evaporator.

2.2. Chemicals and reagents

Flavonoids (quercetin, rutin hydrate) and phenolic acids (gallic, caffeic, and ferulic acids) were sourced from Sigma-Aldrich (UK). Other reagents were procured from Thermo Fisher Scientific (UK). All chemicals were of analytical grade.

2.3. Quantification of bioactive compounds

Quantification of flavonoids and phenolic acids in ELE-CA was performed using an Agilent 1100 Series HPLC system with a ZORBAX SB-C18 analytical column (250 × 4.6 mm, 5 µm). The mobile phase comprised phosphoric acid, methanol, and acetonitrile (72:14:14 v/v/v), with detection at 257 nm. Calibration was conducted using standard compounds (quercetin, kaempferol, and gallic acid).

2.4. Antioxidant assays

2.4.1. DPPH radical scavenging assay

The free radical scavenging activity of ELE-CA was evaluated using DPPH as outlined by Awoke et al. (2020). Various concentrations of the extract and standards (gallic acid, ascorbate) were incubated with DPPH solution, and absorbance was measured at 517 nm. IC50 values were calculated.

2.4.2. Reducing power assay

Reducing power was assessed using sodium phosphate buffer and potassium ferrocyanide, followed by trichloroacetic acid and $FeCl_3$ addition, as per Orji et al. (2020). Absorbance was measured at 700 nm.

2.4.3. Nitric oxide (NO) scavenging assay

The NO scavenging potential of ELE-CA was tested using methods from Igbinosa et al. (2011), with absorbance determined spectrophotometrically.

2.5. Experimental design

Thirty Wistar albino rats (180–200 g) were divided into five groups ($n = 6$). Group 1 (control) received saline, while Group 2 was exposed to lead nitrate (11.25 mg/kg) and mercury chloride (0.4 mg/kg). Groups 3–5 received ELE-CA (200, 400, and 600 mg/kg, respectively) following heavy metal exposure. Treatments were administered orally for 28 days.

2.6. Sample collection

Rats were sacrificed under ether anaesthesia, and blood samples were collected *via* vein puncture. Serum was separated by centrifugation at 3000 rpm for biochemical analyses. Liver tissues were excised, rinsed, and homogenised for further assays.

2.7. Biochemical analyses

2.7.1. Glucose and insulin measurement

Fasting blood glucose was measured using ACCU-CHEK® GO glucometers, while insulin levels were analysed using CUSABIOR ELISA kits. The homeostasis model assessment-insulin resistance (HOMA-IR) index was calculated:

$$\text{HOMA} - \text{IR} = \text{Glucose}(\text{mg/dL}) \times \text{Insulin}(\mu\text{U/mL}) / 405$$

2.7.2. Lipid profile, GLUT-4 and CRP analysis

Serum lipid profiles (total cholesterol, LDL, HDL, triglycerides, and VLDL) were assessed using Randox kits. GLUT-4 and CRP levels were measured using specific ELISA kits following manufacturer protocols.

2.7.3. Oxidative stress markers

Malondialdehyde (MDA) levels were assessed using the Buege and Aust (1978) method. Superoxide dismutase (SOD) and catalase (CAT) activities were measured following methods by Sun and Zigman (1978) and Oyedemi et al. (2010), respectively. Reduced glutathione (GSH) was estimated using Avivar-Valderas et al. (2011).

2.8. Statistical analysis

Results are expressed as mean ± standard deviation. Data were analysed using one-way ANOVA followed by Tukey's post hoc test. Statistical significance was set at $p < 0.05$, using GraphPad Prism 5.

3. Results

3.1. Free radical scavenging activity of ELE-CA using DPPH assay, NO radical-scavenging capacity of ELE-CA and ferric reducing antioxidant potential of ELE-CA

Figure S1(i) illustrates the relationship between antioxidant activity and DPPH, revealing concentration-dependent inhibition activity for the leaf extract, gallic acid, and

ascorbate. The Gallic acid and ascorbate showed similar antioxidative activity (60 – 80%), but both had significantly increased activity when compared to ELE-CA whose activity was < 60% at its highest concentration of 100 µg/ml. The most effective DPPH radical scavenger was gallic acid, with an IC_{50} of 18.88 µg/ml, ascorbate had an IC_{50} of 41.64 µg/ml but ELE-CA had the lowest IC_{50} of 56.49 µg/ml. Similarly, in Figure S1(ii), the gallic acid exhibited the highest nitric oxide radical neutralising potentials with IC_{50} of 35.53 µg/ml, the ascorbate had IC_{50} of 42.76 µg/ml but the ELE-CA has the least IC_{50} of 67.59 µg/ml. The scavenging activities of ELE-CA, vitamin C and gallic acid increased with increasing concentrations of the extracts. As shown in Figures S1(iii), the ELE-CA, Ascorbate and gallic acid exhibited a concentration-dependent increase in ferric-reducing antioxidant power. However, at concentrations of 80 µg/mL and 100 µg/mL, ascorbate and gallic acid exhibited better reducing power than ELE-CA.

Quantitative examination of bioactives by HPLC in ELE-CA revealed the concentration of natural flavonoids like catechin (3.55 x 10^4 mg/100 g), rutin (1.041 x 10^4 mg/100 g), kaempferol (46.12 mg/100 g), apigenin (35.87 mg/100 g), quercetin (22.99 mg/100 g); and certain phenolic acids, such as gallic acid (6263.42 mg/100 g), *P*-coumaric acid (624.45 mg/100) and chlorogenic acid (62.20 mg/100 g), as shown in Table S1.

3.2. Impact of ethanol Leaf-extract of Cnidocolous aconitifolius on glucose, insulin and GLUT levels of Albino rats exposed to mixture of $Pb(NO_3)_2$ and $HgCl_2$

The effects of ELE-CA on glucose and Insulin levels are shown in Figures S2(i) and S2(ii), respectively. In comparison with the Group 1 experimental rats, the administration of the mixture of $Pb(NO_3)_2$ and $HgCl_2$ (at doses of 11.25 and 0.4 mg per kilogram body weight, respectively), daily, increased serum glucose and insulin levels ($p < 0.05$). After ELE-CA intervention, both serum glucose and insulin levels of albino rats exposed to a mixture of $Pb(NO_3)_2$ and $HgCl_2$ decreased substantially ($p < 0.05$). In Figure S2(ii), the impact of the ethanolic leaf extract of *C. aconitifolius* on the insulin level of albino rats exposed to a mixture of lead nitrate and mercury chloride showed no marked difference ($p < 0.05$) in Groups 1 and 5. There was a marked difference ($p < 0.05$) between Group 2 and 1, 3, 4, and 5 experimental rats. In addition, there was a discernible difference between the normal control group 1 and groups (3 and 4) respectively.

The effects of the ethanolic leaf extract of *C. aconitifolius* on GLUT 4 level in albino rats exposed to a mixture of $Pb(NO_3)_2$ and $HgCl_2$ are presented in Figure S2(iii). Group 2 exhibited a marked decline ($p < 0.05$) in GLUT 4 level. Co-administration of the heavy metal mixture with ethanol leaf extract of *C. aconitifolius* produced a remarkable increase ($p < 0.05$) in GLUT 4 level in a dose-dependent manner.

3.3. Impact of ethanol leaf extract of C. aconitifolius on some lipid profile parameters of rats exposed to mixture of $Pb(NO_3)_2$ and $HgCl_2$

The effects of *C. aconitifolius* on total cholesterol, triacylglycerides, HDL-C, LDL-C and VLDL-C in experimental rats administered a combination of $Pb(NO_3)_2$ and $HgCl_2$ are shown in figures S3(i) – (v), respectively. Exposure to the combined solution of $Pb(NO_3)_2$ and $HgCl_2$ caused a marked increase ($p < 0.05$) in the concentrations of total

cholesterol, LDL and VLDL and a significant decline in the amount of HDL (Figures S3(i), (iii) – (v)). Co-administration of the heavy metal mixture with ethanol leaf extract of *C. aconitifolius* produced a profound increase ($p < 0.05$) in HDL levels as well as a marked decrease in total cholesterol, LDL and VLDL.

3.4. Impact of ethanol leaf extract of C. aconitifolius on HCRP level of rats exposed to mixture of $Pb(NO_3)_2$ and $HgCl_2$

The effects of *C. aconitifolius* on HCRP level in experimental rats administered a combination of $Pb(NO_3)_2$ and $HgCl_2$ is shown in figure S4. In comparison with the control group, there was a significant elevation ($p < 0.05$) in the level of HCRP in rats exposed to the combination of $Pb(NO_3)_2$ and $HgCl_2$. In a dose-dependent manner, the elevated increase in the level of HCRP protein of rat exposed to the $Pb(NO_3)_2$ and $HgCl_2$ was significantly ($p < 0.05$) reduced by the administration of alcoholic extract of *C. aconitifolius.*

3.5. Impact of ethanol leaf extract of Cnidoscolus aconitifolius on MDA and GSH contents, and catalase, SOD and GPx activities of albino rats administered $Pb(NO_3)_2$ and $HgCl_2$

The effects of ethanol leaf extract of *C. aconitifolius* on MDA, GSH, Catalase, SOD and GPx activities in experimental rats exposed to combined solutions of $Pb(NO_3)_2$ and $HgCl_2$ are shown in figures S5(i), S5(ii) and S6(i) – S6(iii). There was a marked increase in the level of MDA in rats in group 2 and a marked decline in the MDA amount in albino rats in groups 3, 4 and 5 was observed after co-administration of the heavy metals mixture with ethanol leaf extract of *C. aconitifolius* to the extent that there was no discernible difference in the MDA quantity in rats in the normal control and group 5 (Figure S5(i)). Conversely, there was a profound increase ($p < 0.05$) in GPx, GSH, SOD and catalase activities in rats in group 2 and a substantial increase in GPx, GSH, SOD and catalase activities in rats in groups 3, 4 and 5.

4. Discussion

As shown in Table S1, the high concentrations of natural flavonoids (catechin and rutin) observed in this study strongly suggest that the leaf extract could serve as a good antioxidant source. Natural substances derived from plants can act as antioxidants and reduce oxidative stress. Phenolic compounds derived from natural sources are well known for their abilities as hydrogen donors, chelators for metals, reducing agents, and radical scavengers (Valenzuela Soto et al. 2015). Plants with high quantities of polyphenols (flavonoids and phenolic acids) have attracted substantial attention owing to their antioxidative potential. Valenzuela Soto et al. (2015) and Orji et al. (2016) reported the isolation of quercetin, kaempferol, catechin, and rutin from *C. aconitifolius* leaves. In addition, many bioactive substances, including anthraquinones, flavonoids, triterpenoids, saponins, cardiac glycosides, phlobatannin and phenols, have been found in *C. aconitifolius* leaf extract (Azeez et al. 2010; Otitolaiye and Asokan

2016). Therefore, the results of the HPLC quantitative investigation of the bioactive compounds found in ELE-CA authenticate the reports of previous studies on *C. aconitifolius* leaves as a potential source of antioxidants in the human diet. It could also serve as an efficacious therapeutic agent against heavy metals mixture-induced insulin resistance, redox imbalance, and inflammation in living cells, thereby presenting its pharmacological relevance in the management of type 2 diabetes (T2D) because insulin resistance plays a major pathophysiological role in T2D (Azeez et al. 2010).

The well-established metrics for assessing the antioxidant potential of plant extracts include DPPH radical-scavenging ability, total reducing power, and NO radical scavenging capability. These criteria were adopted in this investigation to assess the antioxidative potential and activity of *C. aconitifolius* leaf extracts. The results of DPPH and NO radical scavenging capabilities, as well as the reducing power of ELE-CA in figures S1(i) – (iii), respectively, showed that ELE-CA, vitamin C and gallic acid were efficacious in inhibiting radicals in a concentration-dependent manner. According to Bulama et al. (2021), antioxidant properties of plants are helpful in the treatment of disorders. The use of *C. aconitifolius* leaves has been linked to some advantages such as the treatment of diabetes, gout, inflammatory swelling, scorpion sting and venereal disease (Ajiboye et al. 2018). Most of these illnesses directly cause oxidative stress as a direct cause (Ajiboye et al. 2018). In a study that focused on the antioxidant properties of *C. aconitifolius* and the ability of plant leaf extract to combat cataracts, *in vitro*, (Bulama et al. 2021) found that the crude extract and the various fractions investigated in the study exhibited outstanding reducing antioxidant potential and radical-scavenging capabilities; the ethyl acetate, n-hexane and dichloromethane fractions had a higher reducing power than L-ascorbate and the crude extract. Paredes et al. assessed comparable outcomes using *C. quercifolius* Pohl, belonging to the genus *C. aconitifolius* (Paredes et al. 2016). The variability in the makeup of the bioactive chemicals in ELE-CA may be responsible for the IC_{50} reported for the radical scavenging capabilities of the substance. The IC_{50} of ELE-CA in this study was higher than those of vitamin C and gallic acid; hence, both gallic acid and vitamin C possess better radical scavenging potentials than ELE-CA.

The impact of ELE-CA on glucose levels in albino rats exposed to a mixture of $Pb(NO_3)_2$ and $HgCl_2$ (Figure S2(i)) showed no marked difference ($p < 0.05$) in experimental rats in Group 1 and those in Groups that received 400 mg per kilogram body weight and 600 mg per kilogram body weight of ELE-CA. There exists a marked difference ($p < 0.05$) between that was administered mixture of $Pb(NO_3)_2$ and $HgCl_2$ and groups (1, 3, 4 and 5). There was also a marked difference between Groups 2 and 3. The report of this study, however, suggests that *C. aconitifolius* leaf extract could be hypoglycaemic at doses of 200 mg/kg body weight and 400 mg/kg body weight, as well as at a dose of 600 mg/kg body weight, but their hypoglycaemic activity increases in a dose-dependent manner, as shown in Figure S2(i). According to Karunaweera et al. (2015), certain polyphenols, including apigenin, quercetin, rutin, and resveratrol, impede the activity of kinases by blocking the translocation and phosphorylation of nuclear factor-kappa B, which is necessary for the production of COX-2. Nevertheless, (Yoshida et al. 2013) found out in their study that toll-like receptors (TLR) play a role in inflammation processes mediated by fat in adipose tissue, which brings about resistance to insulin, as observed in type 2 diabetes. Consequently, it is crucial to understand how TLR expression

or activation is properly regulated to understand the hypoglycaemic effect of ELE-CA. (Yoshida et al. 2017) showed that some flavonoids, such as naringenin, reduce the expression of genes that encode TLR2 in macrophage and adipocyte co-culture, as well as TLR2 expression induced by tumour necrosis factor-alpha (TNF-α), by reducing the rate at which nuclear factor-kappa B is activated. In this study, it could be said that natural flavonoids such as rutin and quercetin, as reported by HPLC quantitative analysis of bioactive compounds in ELE-CA, could inhibit the expression of TLR2 through PPAR activation. A decrease in glycaemia may be due to the comparatively high quantities of rutin, quercetin, and other phenolic compounds present in ELE-CA when these potential contributions by ELE-CA are taken into account. These phenolics was reported to possess brilliant hypoglycaemic characteristics; (Morantes-Caballero et al. 2017) gave explanations on the involvement of nuclear factor-kappa B, nicotinamide adenine dinucleotide phosphate (reduced) oxidase and BAX (a pro-apoptotic gene) in mitochondrial proteins glycosylation and consequent oxidative assaults. They explained how a decrease in hydrogen peroxide (whose level is known to increase in hyperglycaemia and has a direct link with nuclear factor-kappa B) occurs as a result of the NADPH produced by glucose metabolism. Hence, ELE-CA, like metformin, may also exert its hypoglycaemic effects by obstructing these pathways by reducing nuclear factor-kappa B expression and impeding the activity of the kinases responsible for the stimulation of gluconeogenesis in the liver (Rena et al. 2017).

As shown in Figure S2(ii), the results of this study suggest that *C. aconitifolius* leaf extract could reduce the insulin levels in rats administered 200, 400, and 600 mg/kg body weight, dose-dependently to the level that can be compared with the Group 1 experimental rats (i.e. the normal control rats), as shown in Figure S2(ii).

In our study, as shown in Figure S2(iii), AMPK phosphorylation and its consequent activation, which brought about an upsurge in GLUT-4 expression and translocation in the skeletal muscle cell membrane, can be attributed as a possible explanation for the increased GLUT 4 expression after exposure to a mixture of $Pb(NO_3)_2$ and $HgCl_2$ and co-treatment *with C. aconitifolius* ethanol leaf extract (Bao et al. 2016) who investigated the response of diabetic mice to catalpol. In diabetic mice, catalpol improved insulin resistance and diabetes, as well as the expression of GLUT-4. Owing to its function in controlling glucose metabolism, research has indicated the significance of AMPK, as a major therapeutic target in diabetes (Huang et al. 2016). Animal models of diabetes have been shown to have lower peripheral tissue p-AMPK levels (Steinberg and Kemp 2009). When compared to controls, GLUT-4 expression, as well as p-AMPK expression were both observed to be higher after Catalpol therapy. In skeletal muscle, as well as adipose tissue, it is thought to be facilitated by AMPK phosphorylation and its consequent activation (Yamaguchi et al. 2005). (Kumar et al. 2016; Guo et al. 2021) presented similar results. In our study, we also noticed that rats treated with *C. aconitifolius* had higher GLUT-4 expression. Thus, we speculate that the enhanced expression of GLUT-4 in skeletal muscles observed in *C. aconitifolius*-treated rats may be caused by the same mechanism.

The results of the effect of *C. aconitifolius* on total cholesterol, triacylglycerides, HDL-C, LDL-C and VLDL-C in experimental rats administered a combined solution of $Pb(NO_3)_2$ and $HgCl_2$ as presented in Figure S3(i) – (v), revealed that exposure to the combined solution of lead nitrate and mercury chloride caused a marked increase

($p < 0.05$) in the concentrations of total cholesterol, LDL and VLDL, and a marked decline in the amount of HDL. The combination of heavy metals with the ethanol leaf extract of *C. aconitifolius* resulted in a considerable decline in total cholesterol, LDL and VLDL, and a profound increase ($p < 0.05$) in HDL levels. The results of this study suggest that *C. aconitifolius* could effectively improve the impairment of lipid and glucose metabolism as well as the insulin resistance of albino rats after exposure to a combined solution of $Pb(NO_3)_2$ and $HgCl_2$ as presented in Figure S3(i) – (v) above. Administration of extracts obtained from *C. aconitifolius* leaves caused reductions in TC, LDL-C, VLDL-C and TAG levels in a dose-dependent manner, with the lowest dose proving to be the most effective. According to Ademuyiwa et al. (2005), a higher level of cholesterol in plasma can cause atherosclerosis and other heart-related illnesses. As a result, lowering the level of cholesterol associated with the plasma reduces the occurrence of cardiac diseases. Moreover, administration of *C. aconitifolius* leaf extract decreased the total cholesterol levels at a dose-dependent rate, with a dose of 600 mg/kg BW being more efficient. According to this inference, leaf extracts may prevent cardiovascular illnesses. This may have been caused by several bioactive substances that were shown to be polyphenolics and are believed to have the ability to reduce cholesterol in plasma, as well as possessing protective roles against atherosclerosis, according to Ikewuchi et al. (2011). These elements may have contributed to the hypocholesterolemic effects of the extract, alone or in combination. Indicators of independent and synergistic risk factors for cardiovascular illnesses include increased plasma triglyceride levels (Martirosyan et al. 2007). It may also result in hypertension and is caused by improper lipoprotein metabolism (Shen 2007).

As revealed in this study, the reduction in the activities of SOD, GPx, GSH and CAT and increase in the level of MDA, as seen in group 2 rats, could be an indication of high levels of free radicals generated from the mixture of heavy metals, which promotes oxidative stress (Galicia-Moreno and Gutiérrez-Reyes 2014). There were similarities in the reports by Moncada et al. (1994) and (Galicia-Moreno and Gutiérrez-Reyes 2014) regarding the ability of prolonged *in vivo* free radicals to cause tissue damage and oxidative stress in rats, as indicated by elevated MDA levels in group 2 animals (Figure S5(i)) and a subsequent reversal in MDA levels (Figure S5(i)) of rats in the normal control and groups 3 and 4, as well as group 5 following administration of ethanol leaf extract of *C. aconitifolius*. The upsurge in GPx, GSH, SOD and catalase activities as seen in (Figureures S5(ii) and S6(i) – (iii)) could be due to flavonoids like catechin (355 x 10^4 mg/100 g), rutin (1.041 x 10^4 mg/100 g), kaempferol (46.12 mg/100 g), apigenin (35.87 mg/100 g), quercetin (22.99 mg/100 g) and certain phenolic acids like gallic acid (6263.42 mg/100 g), *P*-coumaric acid (624.45 mg/100), chlorogenic acid (62.20 mg/100 g) found in the *C. aconitifolius* leaf extract of as presented in Table S1 of this study. The termination of oxidative reactions by antioxidants could either be due to the inhibition of further oxidative reactions or by the removal of radical intermediates (Galicia-Moreno and Gutiérrez-Reyes 2014).

These findings are consistent with the antioxidant effects of bioactive compounds reported by Ninfali et al. (2017), (Boaventura et al. 2015), (Apostolidou et al. 2015) and (Martinez-Lopez et al. 2020), which have also been presented as antioxidant-rich foods. One study found that a 90-day mate tea intervention significantly increased blood antioxidant activity in the population with dyslipidemia when compared to baseline

values ($p < 0.05$) (Boaventura et al. 2015). Another study showed that daily red wine consumption (125 mL for women and 250 mL for men) for one month significantly increased the overall antioxidant capacity in participants with hypercholesterolaemia (Cardoso et al. 2015). Moreover, 8 weeks of daily orange juice consumption (750 mL) in overweight patients improved antioxidant activity and reduced TC (Martinez-Lopez et al. 2019). This is significant because it has been demonstrated that not all antioxidant meal therapies in participants with dyslipidemia resulted in changes in circulating antioxidant activity (Dourado et al. 2015). These food categories have drawn much attention because of the possible antioxidant activity of the polyphenols' hydroxyl groups and double bonds (Glevitzky et al. 2019). A significant improvement in the lipid profile of experimental individuals with dyslipidemia has also been observed after consuming various foods rich in polyphenols, such as fruits and vegetables (Suwimol et al. 2012), blackberry juice (Kosmala et al. 2017) and red grapes (Razavi et al. 2013). Based on our findings, ELE-CA is a low-cost nutritional therapy for dyslipidemia.

We found that administering ELE-CA to the rat subjects not only boosted antioxidant systems but did well in also reduced MDA content, indicating a protective effect against oxidative stress. Orange juice and coffee are two food substances that have been thoroughly investigated for their antioxidative potential, as well as their ability to reduce oxidative assault on lipids (Martinez-Lopez et al. 2019; 2020). Although oxidative alteration of plasma lipids and lipoproteins is a significant target for many antiatherogenic drugs, it is preferable to avoid the onset of cardiovascular illnesses using the antioxidant activity of polyphenols. Additionally, polyphenols found in ELE-CA, such as quercetin and kaempferol, act by triggering the expression of Nrf2, a transcription factor that subsequently activates reaction cascades that trigger CAT and SOD genes expression (Guevara-Cruz et al. 2021). Our findings showed that ELE-CA administration could induce the expression of genes that encode CAT, which is consistent with prior research showing that dietary interventions with foods rich in antioxidants can help mitigate the prevalence of dyslipidemia, by activating antioxidant enzymes. For instance, *Graptopetalum paraguayense,* a very popular herb home to China, substantially improved glutathione peroxidase and catalase activities. Additionally, these findings imply that polyphenols in ELE-CA help cells maintain their redox equilibrium by activating antioxidant enzymes. As shown in Figure S4, increasing plasma levels of polyphenols at doses of 200, 400, and 600 mg of plant extracts per kilogram body weight may have activated the antioxidant system enough to cause a decline in the inflammatory marker, HCRP.

5. Conclusion

Hence, we can conclude that polyphenol dosages directly ameliorate the inflammatory effects of heavy metals by upregulating antioxidant enzymes, modulating serum glucose and insulin levels and improving lipid profiles in experimental rats.

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