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

Ebunoluwa Racheal Asenuga¹, Temitayo Olabisi Ajibade²*, Omolola Victoria Awoyomi³, Blessing Seun Ogunpolu⁴, Ademola Adetokunbo Oyagbemi² and Temidayo Olutayo Omobowale⁵

¹Department of Veterinary Physiology and Biochemistry, Faculty of Veterinary Medicine, University of Benin, Nigeria
²Department of Veterinary Physiology and Biochemistry, Faculty of Veterinary Medicine, University of Ibadan, Nigeria
³Federal College of Animal Health and Production Technology, Moor Plantation, Ibadan, Nigeria
⁴School of Biomedical Science/Pharmacology, Kent State University, USA.
⁵Department of Veterinary Medicine, Faculty of Veterinary Medicine, University of Ibadan, Nigeria

*Corresponding author Email: toajibade@gmail.com. Tel: +234 907 184 2142

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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 (CoCl₂)-induced oxidative stress in the brain of rats following exposure to cobalt chloride (CoCl₂) 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 (H₂O₂) increased significantly (P<0.05). However, rutin and vitamin E treatment attenuated CoCl₂-induced oxidative stress. Histopathological findings also revealed the ameliorative effect of rutin and Vitamin E on the neuronal damage caused by CoCl₂ 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 B₁₂ 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 anti-cancer, 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 H2SO4, 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 sulphydryl 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_{2}HPO_{4}/KH_{2}PO_{4}, pH = 6.5). The rate of increase in absorbance was measured at 340 nm for 10 min 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 trichloroacetic 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_{2}HPO_{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 °C 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_{2}O_{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 CoCl₂. Results are expressed as Mean ± SD (n=6). a is significantly different from the control (p<0.05), b is significantly different from the CoCl₂ 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 (H₂O₂) present in the brain tissue of rats treated with CoCl₂. Results are expressed as Mean ± SD (n=6). a is significantly different from the control (p<0.05), b is significantly different from the CoCl₂ 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 CoCl₂. Results are expressed as Mean ± SD (n=6). a is significantly different from the control (p<0.05), b is significantly different from the CoCl₂ 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 CoCl₂. Results are expressed as Mean ± SD (n=6). a is significantly different from the control (p<0.05), b is significantly different from the CoCl₂ only (p<0.05).

Figure 7: Representative photomicrographs of Haemoxylin and Eosin-stained sections of brain tissues of rats treated with CoCl₂ and those co-treated with rutin (100 mg/kg; 200 mg/kg) and vitamin E. A= Control, B= CoCl₂ only, C= CoCl₂ with 100 mg/kg Rutin, D= CoCl₂ with 200 mg/kg, E= CoCl₂ with 50 mg/kg Vitamin E. Magnification: Control (x 60), CoCl₂ (x 400) and CoCl₂ treated with CoCl₂; 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 H₂O₂ in the brain, which promotes free radicals that cause oxidative stress; H₂O₂ 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 O₂⁻ 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 CoCl₂ 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 H₂O₂ and MDA also observed in groups treated with CoCl₂ only leading to increased reactive oxygen and hence oxidative stress; this is like the reports of Moustapha et al. (2018).

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 ions, a potent oxidation initiator. With the aid of SOD, superoxide ions are converted directly to H₂O₂ (Sarıkaya and Selami, 2020). In this study, there was a decrease in the level of SOD in rats treated with CoCl₂ 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 radicals 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 CoCl₂ 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 CoCl₂ 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 CoCl₂ 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 CoCl₂ 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 CoCl₂ 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 CoCl₂.
Disclosure statement

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

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