Atrazine-induced/streptozotocin-induced oxidative stress and mitigating effect of virgin coconut oil in male Wistar rats.

Olatunbosun Titilope[a]*, Uka Emmanuel[b]

[a] expensivetiti@gmail.com; [b]emmanueluka@uniuyo.edu.ng

[a]Department of Physiology, University of Uyo, P.M.B. 1017, Uyo, Akwa Ibom State, Nigeria.

[b]Department of Biochemistry, University of Uyo, P.M.B. 1017, Uyo, Akwa Ibom State, Nigeria.

Abstract

This study assessed ameliorative effect of Virgin Coconut Oil (VCO) following atrazine-induced metabolic derangement in rats. Adult male albino Wistar rats weighing 180-200g body weight were used for the study. They were randomly separated into two major experimental groups (The test and recovery groups). Thirty-five (35) rats in the test group were randomly divided into five sub-groups of 7 rats per sub-group (n=7) and were treated thus: Sub-group (SG) 1 served as normal control and received 10ml/kg body weight of distilled water, SG 2 received 10ml/kg of VCO, SG 3 received 123mg/kg of Atrazine (ATZ), SG 4 was the diabetic control that were left untreated and SG 5 was the diabetic group that were treated with 10ml/kg of VCO. Treatment in the test group lasted for 2 weeks, after which the animals were sacrificed and blood collected for analysis. During these 2 weeks' period, thirty-five rats for the recovery group were also divided into 5 subgroups of 7 rats per sub-group (n=7) and were treated as follows: SG 1 served as normal control and received 10ml/kg body weight of distilled water, SG 2 received 10ml/kg of VCO, SG 3, 4 and 5 received 123mg/kg of ATZ. After 2 weeks, the animals were re-treated for recovery and were treated thus: SG 1 received 10ml/kg body weight of distilled water, SG 2 received 10ml/kg of VCO, SG 3 received 123mg/kg of ATZ, SG 4 was treated with 10ml/kg of VCO and SG 5 was given 10ml/kg of distilled water. After 2 weeks, the animals were also sacrificed and blood collected for analysis. In the test groups, on oxidative enzymes; Glutathione (GSH), Superoxide Dismutase (SOD) and Catalase (CAT) levels were significantly reduced ($p<0.05$) in the atrazine and diabetic groups when compared to normal control. Following recovery, GSH was significantly increased ($p<0.05$) in the VCO recovery group when compared to ATZ group. In conclusion, ATZ toxicity caused oxidative stress but its withdrawal significantly reduced the stress; with more pronounced effect following VCO administration.

Keywords: Atrazine; Virgin Coconut oil; oxidative enzymes; Glutathione (GSH); Superoxide Dismutase (SOD); Catalase (CAT) levels

Introduction

Oxidative stress is defined as a disruption in pro-oxidants and antioxidants proportion, favoring the prooxidants because of several reasons which include inflammation, ageing, addiction, and drug toxicity [1]. Generally, it is over-production or deficient disposal of greatly reactive elements, which includes reactive oxygen species (ROS) and reactive nitrogen species (RNS)) [2]. It is well-known that oxygen (O_2) is mainly responsible for life termination; and a significant part in aerobic respiration. Yet, in certain conditions, O_2 might be cell destroyer by producing reactive species which leads to cell injury and finally death of the cells. ROS and RNS likewise oxidize through the formation of certain components which causes interference with normal cell processes [3]. O_2 is exceptionally reactive with the capacity of being a member of the free radicals, which are toxic and destroying elements. Oxidative stress attacks body cells that are healthy and make them loose their framework and capacity.

Oxidative stress has been reported to be involved in the advancement of numerous pathological conditions affecting humans. Nevertheless, reactive oxygen species might be helpful, because they can be utilized by lymphocytes as an approach to combat and destroy pathogenic organisms [4]. Ageing can also be prevented by oxidative stress occurring for a short term by inducing a mechanism called mitohormesis [5]. It is the point at which the antioxidant concentration is limited that this damage can debilitate the cell [6]. Destruction of proteins, DNA, and different large molecules because of oxidative stress have been incriminated in development of a wide array of disorders, especially cardiovascular diseases and cancers [7].

Oxidative stress is believed to be majorly involved in generation of complications associated with diabetes specifically type II diabetes mellitus [8]. Oxidative stress performs a major part in cell damage as a result of high glucose level; this hyperglycemia can facilitate production of ROS or free radicals. The weakened body defence mechanism will not be able to counter the increased production of ROS and subsequently, an imbalance will occur between antioxidant protection and ROS, causing the oxidative stress to dominate [9]. A specific measure of oxidative stress is needed for the physiological procedures because ROS carry out different control functions in the body tissues [10]. Free radicals or ROS are generated by macrophages and neutrophils when respiratory burst occurs, so as to cause antigen elimination [11]. In addition, they send stimulating alerts to some genes encoding transcription components, cellular signaling, differentiation, improvement and stimulation of cellular-cellular adhesion, proliferation of fibroblast, vasoregulation inclusion, and elevated antioxidant enzymes expression [12]. However, over and/ uncontrolled generation of ROS is destructive. Elevation of ROS level in diabetes mellitus might be because of diminishes in elimination or increment in generation by antioxidants such as, superoxide dismutase, catalase, and glutathione peroxidase. The difference in the antioxidant level renders the body tissues vulnerable to oxidative stress causing advancement of complications associated with diabetes [13]. According to epidemiological studies, diabetic mortalities can be explained notably by an increase in vascular diseases other than hyperglycemia [8]. The mitochondria are the principal origin of oxidative stress in diabetes mellitus.

Atrazine (ATZ) is a known persistent organic pollutant which is commonly used as an herbicide worldwide; which has been widely accepted in Nigeria [14]. Humans are exposed to ATZ in the air [15], water [16] and food. Atrazine effect in human and animals primarily involve the endocrine system. Atrazine has been reported to have oxidative stress ability in a research carried out on various species, including rats [17]. ATZ was reported to reduce various antioxidative enzymes in mice and rats, thus leaving the organism prone to oxidative stress. An association has been reported between prolonged oxidative stress and insulin resistance – a characteristic of type 2 DM [18]. The harmful impacts of ATZ can be due to its production of reactive oxygen species which initiate oxidative stress in different tissues. Increase in oxidative stress has been incriminated in the development of liver damage induced by herbicide [19]. Exposure to atrazine brings about diminished kidney and liver glutathione (GSH) levels Glutathione (GSH) is the most abundant antioxidant in body fluids and tissues. It scavenges the free radicals and thus protects the tissues from oxidative stress; GSH also participates in the detoxification of hydrogen peroxide by various glutathione peroxidases. However, ATZ reduces the levels of GSH in the liver and kidney. The impact of this ATZ in distorting the GSH and MDA levels brings about oxidative stress and thus diabetic complications and tissue damage [20].

The antioxidant polyphenols have been observed to be abundant in VCO than copra oil (CO) [21]. Estimation of absolute polyphenol substance demonstrated that VCO contained 84 mg/100g oil while CO contained 64.4 mg/100 g oil [22]. Diet enhanced by VCO addition boosted the antioxidant levels in experimental rodents [23]. Despite the fact that the fatty acid analysis of CO and VCO showed that they possess equal saturated fatty acids quantity, the more the unsaponifiable parts such as polyphenols and vitamin E might contribute to these VCO effects. Another research demonstrated that virgin coconut oil reduced oxidative stress induced by alcohol by decreasing level of testicular malondialdehyde (tMDA), thus reducing the harmful impacts of the liquor on testosterone concentration in experimental rodents [23]. [23] observed the actions of antioxidant enzymes concentration in male experimental rodents administered with groundnut oil (GO), VCO, and CO. Their outcomes demonstrated that actions of catalase (CAT) and superoxide dismutase (SOD) were observed to be significantly higher in VCO and CO treated rats when compared to GO but VCO treated rats indicated more beneficial impact than CO. [24] observed a critical decrease in protein carbonyl adducts, conjugated dienes and serum and hepatic thiobarbituric acid reactive substances concentration in rats fed with VCO containing diet which implies a decrease in oxidative and carbonyl stress when compared to CO nourished rats.

Additionally, VCO fed rats indicated improved redox status of the liver. They conclusively reported that VCO could be an effective nutraceutical in avoiding insulin resistance activated by food, including complications related to it via its antioxidative capacity.

MATERIALS AND METHODS

Experimental animals

Adult male albino Wistar rats (180-200g body weight) were purchased and maintained at the animal house Unit of the Department of Physiology, Faculty of Basic Medical Sciences, University of Calabar. The animals were kept in a well-ventilated space to acclimatize for two weeks. The animals were fed with rat chow and allowed drinking water ad libitum. After the acclimatization period, the animals were weighed, their fasting blood glucose level were measured and reassigned before the commencement of the experimental treatment. The cages were cleared and kept clean throughout the period of the experiment.

Experimental design and treatment of animals

The rats were randomly separated into two major experimental groups (the test and recovery groups) of 35 rats in each major group. Experiment for the test group lasted for two weeks while experiment for the recovery group lasted for four weeks.

Thirty-five (35) rats in the test group were randomly divided into five sub-groups of 7 rats per subgroup (n=7) and were oral gavaged and treated thus: sub-group (SG)1 served as normal control and received 10ml/kg body weight of distilled water, SG 2 received 10ml/kg of Virgin Coconut Oil (VCO), SG 3 received 123mg/kg (20% of lethal dose) of Atrazine (ATZ), SG 4 was the diabetic control that were left untreated and SG 5 was the diabetic group that were treated with 10ml/kg of VCO. Treatment in the test group lasted for 2 weeks, after which the animals were sacrificed and blood collected for analysis.

During these 2 weeks' period, thirty-five rats for the recovery group were also divided into 5 subgroups of 7 rats per sub-group (n=7) and were treated as follows: (SG) 1 served as normal control and received 10ml/kg body weight of distilled water, SG 2 received 10ml/kg of Virgin Coconut Oil (VCO), SG 3, 4 and 5 received 123mg/kg of ATZ. After 2 weeks, the animals were re-treated for recovery and were treated thus: (SG) 1 served as normal control and received 10ml/kg body weight of distilled water, SG 2 received 10ml/kg of Virgin Coconut Oil (VCO), SG 3 received 123mg/kg of ATZ, SG 4 was treated with 10ml/kg of VCO and SG 5 was given 10ml/kg of distilled water. Treatment for recovery also lasted for 2 weeks, after which the animals were sacrificed and blood collected for analysis

EXPERIMENTAL GROUPING AND TREATMENT TEST GROUP (2 WEEKS)

GROUPS	TREATMENT
Normal Control + H_20	10ml/kg of distilled water (H_2O)
Normal Control + VCO	10ml/kg of Virgin Coconut Oil (VCO)
Atrazine Treated	123mg/kg (20% of lethal dose) of Atrazine
Diabetic Control	10ml/kg of distilled water (H_2O)
Diabetic +VCO	10ml/kg of Virgin Coconut Oil (VCO)

RECOVERY GROUP (4 WEEKS)

GROUPS	TREATMENT (1st 2 weeks)	TREATMENT (2nd 2 weeks)
Normal Control + H_20	10ml/kg of distilled water (H_2O)	10ml/kg of distilled water (H_2O)
Normal Control + VCO	10ml/kg of Virgin Coconut Oil (VCO)	10ml/kg of Virgin Coconut Oil (VCO)
Atrazine Treated	123mg/kg (20% of lethal dose) of Atrazine	123mg/kg (20% of lethal dose) of Atrazine
VCO after ATZ	123mg/kg (20% of lethal dose) of Atrazine	10ml/kg of Virgin Coconut Oil (VCO)
Untreated after ATZ	123mg/kg (20% of lethal dose) of Atrazine	10ml/kg of distilled water (H_2O)

Induction of Diabetes mellitus (DM)

Diabetes was induced intraperitoneally using 150mg/kg body weight of alloxan monohydrate [25, 26]. The diabetic state was observed from about 48 hours by the symptoms of polyuria and glucosuria. After 72 hours, DM was confirmed with blood glucose level of 180-200mg/dL and above [27] using a glucometer (ACCU-CHECK Active) and ACCU-CHECK compatible glucose test strips.

Preparation of Virgin Coconut Oil (VCO)

Mature dried coconuts were purchased from the local market and VCO was extracted using the modified wet extraction method [23]. The solid endosperm of mature coconut was crushed and made into thick slurry. About 500 ml of water was added to the slurry obtained and squeezed through a fine sieve to obtain coconut milk. The resultant coconut milk was left for about 18 hours to facilitate the gravitational separation of the emulsion. Demulsification produced layers of an aqueous phase (water) at the bottom, an oil phase in the middle layer and emulsion phase (cream) on top. The cream on top was removed and the oil was scooped and warmed for about 5 minutes to remove moisture. The oil obtained was then filtered and stored at room temperature.

Evaluation of GSH concentration

My BioSource Rat Glutathione (RGLU) ELISA kit (USA) was used. It is a 1.5 hour solid-phase ELISA designed for the quantitative determination of RGLU as applied by Noeman et al, (2011) [28].

100 µL of Standards or Samples was added to the appropriate well and 100 µL of PBS was added in the blank control well.50 µL of conjugate was added to each well except the blank control well and mixed very well. The plate was covered and incubated for 1 hour at 37°C. Finally, a stop solution was added to stop the reaction, which will then turn the solution yellow. The intensity of color is measured spectrophotometrically at 450nm in a microplate reader. The intensity of the color is inversely proportional to the RGLU concentration since RGLU from samples and RGLU-HRP conjugate compete for the anti-RGLU antibody binding site. Evaluation of serum SOD concentration.

Elabscience® Rat ELISA kit (USA) was used for determination of serum SOD concentration as applied by Fujita et al., (2011) [29].

50µl Sample dilution buffer and 50µl sample are added and loaded onto the well bottom, 50 µL Biotinylated Detection Ab was immediately added to each well, mixed and incubated for 45 min at 37ºC after being sealed with closure plate membrane. After incubation, plates are washed gently, aspirate and refilled with wash solution and washed three times. 100 µL HRP-conjugate reagent was added to each well and incubated for 30 min at 37ºC, it was aspirated and washed 5 times. 90 µL of TMB Substrate Reagent was added and incubated for 15 min at 37°C. 50 µL stop solution was added to each well to terminate the reaction. The colour in the well changed from blue to yellow. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm.

Evaluation of serum CAT concentration

Elabscience® Rat ELISA kit (USA) was used for determination of serum CAT concentration as applied by [29].

50µl Sample dilution buffer and 50µl sample are added and loaded onto the well bottom, 50 µL Biotinylated Detection Ab was immediately added to each well, mixed and incubated for 45 min at 37ºC after being sealed with closure plate membrane. After incubation, plates are washed gently, aspirate and refilled with wash solution and washed three times. 100 µL HRP-conjugate reagent was added to each well and incubated for 30 min at 37ºC, it was aspirated and washed 5 times. 90 µL of TMB Substrate Reagent was added and incubated for 15 min at 37°C. 50 µL stop solution was added to each well to terminate the reaction. The colour in the well changed from blue to yellow. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm.

Statistical analysis

Statistical analysis was carried out using windows SPSS package (SPSS 20.0). Data were analyzed using one-way ANOVA followed by Tukey's post hoc test. Data were expressed as mean + standard error of mean (Mean ± SEM). Results with values of $p<0.05$ were considered significant.

RESULTS

GSH levels (ng/mL) in normal control, ATZ and diabetic groups

The mean values for GSH in the NC + H_2O, NC + VCO, ATZ treated, Diabetic control and Diabetic +VCO groups are 0.57±0.06, 0.74±0.04, 0.47±0.01, 0.43±0.02 and 0.68±0.07 respectively. There was a significant ($p<0.05$)

increase in GSH level in NC+VCO group when compared with NC + H_2O group. GSH level was significantly ($p < 0.05$) decreased in the ATZ treated group when compared with the NC + H_2O and NC + VCO groups. In the diabetic untreated group, GSH level was significantly ($p < 0.05$) lowered when compared with the NC + H_2O and NC + VCO groups but not significantly different from the ATZ treated group. The diabetic + VCO showed a significant ($p < 0.05$) increase in GSH level when compared with the NC + H_2O, diabetic control and ATZ treated groups but a significant decrease was observed when compared with the NC + VCO groups (Figure 1).

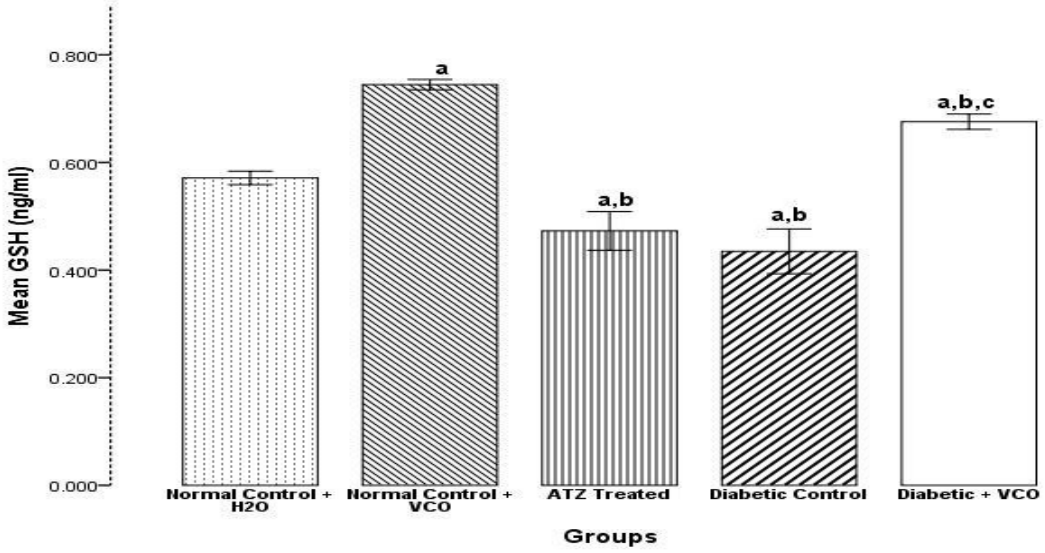

Fig 1: Comparison of GSH levels in Control, ATZ and Diabetic groups. Values are mean ±SEM. n=7. a=p<0.05 vs NC, b= p<0.05 vs NC+VCO, c= p<0.05 vs ATZ, d= p<0.05 vs diabetic control.

GSH levels in normal control and ATZ recovery groups

In figure 2, it was observed that there was a significant ($p < 0.05$) decrease in GSH levels in the ATZ continued group (0.53±0.02) when compared with the NC + H_2O (0.72±0.02) and NC + VCO (0.60±0.01) groups. In VCO recovered group (0.53±0.02), GSH level was significantly ($p < 0.05$) lower when compared with the NC + H_2O and NC + VCO groups but significantly lower when compared with the ATZ continued group. GSH level was significantly ($p < 0.05$) lower in the ATZ untreated group (0.52±0.06) when compared with NC + H_2O, NC + VCO groups but significantly higher when compared with the ATZ continued group and there was no significant change when compared with the VCO untreated.

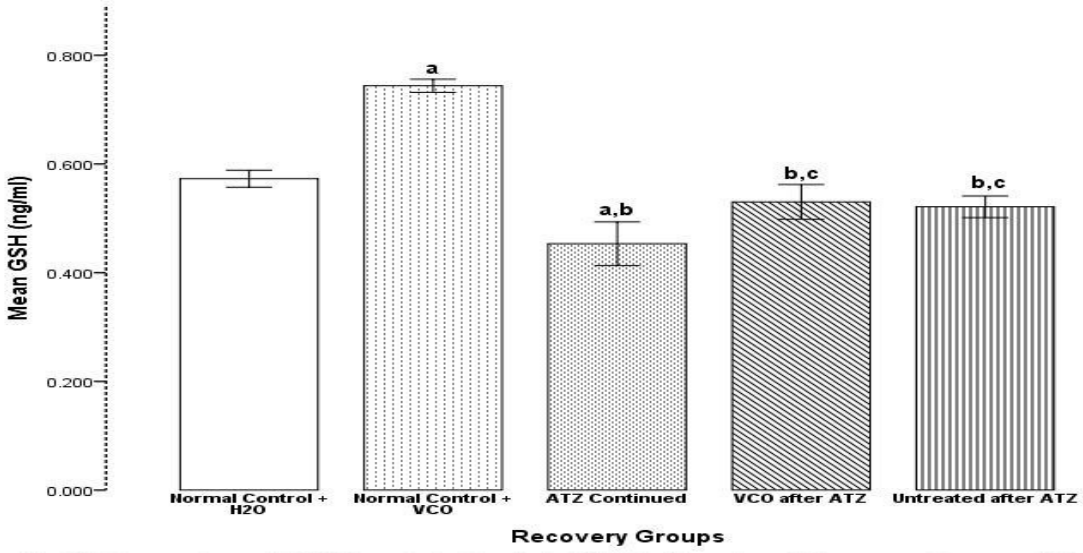

Fig 2: Comparison of GSH levels in Control, ATZ continued and Recovery groups. Values are mean ±SEM. n=7. a=p<0.05 vs NC, b= p<0.05 vs NC+VCO, c= p<0.05 vs ATZ continued, d= p<0.05 vs VCO after ATZ.

SOD levels (mg/dL) in normal control, ATZ and diabetic groups

Figure 3 showed a significant increase in SOD levels in the NC + VCO (4.19±0.06) group when compared with the NC + H_2O (3.04±0.03). SOD levels in ATZ treated group (3.01±0.05) were significantly lower than NC + VCO group but not significantly different from NC + H_2O group. There was a significant decrease in SOD levels in the diabetic control (1.03±0.02) when compared with the NC + H_2O, NC + VCO and ATZ treated groups. With VCO treatment in the diabetic group (2.35±0.05), SOD levels significantly increase when compared to the diabetic control but was significantly lower than the NC + H_2O, NC + VCO and ATZ treated groups.

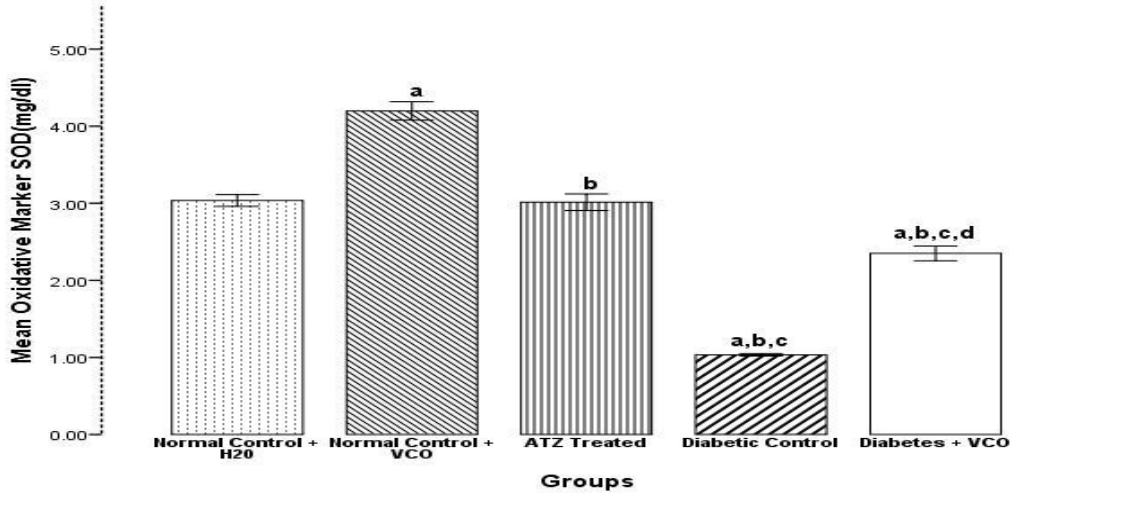

Fig 3: Comparison of SOD levels in Control, ATZ and Diabetic groups. Values are mean ±SEM. n=7. a=p<0.05 vs NC, b= p<0.05 vs NC+VCO, c= p<0.05 vs ATZ, d= p<0.05 vs diabetic control.

SOD levels in normal control and ATZ recovery groups

The mean values for SOD in the recovery group are 3.20±0.05, 4.20± 0.06, 2.28±0.07, 3.22±0.09 and 2.95±0.03 for NC + H_2O, NC + VCO, ATZ continued, VCO after ATZ and untreated after ATZ groups respectively. With continuous ATZ administration, the SOD levels in the ATZ continued group became significantly lower when compared with the NC + H_2O and NC + VCO groups. SOD levels in the VCO recovered and untreated groups were significantly higher when compared with the ATZ continued group but significantly lower than NC + VCO groups and not significantly different from the NC + H_2O group. There was no significant difference between the VCO recovered and untreated group, though a marginal increase in SOD levels was observed in the VCO recovered group (Figure 4).

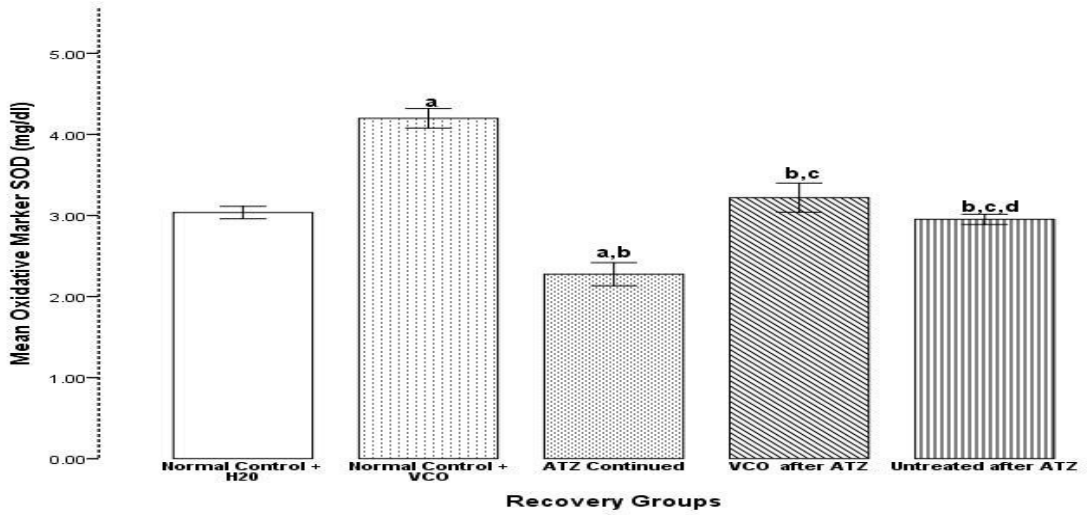

Fig 4: Comparison of SOD levels in Control, ATZ continued and Recovery groups. Values are mean ±SEM. n=7. a=p<0.05 vs NC, b= p<0.05 vs NC+VCO, c= p<0.05 vs ATZ continued, d= p<0.05 vs VCO after ATZ.

Catalase (CAT) levels (mg/dl) CAT levels in normal control, ATZ and diabetic groups

As shown in Figure 5, there was a significant (p<0.05) increase in CAT levels in the NC + VCO group (1.81±0.03) when compared with the NC + H_2O group (1.43±0.21). CAT level in the ATZ treated group (1.51±0.03) was not significantly (p<0.05) different from the NC + H_2O group but significantly (p<0.05) lower than the NC + VCO group. Diabetes induction significantly (p<0.05) reduced CAT level in the diabetic control group (0.45±0.03) when compared with the NC + H_2O, NC + VCO and ATZ treated groups.

In the diabetic group (1.50±0.04), there was a significant (p<0.05) increase in CAT level in the diabetes +VCO group when compared to the diabetic control group.

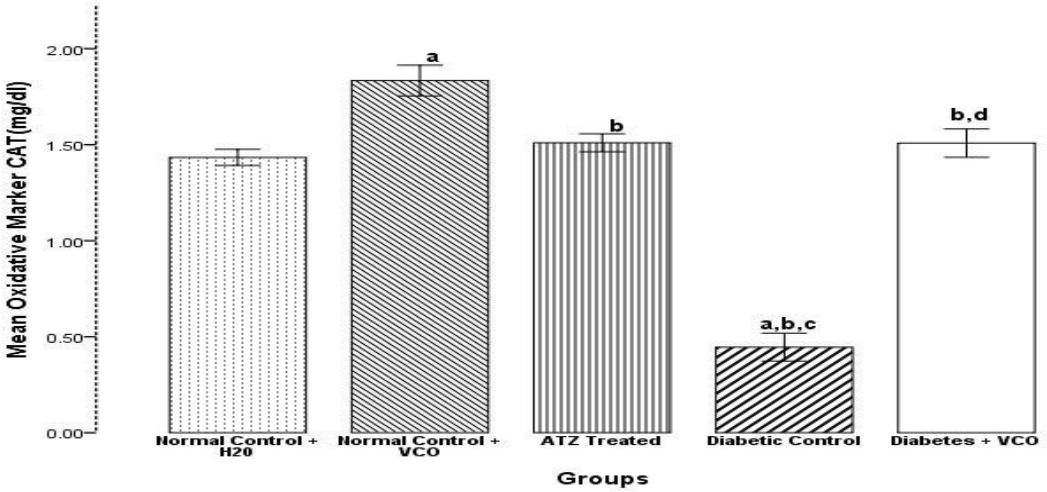

Fig 5: Comparison of CAT levels in Control, ATZ and Diabetic groups. Values are mean ±SEM. n=7. a=p<0.05 vs NC, b= p<0.05 vs NC+VCO, c= p<0.05 vs ATZ, d= p<0.05 vs diabetic.

CAT levels in normal control and ATZ recovery groups

In figure 6, a significant (p<0.05) decrease in CAT levels was observed in ATZ continued group (0.76±0.06) when compared with the NC + H_2O (1.45±0.04) and NC + VCO (1.86±0.06) groups. With VCO administration after ATZ (2.12±0.03), the CAT levels significantly (p<0.05) increased when compared with the NC + H_2O, NC + VCO and ATZ continued groups. The CAT level in the untreated group (1.76±0.02) was significantly (p<0.05) higher when compared with the ATZ continued group.

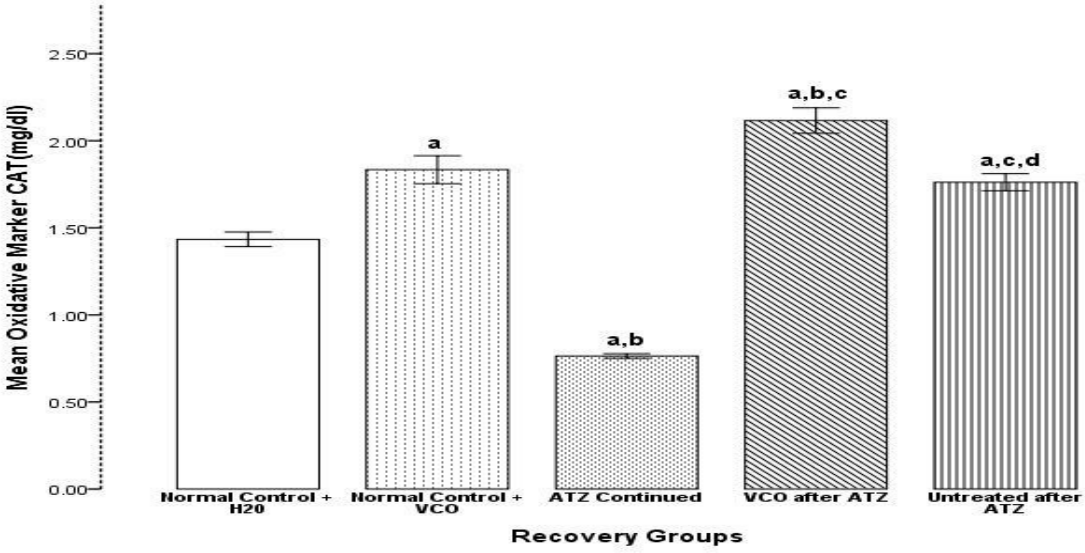

Fig 6: Comparison of CAT levels in Control, ATZ continued and Recovery groups. Values are mean ±SEM. n=7. a=p<0.05 vs NC, b= p<0.05 vs NC+VCO, c= p<0.05 vs ATZ continued, d= p<0.05 vs VCO after ATZ.

DISCUSSION

Oxidative stress contributes significantly to the pathophysiology of several diseases which include diabetes [30]. Several studies have reported elevations in specific oxidant stress markers in both experimental alloxan and human diabetes mellitus, together with reduced total antioxidant defense and depletion in individual antioxidants [31, 32]. Oxidative cellular injury caused by free radicals is said to contribute to the development of diabetes mellitus [33]. Reactive oxygen species occur naturally, but their accumulation is a marker for oxidative stress. Moreover, diabetes also induces changes in the tissue content and activity of the antioxidant enzymes [34]. Increased oxidative stress and lipid peroxidation are implicated in the pathogenesis of herbicide induced hepatic injury [35]. Several studies have shown that oxidative stress (OS) has been implicated in ATR toxicity through the evaluation of specific biomarkers in tissues such as liver, erythrocytes, testis, and epididymis in the rat [36, 37, 38]. ATZ induces oxidative damage, cytotoxicity and apoptosis in several *in-vivo* and *in-vitro* models system. The whole story of oxidative stress in diabetes mellitus is attributed to hyperglycemia as the cause; hyperglycemia generates reactive oxygen species which in turn causes oxidative stress and consequently resulting in secondary complications in diabetes mellitus [39].

GSH is an efficient antioxidant present in almost all living cells and is also considered as a biomarker of redox imbalance at cellular level. GSH also functions as free radical scavenger and in the repair of free radical caused biological damage [40]. Result showed a decrease in the GSH level in both the alloxan induced diabetic. Studies have reported reduced level of GSH in diabetes and may be one of the leading factors in the oxidative DNA damage in type II diabetes [41, 42]. It appears that generation of oxygen radicals by increased levels of glucose causes increased utilization of GSH [43]. The important mechanism implicated in the diabetogenic action of diabetogenic agents is by increased generation of oxygen free radicals, which causes a decrease in plasma GSH concentration, and plasma GSH/GSSG ratio [44]. The reduced level of blood GSH observed in the diabetic rats could be due to the destruction of pancreatic ß cells by alloxan probably through the generation of oxygen free radicals [45]. The result of this study showed a reduction in the GSH content in the atrazine treated rats when compared to the normal control and normal control +VCO groups. The significant decrease in the GSH content in erythrocytes of rats after atrazine exposure show the pro-oxidant activities going on in the erythrocytes, which could result in oxidative damage. The reduced levels of GSH in atrazine-treated rats could be due to either increased utilization of GSH for conjugation and/or participation of GSH as an antioxidant in terminating free radicals produced due to atrazine toxicity [46]. These results are consistent with the report by [47] who also indicated that the reduced glutathione (GSH) content and antioxidant power of the hepatocytes of atrazine treated mice were significantly decreased as compared to the control group. As a consequence of increased oxidative stress, GSH showed a frequent alteration in its concentration. Plasma GSH showed a significant decrease in diabetes as compared to normal [41]. Reduced glutathione (GSH), a nonenzymatic antioxidant plays an excellent role by protecting cells from oxidative damage keeping up the cellular levels of the active forms of vitamins C and E by neutralizing the free radicals. [48] reported that animals induced with atrazine shows a significant decrease in GSH levels. In the second phase of the study, we observed that supplementation of the atrazine induced group with VCO showed a reversal of the oxidative markers derangement. A study by [49] showed that administration of VCO polyphenols before and along with cadmium notably increased GSH content and activities of SOD and CAT while decreased MDA level in the liver of rats comparable to the normal rats. They attributed the improvement they

observed to the potent antioxidant and membrane-stabilizing properties of VCO polyphenols. These could also be responsible for the observed mitigating effect of VCO on atrazine induced oxidative stress in this study. [47] also reported that co-administration of ginger along with atrazine restored the GSH content and antioxidant power level of liver tissue nearly to control levels. Therefore, the recovery of the GSH content in our study was possibly achieved through the effect of VCO administered.

SOD is the primary step of the defense mechanism in the antioxidant system against oxidative stress by catalyzing the dismutation of 2 superoxide radicals (O_{2-}) into molecular oxygen (O_2) and H_2O_2 [50]. In this study, the SOD level in the normal control (VCO) group increased significantly when compared to the normal control group. This shows that there is an increase the antioxidant defense system of the body targeted to fight the reactive oxygen species of the body which causes oxidative stress in the body. This also proves the antioxidant property of virgin coconut oil. Reports have shown that the activities of SOD, CAT and GPx were lowered in tissues of diabetic rats [51]. [52] also reported that the activities of SOD, CAT and GPx were significantly reduced in liver, kidney and pancreas of diabetes induced rats. In diabetes, the oxidative stress damages the pancreatic tissue thereby further reducing insulin secretion. The observed decrease may be due to the utilization of non-protein thiols by increased oxygen free radicals produced in hyperglycemia conditions [52]. [27] also observed a decrease in the activity of all measured antioxidants enzymes (SOD, CAT and GSH) in diabetic rats. This decrease indicates a reduction in the anti-oxidant defense system. Since oxidative stress significantly contributes to the pathogenesis of diabetes [53] substances that suppress or reduces oxidative stress might be therapeutically beneficial. We observed that when the VCO was administered to the diabetes (VCO) group, a significant increase of SOD enzyme was observed when compared to the diabetes control group. This also supports the report that VCO has a hypoglycemic and anti-oxidative effect in diabetes [27, 54]. [55] also reported that VCO was able to reduce lipid peroxidation and increase the activity of superoxide dismutase in the serum of mice. This anti-stress activity may be attributed to the polyphenols and medium-chain fatty acids present in VCO [55]. Studies suggest that overexpression of SOD targeted to overcome oxidative stress, reduce ROS and increase antioxidant enzymes have been shown to prevent diabetes mellitus [56]. Studies have shown that exogenously administered antioxidants have protective effects on diabetes, thus providing insight into the relationship between free radicals and diabetes [57, 58]. It was also observed in this study that the administration of ATZ caused a significant decrease in the SOD levels which suggests an increase in production of superoxide radical and other reactive oxidants therefore inducing oxidative damage. [59] reported a significant decrease in the specific activity of SOD in mice administered atrazine. On the contrary, a study by [46] showed an increase in the activity of SOD was observed in the erythrocytes of atrazine-treated rats. They attributed this increase SOD levels to increased generation of reactive oxygen species which reflects an activation of the compensatory mechanism through the effects of atrazine on various tissues, and its extent depends on the magnitude of the oxidative stress and hence up on the duration of the dose of stressor [46]. However, in the recovery groups we observed that the SOD level in the VCO recovered atrazine groups increased significantly when compared to the groups they were left to recover on their own. This further suggests and probably confirms the antioxidant capacity of VCO even against atrazine-induced oxidative stress. [47] also observed that ginger administration ameliorated the effects of atrazine on experimental animals. [46] further

reported that Vitamin E, being an antioxidant, reduces the oxidative stress and hence normalization of SOD activities were observed to some extent in their study.

Catalase plays a main role in the regulation of hydrogen peroxide metabolism. It enzymatically neutralizes hydrogen peroxide by converting it into oxygen and water. Catalase protects pancreatic -cells from damage by hydrogen peroxide [60]. Deficiency of CAT increases oxidative stress in the -cells mitochondria thereby increasing mitochondria ROS in response to free fatty acids [61], this leads to -cell dysfunction and ultimately diabetes. The CAT level significantly increased in the Normal control + VCO group when compared with the normal control + H_20 group. This further confirms the antioxidative property of VCO that has been reported in several studies [27, 55]. There are variations in the catalase activity after different treatments in rats. Both increase and decrease in CAT activity and levels have been reported in both diabetes and atrazine administration which could be due to the body's adaptive response. In the present study, we observed a significant decrease in the catalase activity in untreated alloxan induced diabetic group. This result is in support to other reports which have indicated the reduction in the activity of CAT in alloxan and streptozotocin induced rats [27, 62]. [63] reported that there was no significant increase in hepatic CAT activity in their alloxan induced diabetic rat model. They suggested that whereas there was peroxidation of the membrane lipids in the liver and kidneys of these groups of rats, their antioxidant defence mechanisms may not been totally overwhelmed by the effect of peroxidation of their lipids due perhaps to the duration of the experiment (21 days). In addition, the non-effect alloxan had on the hepatic catalase activity of the DC may also suggest that there may have been less hydrogen peroxide generated from the action of SOD on superoxide radical for degradation by GPx and catalase. In contrast to our report, [64] showed that catalase activity increased significantly in the erythrocytes of STZ-induced diabetic rats. They attributed this increase to the presence of some alteration in oxidant–antioxidant balance in the erythrocytes of diabetic rats [64]. The elevated activity of catalase appears to be an adaptive response to increased generation of reactive oxygen species or free radicals indicating the failure of the total antioxidant defense mechanism to protect the tissues from mechanical damage caused by alloxan [65]. However, the reduced catalase level was elevated significantly in the diabetic group treated with VCO when compared with the diabetic untreated group. This shows the protective role of VCO in decreasing lipid peroxidation and by normalizing the deranged antioxidant system. Atrazine, like diabetes can promote oxidative stress by increasing the concentration of ROS and products of oxidative damage and at the same time influence the activity of antioxidant enzymes [66, 67]. We observed a significant decrease in CAT activity in the atrazine group when compared to the normal control groups and diabetic groups. This reduction could be attributed to the toxicity of atrazine. [68] also observed a reduced activity of catalase following atrazine exposure in their study. CAT activity was also decreased in atrazinetreated mice after 14 days of treatment, which is indicated the presence of superoxide radical [69]. The antioxidant enzyme CAT protects SOD against inactivation by hydrogen peroxide. Reciprocally, the SOD protects the CAT against inhibition by superoxide anion that could be formed during the treatment mice with atrazine. The CAT levels were restored back following recovery by VCO which further support the antioxidative capacity of VCO. [68] also reported that Andrographis paniculata treatment ameliorated the effects of atrazine suggesting it as potential antioxidant against atrazine induced

oxidative stress. Therefore, VCO could also be said to possess protective antioxidant capacity against atrazine induced oxidative stress.

CONCLUSION

Diabetes disrupted the oxidative balance by significantly reducing the levels of the oxidative enzymes but the anti-oxidative property of VCO was able to restore them. ATZ did not significantly alter the oxidative stress enzymes in the first phase of the experiment, but with the continuous use in the recovery period, it significantly reduced them. Therefore, VCO supplementation did not significantly affect them during recovery.

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