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Protective Effect of Rutin and Vitamin E on Cobalt Chloride Induced Neurotoxicity

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ABSTRACT: Toxic exposures to cobalt chloride could occur from dietary, occupational, and medical sources. This study evaluated the ameliorative effect of oral administration of rutin and vitamin E on cobalt chloride ($CoCl_2$)-induced oxidative stress in the brain of rats following exposure to cobalt chloride ($CoCl_2$) toxicity. Wistar rats were randomly divided into five groups. Group 1 received only water, while group 2 received cobalt chloride (300 ppm) in drinking water. Groups 3, 4 and 5 also received cobalt chloride, but were treated with rutin (100 mg/kg body weight), rutin (200 mg/kg BW) and vitamin E (50 mg/kg BW) respectively for seven consecutive days. The rats were euthanized 24 hours after the last treatment. The brain was collected for biochemical and histopathological evaluations. The study demonstrated that cobalt chloride caused a significant (P<0.05) decrease in the activities of superoxide dismutase (SOD), glutathione S-transferase (GST), glutathione peroxidase (GPx) and glutathione level, whereas the levels of malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) increased significantly (P<0.05). However, rutin and vitamin E treatment attenuated $CoCl_2$-induced oxidative stress. Histopathological findings also revealed the ameliorative effect of rutin and Vitamin E on the neuronal damage caused by $CoCl_2$ treatment.

Keywords: Antioxidant, Oxidative stress, Cobalt chloride, Rutin, Vitamin E

Introduction

Heavy metals are toxic, and nearly all organs are susceptible to their toxicity. The industrial revolution has led to heavy metal poisoning, a major serious health problem (Gilani *et al*., 2015). Commonly affected systems include the central nervous system, peripheral nervous system, hematopoietic, renal, and cardiovascular systems. Other problems associated with heavy metal toxicity include carcinogenicity, respiratory system toxicity, endocrine and reproductive effects. Heavy metals hinder normal brain development and neurotransmitter function (Gilani *et al*., 2015).

Cobalt is a heavy metal that occurs naturally in low concentrations. It is an essential component of vitamin B_{12} required to produce red blood cells (Danzeisen *et al.*, 2020). The most typical route of exposure to cobalt is diet. However, environmental, and occupational exposure could occur in industrial settings since cobalt is used as a pigment in glass, ceramics, and paints. In addition, cobalt alloys are used to produce aircraft engine magnets and artificial joints. Workers involved in metal mining, smelting, and refining may also be subject to higher levels of cobalt (Permenter *et al*., 2013; Petrova *et al*., 2019).

Rutin is a naturally occurring polyphenol flavonoid found in various leaves, fruits, and seeds. Rutin, a glycoside derivative of quercetin, possesses pharmacological activities such as anticancer, anti-inflammatory, antiviral, and antioxidant effects (Magalingam *et al*., 2013; Ragheb *et al*., 2019). Rutin reportedly scavenge reactive oxygen species (ROS) directly and potentiates oxidative cellular defence (Enogieru *et al*., 2018). Likewise, vitamin E is an antioxidant found in the cellular membrane. Vitamin E prevents the release of reactive oxygen species and inhibits the peroxidation of unsaturated fatty acids, and mitigates oxidative stress, which is the major cause of neurodegenerative diseases (Fritsche *et al*., 2017). This study was designed to evaluate the protective effects of rutin and vitamin E on cobalt chloride induced neurotoxicity in rats.

Materials and methods

Chemicals: Cobalt Chloride, Rutin (Quercetin-3-rutinoside hydrate), Vitamin E, Thiobarbituric acid (TBA), Tris KCl, Epinephrine, 5',5'-dithiobis-(2-nitrobenzoic acid) (DTNB or Ellman's reagent), 1-chloro-2,4-dinitrobenzene (CDNB), Reduced Nicotinamide adenine dinucleotide phosphate (NADPH) were purchased from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals and reagents used for this work were of analytical grade.

Animals: Thirty (30) male Wistar rats (170–200 g) were obtained from the Department of Veterinary Physiology and Biochemistry, University of Ibadan, Nigeria, and housed in the Department of Veterinary Physiology and Biochemistry's animal house, University of Ibadan, Nigeria, under standard conditions. The rats were divided into five groups of six rats per group. The animals were fed food liberally and water *ad libitum* and were handled and used following the National Institutes of Health (NIH) guide for the care and use of laboratory animals.

Animal treatment and grouping: The animals were randomly selected and divided into control group, cobalt chloride group (300 ppm), groups of cobalt chloride animals treated with rutin (100 mg/kg, body weight), rutin (200 mg/kg, body weight) and Vitamin E (50 mg/kg, body weight). Rutin and Vitamin E were administered orally while cobalt chloride was in the drinking water for 7 days. On day 8, the animals were euthanised, and the brain was dissected, and subsequently used for the evaluation of oxidative stress markers and histopathological studies.

Oxidative stress markers

Determination of malondialdehyde content (MDA): According to Varshney and Kale (1990), the concentration of malondialdehyde was assessed by considering the rate of the formation of thiobarbituric acid reactive substances (TBARS). Under the acidic condition, malondialdehyde (MDA) produced from the peroxidation of fatty acid membranes and food products react with the chromogenic reagent, 2-thiobarbituric acid, to yield a pink-coloured complex with maximum absorbance at 532 nm. The method used involved the addition of 1.6 ml of Tris-KCl to 0.5 ml of 30 % TCA, 0.4 ml of samples, and 0.5 ml of 0.75% thiobarbituric acid (in 0.2 M HCl). The mixture was incubated at 80 °C for 45 min. subsequently, the mixture was cooled on ice and centrifuged at 4,000 rpm for 15 min. Then, the absorbance was measured against a blank (distilled water) at 532 nm.

Determination of hydrogen peroxide: The concentration of hydrogen peroxide was measured by ferrous oxidation in xylenol orange (Wolff, 1994). This method is highly sensitive and consists of peroxide-mediated oxidation of ferrous ions in an acidic medium containing the dye xylenol orange, which binds the resulting ferric ions to produce a blue-purple complex with an absorbance of between 540 and 580 nm. To 10 uL of standard hydrogen peroxide solution 2.0 mL of HC1, 0.2 mL of KI, 0.2 mL of ammonium molybdate in H_2S0_4, and 0.2 mL of starch solution were added. The absorbance of the mixture was then measured in 1.0 cm cuvets at 570 nm.

Evaluation of reduced glutathione (GSH) level: This method, as described by Beutler *et al*. (1963), is based upon the formation of a relatively stable (yellow) colour resulting from the addition of 5′, 5′ – dithiobis - (2-nitrobenzoic acid, DTNB) to sulfhydryl compounds. The chromophoric product resulting from the reaction of Ellman reagent with the reduced glutathione, 2-nitro-5-thiobenzoic acid, possesses a molar absorption at 412 nm. 0.5 ml of 4% sulfosalicylic acid was added to 0.5 ml of post mitochondrial fraction of sample. The mixture was centrifuged for 5 min at 4,000 rpm. The supernatant (0.5 ml) was subsequently decanted and mixed with 4.5 ml of Ellman's reagent. The absorbance was read at 412 nm wavelength.

Evaluation of superoxide dismutase activity (SOD): SOD activity was determined spectrophotometrically according to the method of Misra and Fridovich, (1972) with slight modification from Oyagbemi *et al.* (2018). Briefly, 100 mg of epinephrine was dissolved in 100 ml distilled water that was acidified with 0.5 ml concentrated hydrochloric acid. Thereafter, 10 µl of the post mitochondrial fraction of samples was added to 2.5 ml 0.05 M carbonate buffer, and 300 µl of 0.3 mM adrenaline was added. The increase in absorbance at 480 nm was monitored every 30 s for 150 s.

Determination of glutathione S-transferase (GST): GST activity was assayed spectrophotometrically at 25 °C with reduced glutathione (GSH) and 1-chloro-2, 4-dinitrobenzene (CDNB) as substrates according to the method described by Habig *et al*. (1974). Briefly, 0.02 ml of 0.5 mM 1-Chloro-2,4 dinitrobenzene (CDNB) was added to 0.05 ml of 1.0 mM reduced glutathione (GSH), 0.68 ml of distilled water and (0.2 ml) 100 mM phosphate buffer (K_2HPO_4/KH_2PO_4; pH = 6.5). The rate of increase in absorbance was measured at 340 nm for 10min at 37 °C against a blank solution.
Determination of glutathione peroxidase (GPx): According to Beutler *et al*. (1963), the enzymatic reaction was initiated by adding hydrogen peroxide. A solution containing 0.5 ml potassium phosphate buffer, 0.1 ml sodium azide, 0.2 ml reduced glutathione solution, 0.1 ml hydrogen peroxide, 0.5 ml post mitochondrial fraction of brain sample and 0.6 ml of distilled water was prepared. The mixture was incubated at 37 °C for 5 min and 0.5 ml of trichloro acetic acid was added. Centrifugation was carried out at 4,000 rpm for 5 min. Subsequently, 1 ml of the supernatant was added to 2 ml of dipotassium phosphate (K_2HPO_4) and 1 ml Ellman's reagent. The absorbance of the mixture was read at 412 nm wavelength.
Total protein concentration: This was done according to the Biuret method described by Gornall *et al*. (1949). Briefly, 0.1 ml of serum was placed into a test tube, 2.9 ml distilled water was added, and 3 ml of working biuret reagent was also added. The tubes were placed in a water bath at 37 ºC for 10 min. Thereafter the mixture was allowed to cool, and a spectrometer (SP600) reading was done at 540 nm wavelength.
Histopathological assessment: The brain tissues were fixed in 10 % formalin, and then dehydrated with grades of ethanol (70, 80, 90, 95 and 100 %). Dehydration was then followed by clearing the samples in 2 changes of xylene. Samples were then impregnated with 2 changes of molten paraffin wax, then embedded and blocked out. Paraffin-embedded, 5-6 mm thick, transverse sections of the brain were cut using a rotary microtome and mounted on glass slides. The slides were stained with Haematoxylin and Eosin (H&E). Stained sections of control and treated rats were examined under the light microscope (Olympus CH Japan) for presence or absence of histopathological lesions (Drury *et al*., 1976).
Statistical analysis: All values are expressed as mean±SD. One-way analysis of variance (ANOVA) and Turkey's multiple comparison tests were done using GraphPad Prism version 8.0, with the level of statistical significance considered as $p<0.05$.

Results

Oxidative stress: The results showed that the brain tissue of rats treated with cobalt chloride exhibited a significant increase in oxidative stress markers such as MDA and H_2O_2 (Figures 1 and 2) compared with the control group. Meanwhile, reduction in the oxidative stress marker was observed in groups co-treated with rutin (100 mg/kg; 200 mg/kg) and vitamin E compared with $CoCl_2$ only treated group.
The concentration of GSH, a primary cellular non-enzymatic antioxidant, was significantly reduced in $CoCl_2$ only treated group, and the group co-treated with rutin (100 mg/kg) in contrast to the control group as shown in Figure 3 while it was significantly increased in the groups co-treated with rutin (200 mg/kg) and vitamin E (50 mg/kg) when compared with the $CoCl_2$ only treated group.
Superoxide dismutase activity (Figure 4) in the brain tissue of rats was significantly reduced in the group treated with $CoCl_2$ only, group co-treated with rutin (100 mg/kg) and vitamin E (50 mg/kg) while there was significant increase in the group co-treated with rutin (200 mg/kg) and vitamin E (50 mg/kg).
In Figure 5, glutathione peroxidase activity was significantly reduced in the group treated with $CoCl_2$ only, group co-treated with rutin (100 mg/kg) and vitamin E (50 mg/kg) when compared to control. In comparison, there was a significant increase in the activity in the groups treated with rutin at both the100 mg/kg, 200 mg/kg doses, and vitamin E (50 mg/kg) compared to the $CoCl_2$ only treated group.
The activity of glutathione-S-transferase in Figure 6 decreased in the brain tissue of rats treated with $CoCl_2$ only, group co-treated with rutin (100 mg/kg) and vitamin E (50 mg/kg); however, a significant increase in the activity was observed in groups co-treated with rutin (100 mg/kg, 200 mg/kg) and vitamin E (50 mg/kg) when compared to $CoCl_2$ only treated group.
Histopathological changes: Figure 7 showed the morphology of the brain tissue of rats in the control group, $CoCl_2$ treated group and groups treated with rutin (100 mg/kg; 200 mg/kg) and vitamin E. Normal and organised cells with no visible lesions were observed in the control group. Meanwhile, distinct structural changes with marked disruption of ependymal cells, ventricular haemorrhage, atrophy of neurons, and marked astrocytosis was observed in $CoCl_2$ only treated group, but there was an improvement in the groups treated with rutin (100 mg/kg; 200 mg/kg) and vitamin E. With groups co-treated with rutin (100 mg/kg, 200 mg/kg), severe meningeal haemorrhage (black arrows) and focal haemorrhages in the neutrophil (Black arrows) was observed,

respectively, while focal meningeal haemorrhage (black arrow) was observed in groups co-treated with (50 mg/kg) vitamin E.

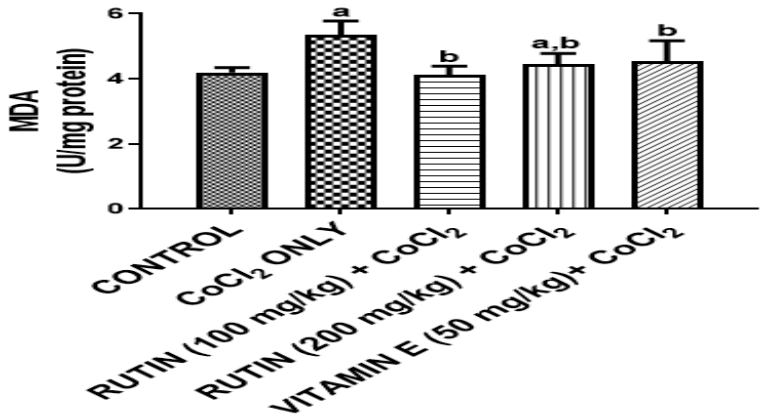

Figure 1: Ameliorative effect of Rutin (100 mg/kg; 200 mg/kg) and Vitamin E on the concentration of Malondialdehyde (MDA) present in the brain tissue of rats treated with $CoCl_2$. Results are expressed as Mean ± SD (n=6). a is significantly different from the control ($p<0.05$), b is significantly different from the $CoCl_2$ only ($p<0.05$).

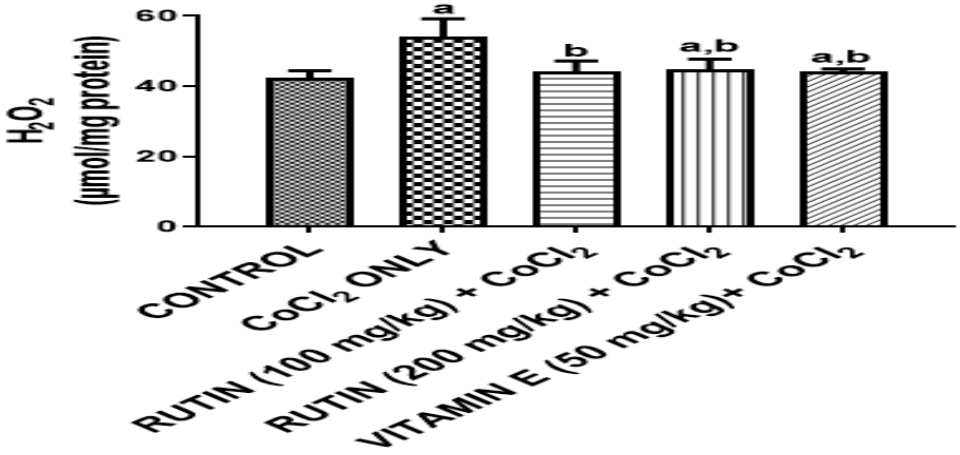

Figure 2: Ameliorative effect of Rutin (100 mg/kg; 200 mg/kg) and Vitamin E on the concentration of hydrogen peroxide (H_2O_2) present in the brain tissue of rats treated with $CoCl_2$. Results are expressed as Mean ± SD (n=6). a is significantly different from the control ($p<0.05$), b is significantly different from the $CoCl_2$ only ($p<0.05$).

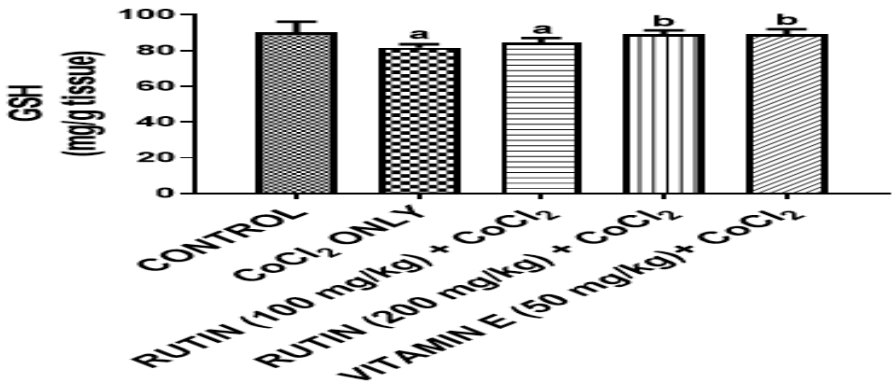

Figure 3: Ameliorative effect of Rutin (100 mg/kg; 200 mg/kg) and Vitamin E on the concentration of Glutathione (GSH) present in the brain tissue of rats treated with $CoCl_2$. Results are expressed as Mean ± SD (n=6). a is significantly different from the control ($p<0.05$), b is significantly different from the $CoCl_2$ only ($p<0.05$).

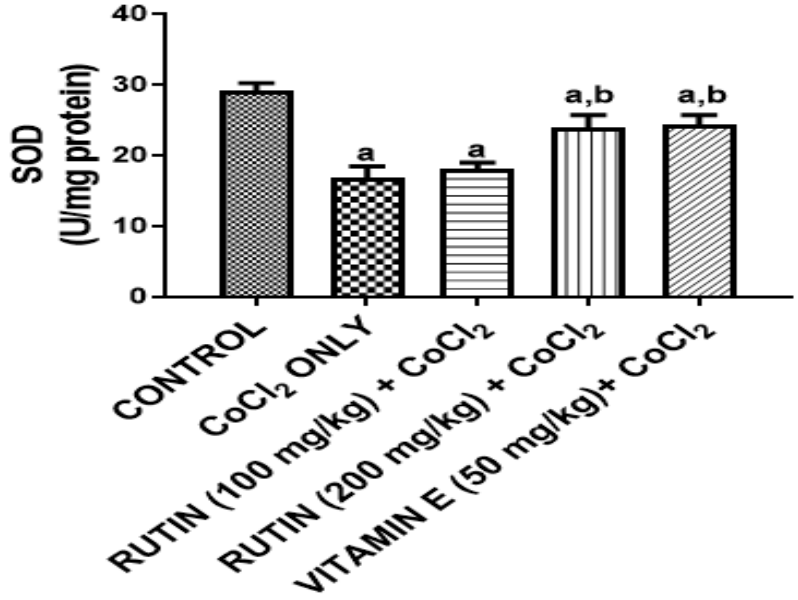

Figure 4: Ameliorative effect of Rutin (100 mg/kg; 200 mg/kg) and Vitamin E on the activity of superoxide dismutase in the brain tissue of rats treated with $CoCl_2$. Results are expressed as Mean ± SD (n=6). a is significantly different from the control ($p<0.05$), b is significantly different from the $CoCl_2$ only ($p<0.05$).

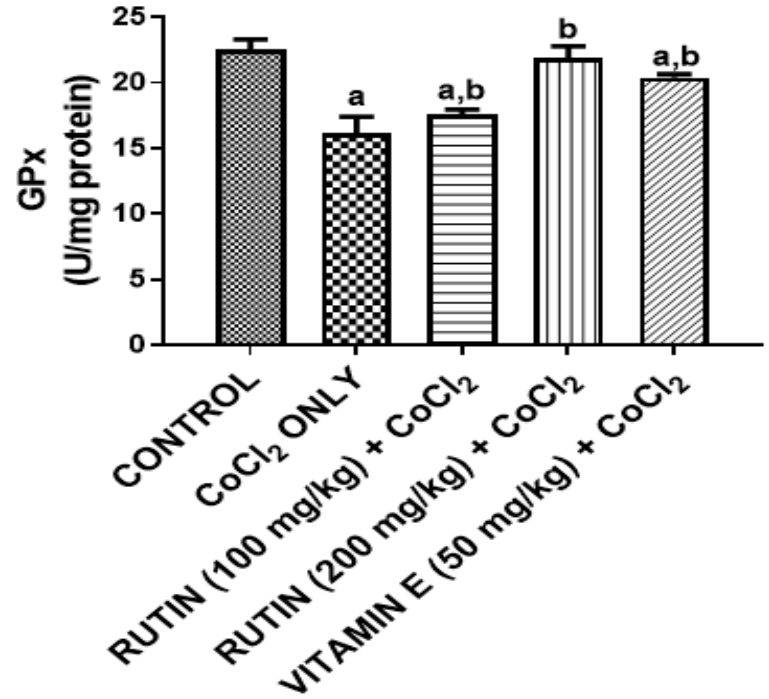

Figure 5: Ameliorative effect of Rutin (100 mg/kg; 200 mg/kg) and Vitamin E on the activity of Glutathione peroxidase in the brain tissue of rats treated with $CoCl_2$. Results are expressed as Mean ± SD (n=6). a is significantly different from the control ($p<0.05$), b is significantly different from the $CoCl_2$ only ($p<0.05$).

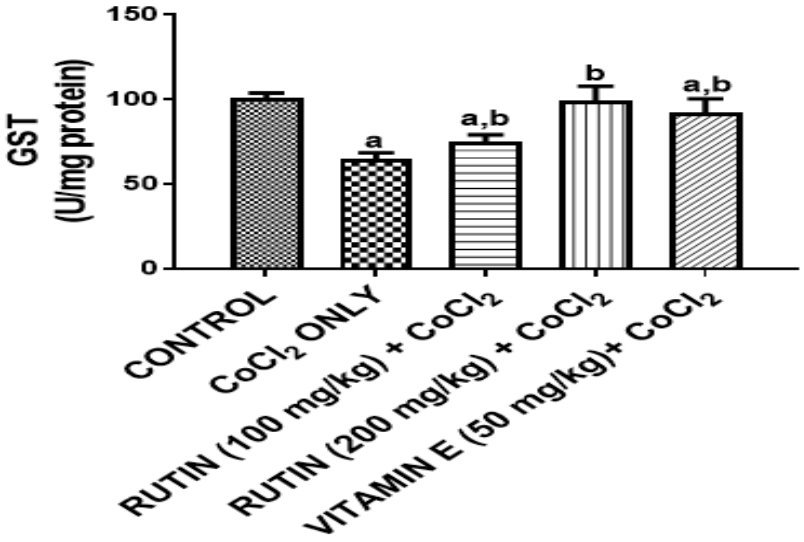

Figure 6: Ameliorative effect of Rutin (100 mg/kg; 200 mg/kg) and Vitamin E on the activity of Glutathione peroxidase in the brain tissue of rats treated with $CoCl_2$. Results are expressed as Mean ± SD (n=6). a is significantly different from the control ($p<0.05$), b is significantly different from the $CoCl_2$ only ($p<0.05$).

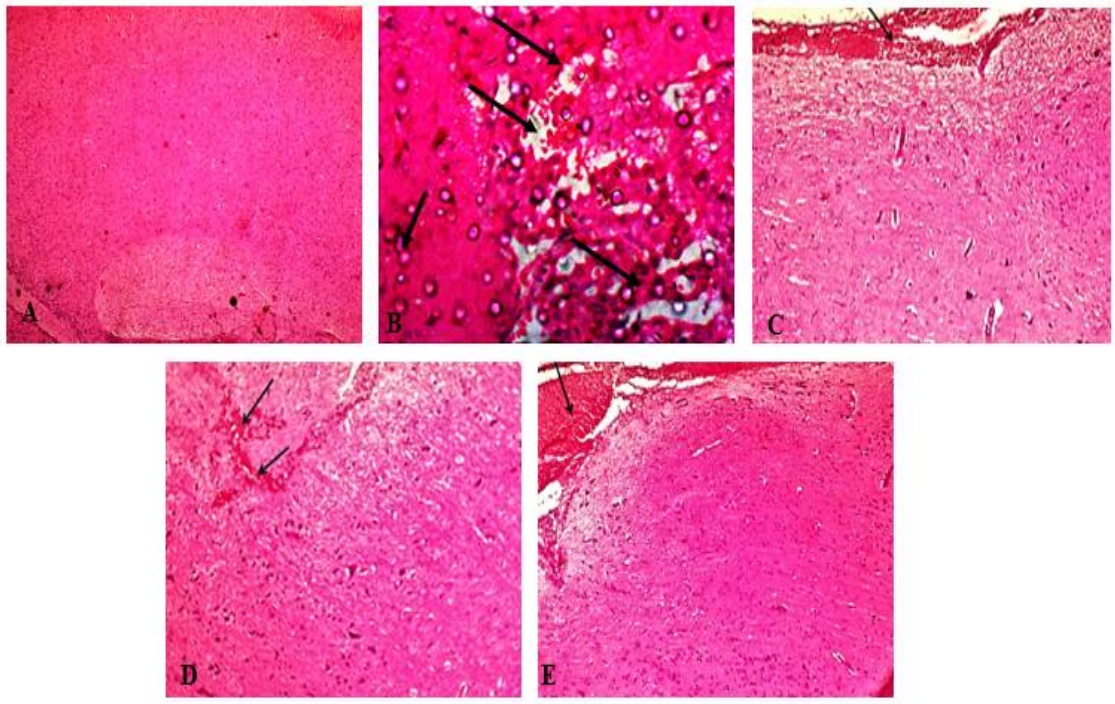

Figure 7: Representative photomicrographs of Haemoxylin and Eosin-stained sections of brain tissues of rats treated with $CoCl_2$ and those co-treated with rutin (100 mg/kg; 200 mg/kg) and vitamin E. A= Control, B= $CoCl_2$ only, C= $CoCl_2$ with 100 mg/kg Rutin, D= $CoCl_2$ with 200 mg/kg, E= $CoCl_2$ with 50 mg/kg Vitamin E. Magnification: Control (x 60), $CoCl_2$ (x 400) and $CoCl_2$ treated with $CoCl_2$ rutin (100 mg/kg; 200 mg/kg) and vitamin E (x 150).

Discussion

Exposure to cobalt leads to the generation of free radicals and reactive oxygen species (ROS). Rutin and vitamin E are potent antioxidants that have been reported to ameliorate damages induced by oxidative stress (Hamza *et al*., 2017; Ragheb *et al*., 2020). In the brain, oxidative stress is induced by elevation of free radicals and reactive oxygen species (Ojo *et al*., 2019). Reactive oxygen species (ROS) are produced in the body continually via oxidative metabolism (Bentley *et al*., 2015). Exposure to cobalt chloride resulted in increased MDA and H_2O_2 in the brain, which promotes free radicals that cause oxidative stress; H_2O_2 occurs as functional reactive species (Konno *et al*., 2021); our findings was consistent with the result of Han *et al*., (2021). Although treatment with vitamin E and rutin led to the reduction of this free radical being generated, this can be associated with the fact that vitamin E and rutin are essential antioxidants that aid the decreasing oxidative stress (Kandemir *et al*., 2015; Fagan *et al*., 2020).

Antioxidants help protect cellular organs from oxidative damage incurred via free radicals (Madalyn *et al*., 2020). Glutathione peroxidase (GPx) catalyses the mopping-up of hydrogen peroxides while reduced glutathione (GSH) scavenges O^{-2} and OH and donates electrons for the GPx reaction to occur (Enogieru *et al*., 2018; Sarıkaya and Selami, 2020). Ideally, the oxidised to reduced glutathione ratio is reported as a measurement of oxidative stress (Fagan *et al*., 2020). However, due to some limitations, only total glutathione is reported for this study. A decrease in the activity of GPx and the level of GSH in the $CoCl_2$ treated group compared with the control was observed in this study; this agrees with the study of Oyagbemi *et al*. (2018). This could be the cause of the increase in the level of H_2O_2 and MDA also observed in groups treated with $CoCl_2$ only leading to increased reactive oxygen and hence oxidative stress; this is like the reports of Moustapha (2020).

The brain generates a lot of reactive species, a few of which are consumed by the powerhouse of the cells and hence converted to superoxide ion, a potent oxidation initiator. With the aid of SOD, superoxide ions are converted directly to H_2O_2 (Sarıkaya and Selami, 2020). In this study, there was a decrease in the level of SOD in rats treated with $CoCl_2$ compared with the control group. This could result from the decrease observed in the activity of other cellular enzymatic antioxidants. This would result in high ROS and hence oxidative stress. Konno *et al*., (2021) reported that SOD is crucial in cellular health and the prevention of oxidative stress; therefore, as the activity decreases, the free radical increases. However, there was an increase in the activity of SOD in groups treated with rutin and vitamin E; this corroborates the findings from Sharma *et al*. (2020).

The cellular activity of GST is like that of GPx. It promotes the detoxification of organic hydrogen peroxides produced by lipid peroxidation and oxidative stress to alcohol (Allocati *et al*., 2018). The reduction in its activity compared to the control group was observed in this study and could be the cause of the increase in hydrogen peroxide concentration and lipid peroxidation.

The study reveals that the co-treatment of $CoCl_2$ with rutin and vitamin E modulates the oxidative stress markers, improves the activity of the enzymatic antioxidant, and amends the decrease in the level of non-enzymatic antioxidant observed in $CoCl_2$ only treated group. Furthermore, the significant increase in the activities of antioxidant enzymes following treatment with rutin and vitamin C agrees with the findings of Kandemir *et al*. (2015). The ability of rutin to ameliorate the effect of $CoCl_2$ can be attributed to its structure with its ability to promote the release of GSH and abort the generation of ROS through xanthine oxidase (Enogieru *et al*., 2018). Likewise, Vitamin E inhibits ROS generation, and it can convert peroxyl radicals into a tocopheroxyl radical, thereby inhibiting lipid peroxidation (Hamza *et al*., 2017).

The control group observed normal and organised cells with no visible lesions from the histopathological slides. Meanwhile, distinct structural changes with marked disruption of ependymal cells, ventricular haemorrhage, atrophy of neurons, and marked astrocytosis was observed in $CoCl_2$ only treated group, but there was an improvement in the groups treated with rutin (100 mg/kg; 200 mg/kg) and vitamin E though some lesions were still seen. The observed brain cells histopathological changes due to $CoCl_2$ induced neurotoxicity are like an earlier report of Oyagbemi *et al*. (2018), and the ability of rutin and vitamin C to negate such alterations have been reported by several researchers (Kandemir *et al*., 2015; Al-Attar, 2011).

Conclusion

Cobalt chloride causes oxidative stress in the brain tissue due to the alterations in the enzymatic antioxidant activity and the non-enzymatic antioxidant level (Hamza *et al*., 2017). From this study, rutin, at two different doses, and vitamin E possess an ameliorative effect against brain damages induced by exposure to $CoCl_2$.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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