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# Neuroprotective Mechanism of Ethanolic Extract of *Irvingia gabonensis* Stem Bark against Cadmium-induced Neurotoxicity in Rats

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## Authors' contributions

This work was carried out in collaboration between all authors. Author OAO designed the study, performed the statistical analysis. Author BEO wrote the protocol, and wrote the first draft of the manuscript. Author BOA managed the analyses of the study. Author SAO managed the literature searches. All authors read and approved the final manuscript.

## Article Information

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## ABSTRACT

**Objective:** To explore the neuroprotective effect of *Irvingia gabonensis* (IG) against cadmium-induced oxidative damage in rats brain.

**Place and Duration of Study:** Department of Chemical sciences, (Biochemistry laboratory), Afe Babalola University and Department of Biochemistry, Ekiti State University, Ado Ekiti, Nigeria between February 2014 and May 2014.

**Methods:** The study was performed on twenty (20) male rats divided into four groups: a

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control group, cadmium group ( $4\text{mgkg}^{-1}\text{day}^{-1}$ , intraperitoneally [i.p.]) and cadmium toxication groups received 200 and  $400\text{mgkg}^{-1}$  body weight of extract by oral gavage for 28 days. The degree of protection in brain tissue was evaluated by the levels of malondialdehyde (MDA), glutathione (GSH), superoxide dismutase, and catalase. The aminotransferase (ALT), aspartate aminotransferase (AST) activities and histological examination were monitored.

**Results:** *Irvingia gabonensis* showed a significant ( $P>.05$ ) brain-protective effect by decreasing the level of lipid peroxidation and elevate the activities of antioxidative enzymes and level of GSH. Furthermore, histological alterations in brain were observed in cadmium untreated rats and were ameliorated in cadmium-induced treated rats with IG.

**Conclusions:** Consequently *Irvingia gabonensis* blocked oxidative brain damage induced by cadmium in rats. These data suggest that *Irvingia gabonensis* extract may play a very useful role in reduction of the neurotoxicological damage induced by cadmium.

**Keywords:** Cadmium; antioxidant; neuropharmacology; *Irvingia gabonensis*; lipid peroxidation.

## 1. INTRODUCTION

Cadmium (Cd) is a toxic heavy metal in the environment. Cd is a highly accumulative toxicant with very long biological half-life [1]. Cd is not biodegradable and its levels in the environment are increasing due to industrial activities and human exposures to Cd are inevitable [1,2]. Acute Cd exposure produced toxicities to the lung, liver, testes, and brain, while chronic exposure to Cd often leads to renal dysfunction, anemia, osteoporosis, and bone fractures [1-3]. Cd is a potent carcinogen in a number of tissues of rodents and classified as a human carcinogen [4]. The neurotoxic effects of Cd have been reported in neonatal mouse brain [5] and young rat brain [6]. Cd produces oxidative damage to isolated rat optic nerve [7] and cultured rat cortical neurons [8]. In humans, occupational exposure to Cd is associated with neuropsychological disorders [9], and Cd exposure is reported to be a cause of amyotrophic lateral sclerosis [10]. Cd is shown to selectively damage striatum [11], and Parkinsonism has been reported in a 64-year-old man exposed to Cd at a high dose [12]. Thus, accumulating evidence clearly indicates that Cd is neurotoxic in a number of settings. The mechanisms involved in neurotoxicity of Cd are poorly understood. Oxidative stress has been proposed as a mechanism for Cd toxicity in a number of tissues such as the kidney [13], liver [14], and brain [15]. However, little is known about the neurotoxic effects of Cd to dopaminergic neurons, and little is known about the role of microglia in Cd-induced radical generation and oxidative stress in the brain. To fill these gaps would be helpful in understanding of Cd neurotoxicity and would be useful in setting appropriate measures to prevent and or reduce Cd toxicity. *Irvingia gabonensis* Baill. Ex Lanen. (Irvingiaceae) is a commercial and indigenous fruit tree of West and Central Africa, which identified as the most important tree for domestication [16,17]. It's called bush mango in Nigeria. The plant occurs freely in many parts of Africa. Local names include *goronbiri* (Hausa), *Ogbono* (Ibo). The plant is a food supplement. As there has been no scientific report on the protective or therapeutic effect of *Irvingia gabonensis* against Cd induced oxidative damage in brain of animals. Therefore, in this study, we aimed to evaluate the neuroprotective effect of the *Irvingia gabonensis* stem bark by using the Cd-induced oxidative damage in rats.

## **2. MATERIALS AND METHODS**

### **2.1 Chemicals**

Cadmium chloride was bought from a local chemist in Ibadan, Nigeria. Thiobarbituric acids (TBA) were bought from Aldrich Chemical Co. (Milwaukee, WI, USA). Glutathione, hydrogen peroxide, 5, 5'-dithio-bis-2-nitrobenzoic acid (DNTB) and Epinephrine bought from Sigma Chemical Co., Saint Louis, MO USA. Trichloroacetic acid (TCA) and Thiobarbituric acid (TBA) was bought from British Drug House (BDH) Chemical Ltd., Poole, UK. Other reagents were of analytical grade and the purest quality available.

### **2.2 Collection and Extraction of *Irvingia gabonensis* Stem Bark**

The stem bark of *Irvingia gabonensis* was collected in Ado-Ekiti (Ekiti State) and authenticated at the Department of Plant Science, Ekiti State University. The stem bark of *Irvingia gabonensis* air-dried and crushed into fine powder. The powdered part extracted with ethanol using maceration and the extract concentrated in vacuum at 40°C with rotary evaporator and water bath to dryness. The yield of the extraction was 5.01%.

### **2.3 Preliminary Phytochemical Analyses**

The preliminary phytochemical analyses were carried out with ethanolic extracts of *Irvingia gabonensis* stem bark for the detection of various phytochemicals. Tests for common phytochemicals were carried out by standard methods [18].

#### **2.3.1 Test for tannins**

One ml of extract was boiled in 20ml of water in a test and then filtered. A few drops of 0.1% ferric chloride was added and observed green or a blue-black coloration which confirms the presence of tannin.

#### **2.3.2 Test for phlobatannin**

Deposition of a red precipitate when 2ml of extract of each leaf samples was boiled with 1% aqueous hydrochloric acid was taken as evidence for the presence of phlobatannins.

#### **2.3.3 Test for saponin**

Five ml of the extract was boiled in 20ml of distilled water in a water bath and filtered. 10ml of the filtrate was mixed with 5ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, then observed for the formation of emulsion which confirms a positive presence of saponin.

#### **2.3.4 Test of flavonoid**

Three ml of 1% aluminium chloride solution were added to 5ml of each extract. A yellow coloration was observed indicating the presence of flavonoids. 5ml of dilute 0.1M ammonia solution were added to the above mixture followed by addition of 0.1M concentrated H<sub>2</sub>SO<sub>4</sub>. A yellow coloration disappeared on standing. The yellow coloration which disappeared on standing indicates a positive test for flavonoids.

### **2.3.5 Test for cardiac glycosides**

Five ml of each extracts was treated with 2ml of glacial acetic acid containing one drop of ferric chloride solution. This was underplayed with 1ml of 0.1M concentrated H<sub>2</sub>SO<sub>4</sub>. A brown ring at the interface indicates a deoxysugar characteristic of cardenolides which confirms a positive presence of cardenolides. A violet-green ring appearing below the brown ring, in the acetic acid layer, indicates the positive presence of glycoside.

### **2.3.6 Test for alkaloids**

One ml of the extract was stirred with 5ml of 1% aqueous HCL on a steam bath and filtered while hot. Distilled water was added to the residue and 1ml of the filtrate was treated with a few drops of Mayer's reagent (Potassium mercuric iodide-solution). The formation of a cream colour with Mayer's reagent gives a positive test for alkaloids.

### **2.3.7 Test for anthraquinone**

Five ml of extract was mixed with 10ml benzene, filtered and 5ml of 10% NH<sub>3</sub> solution added to the filtrate. The mixture was shaken and the presence of pink, red or violet colour in the ammoniac (lower) phase indicated the presence of anthraquinones.

### **2.3.8 Test for phenol**

Five ml of the extract was pipetted into a 30ml test tube and 10ml of distilled water was added. 2ml of 0.1M ammonium hydroxide solution and 5ml of concentrated amyl alcohol were also added and left to react for 30min. Development of bluish green colour was taken as a positive presence of phenol.

## **2.4 Animals**

Male Wistar rats (*Rattus norvegicus*) weighing between 80-120g bought from the animal house of the Department of Chemical Sciences, Biochemistry Unit, Afe Babalola University, Nigeria. Animals kept in aired cages at room temperature (28-30°C) and preserved on normal laboratory chow (Ladokun Feeds, Ibadan, Nigeria) and water *ad libitum*.

### **2.4.1 Ethical approval**

Rats handling and treatments conform to guidelines of the National Institute of Health (NIH publication 85-23, 1985) for laboratory animal care and use. The ethical committee of the Afe Babalola University approved this study. All animals in this study follow the institutional Animal Ethical Committee according to guidelines given by Committee for Control and Supervision of Experiments on animals (CPCSEA).

### **2.4.2 Induction of experimental animal**

Cadmium induced in groups II, III and IV. Briefly, Cadmium dissolved in distilled water and after that managed by intravenous injection (through tail vein) at a dose of 4mgkg<sup>-1</sup> body weight.

## **2.5 Study Design**

Twenty male rats divided into four groups of five rats each. Group I served as the negative control and accessed to normal rat diet and water *ad libitum* only, Group II served as the

positive control and given  $4\text{mgkg}^{-1}$  body weight of cadmium chloride. Group, III and IV given  $200\text{mgkg}^{-1}$  and  $400\text{mgkg}^{-1}$  body weight of the stem bark extracts daily after exposure to cadmium chloride for 28 days respectively.

## **2.6 Preparation of Brain Tissues**

Animals were sacrificed by cervical decapitation. The brain was removed, washed with normal saline and all the extraneous materials were removed before weighing. The rat brain tissue was minced and homogenized in  $500\mu\text{l}$  of buffer A (20mM HEPES, pH 7.5, 50mMKCl, 1 mM EDTA, 2mM  $\text{MgCl}_2$ , 220mMmannitol, 68mM sucrose, 1mMleupeptin,  $5\mu\text{g/ml}$  pepstatin A,  $5\mu\text{g/ml}$  aprotinin, 0.5mM PMSF). The brain was kept at ice-cooled conditions all the time.

## **2.7 Preparation of Serum**

Following daily exposure for 28 days, the animals were sacrificed 24 hours after the last dose by cervical dislocation. Blood samples were then collected by retro-orbital puncture and allowed to coagulate at room temperature for half an hour, after which the serum was obtained by blood centrifugation at 3,000 g for 15 minutes in a Beckman bench centrifuge and kept at  $20^\circ\text{C}$  until analyses were done.

## **2.8 Biochemical Tests**

Protein contents of the samples tested by the method of [19] using bovine serum albumin as standard. The alanine and aspartate aminotransferases (ALT and AST) tested by the combined methods of [20] and [21]. Lipid peroxidation level was tested by the reaction between 2-thiobarbituric acid (TBA) and malondialdehyde (MDA), a product of lipid peroxides as described by [22]. The tissue superoxide dismutase (SOD) measured by the nitro blue tetrazolium (NBT) decrease method of [23]. Catalase (CAT) tested spectrophotometrically by measuring the rate of decomposition of hydrogen peroxide at 240 nm as described by [24]. Reduced glutathione level by the method of [25], this method is on developing a stable (yellow) colour when 5', 5'-dithiobis-(2-nitrobenzoic acid) (Ellman's reagent) mix to sulfhydryl compounds. The chromophoric product resulting from Ellman's reagent with the reduced glutathione (2-nitro-5-thiobenzoic acid) holds a molar absorption at 412nm which is part to the reduced glutathione in the test sample. The glutathione peroxidase (GPx) tested by the method of [26]. When this substance is mixed with reduced glutathione, its absorption shifts to a longer wavelength 340 nm and increase at this wavelength provides a direct measurement of the enzymatic reaction.

## **2.9 Histopathology of Tissues**

The brains from control and experimental groups were fixed with 10% formalin and embedded in paraffin wax and cut into longitudinal section of  $5\mu\text{m}$  thickness. The sections were stained with haematoxylin and eosin dye for histopathological observation.

## **2.10 Statistical Analysis**

All the data are expressed in  $\text{mean}\pm\text{SEM}$ . The significance of difference in means between control and treated animals was determined by One-way analysis of variance (ANOVA) followed by the Duncan multiple range test for analysis of biochemical data using SPSS (16.0). Values considered statistically significant at  $p=.05$ .

### 3. RESULTS AND DISCUSSION

#### 3.1 Phytochemicals Investigation

It was found that ethanolic extract contained compounds known to have antioxidant activity like tannins, phlobatannins, flavonoids, anthocyanin, cardiac glycosides and alkaloids (Table 1).

**Table 1. Phytochemical screening of ethanolic extract of *Irvingia gabonensis* stem bark**

Phytochemical	Extract content
Alkaloids	+++
Tannin	++
Phlobatannins	++
Saponin	+
Flavonoids	+++
Anthraquinones	++
Phenol	+++
Cardiac glycosides	++

+ = Trace amount Present ++ = Moderate amount present, +++ = Noticeable amount present

#### 3.2 Effects of *Irvingia gabonensis* Stem Bark on Body Weight and Relative Weight of Organs of Cadmium-induced Oxidative Damage in Rats

In (Table 2), there was significant increases ( $p < .05$ ) in the relative weight of brain of cadmium untreated rats when compared with the control, while treatment with *Irvingia gabonensis* stem bark (100 and 200mgkg<sup>-1</sup>) significantly decrease the relative weight of brain of cadmium-induced rats to values that statistically similar ( $p > .05$ ) to the control. All these changes induced by cadmium intoxication significantly ( $p < .05$ ) restored to near normal levels on administration of *Irvingia gabonensis* stem bark.

**Table 2. Changes in the body weight and relative weight of organs of Cadmium-induced oxidative damage in rats treated with ethanolic extract of *Irvingia gabonensis***

Treatment	Body weight (g)		Weight of organs(g)	Relative weight of organs
	Initial	Final	Brain	Brain
Control	100.25±0.21	117.46±5.32	6.35±0.27	0.78±0.05
Cadmium untreated	112.08±1.12	128.10±4.96	5.24±0.60	1.35±0.08*
Cadmium+200mgkg <sup>-1</sup>	86.45±2.23	125.55±3.11	4.40±0.22	0.65±0.03**
Cadmium+400mgkg <sup>-1</sup>	98.02±3.35	131.20±2.09	5.34±0.40	0.72±0.03**

Values are means±S.D. of 5 animals per group, cadmium=at 4mgkg<sup>-1</sup>, cadmium Treated=*Irvingia gabonensis* at 200mgkg<sup>-1</sup>, cadmium treated=*Irvingia gabonensis* at 400mgkg<sup>-1</sup>, \*significantly different from Control ( $P = .05$ ), \*\* significantly different from cadmium untreated ( $P = .05$ )

### 3.3 Effects of *Irvingia gabonensis* Stem Bark on Antioxidant Parameters and Marker Enzymes in Cadmium-induced Toxicity in Rats

Administration of cadmium chloride significantly increased ( $p < .05$ ) serum, and brain lipid peroxidation (LPO) products measured as thiobarbituric acid reactive substances respectively (Table 3). However, treatment with *Irvingia gabonensis* extract completely ameliorated cadmium chloride-induced increase in LPO. In cadmium-induced rats, the activities of brain GSH, SOD and CAT as well as GPx decreased significantly relative to the control (Table 4). Excellent performance of extract at ( $400\text{mgkg}^{-1}$ ) reversed the adverse effect of cadmium chloride by normalizing this enzymatic antioxidant. *Irvingia gabonensis* treatment to cadmium treated groups caused a significant increase in GPx activities as well as a noticeable increase in GSH level. However, in serum, *Irvingia gabonensis* treatment to cadmium treated groups caused a significant increase in the antioxidant enzymes (Table 5). In cadmium-induced rats, serum ALT and AST were significantly increased (Table 6) relative to the control. Treatment with *Irvingia gabonensis* resulted in significant protection of the brain, as indicated by reductions in the elevated levels of ALT and AST; however, there was evidence of amelioration in the treated group.

**Table 3. Changes in the levels of lipid peroxidation in cadmium-induced toxicity rats treated with ethanolic extract of *Irvingia gabonensis***

Treatments	Brain ( $\mu\text{mol MDA/mgprotein}$ )	Serum( $\mu\text{mol MDA/mgprotein}$ )
Control	6.05 $\pm$ 0.02	6.82 $\pm$ 0.08
Cadmium untreated	8.62 $\pm$ 0.03*	8.26 $\pm$ 0.06*
Cadmium+200mg kg <sup>-1</sup>	5.53 $\pm$ 0.04**	5.52 $\pm$ 0.56**
Cadmium+400mg kg <sup>-1</sup>	5.85 $\pm$ 0.02**	5.04 $\pm$ 0.35**

Values are means $\pm$ S.E.M. of 5 animals per group, cadmium Treated=*Irvingia gabonensis* at  $200\text{mgkg}^{-1}$ , cadmium treated=*Irvingia gabonensis* at  $400\text{mgkg}^{-1}$ , \*significantly different from control ( $P=0.05$ ), \*\* significantly different from cadmium untreated ( $P=0.05$ )

**Table 4. Changes in the levels of brain antioxidant parameters in cadmium-induced rats treated with ethanolic extract of *Irvingia gabonensis***

Treatment	Brain			
	GSH	GPx(mg /gtissue)	SOD	CAT(U/mg protein)
Control	39.85 $\pm$ 0.15	45.55 $\pm$ 0.83	47.26 $\pm$ 1.01	45.77 $\pm$ 1.08
Cadmium untreated	18.23 $\pm$ 0.21*	20.75 $\pm$ 0.41*	28.53 $\pm$ 0.68*	26.22 $\pm$ 0.58*
Cadmium+200mgkg <sup>-1</sup>	33.33 $\pm$ 0.24**	36.23 $\pm$ 0.45**	38.88 $\pm$ 0.03**	36.89 $\pm$ 0.41**
Cadmium+400mgkg <sup>-1</sup>	35.21 $\pm$ 0.27**	41.48 $\pm$ 0.21**	42.34 $\pm$ 1.00**	41.12 $\pm$ 0.81**

Values are means $\pm$ S.E.M. of 5 animals per group, cadmium Treated=*Irvingia gabonensis* at  $200\text{mgkg}^{-1}$ , cadmium treated=*Irvingia gabonensis* at  $400\text{mgkg}^{-1}$ , \*significantly different from control ( $P=0.05$ ), \*\* significantly different from cadmium untreated ( $P=0.05$ )

### 3.4 Effects of *Irvingia gabonensis* Stem Bark on the Histology of Brain

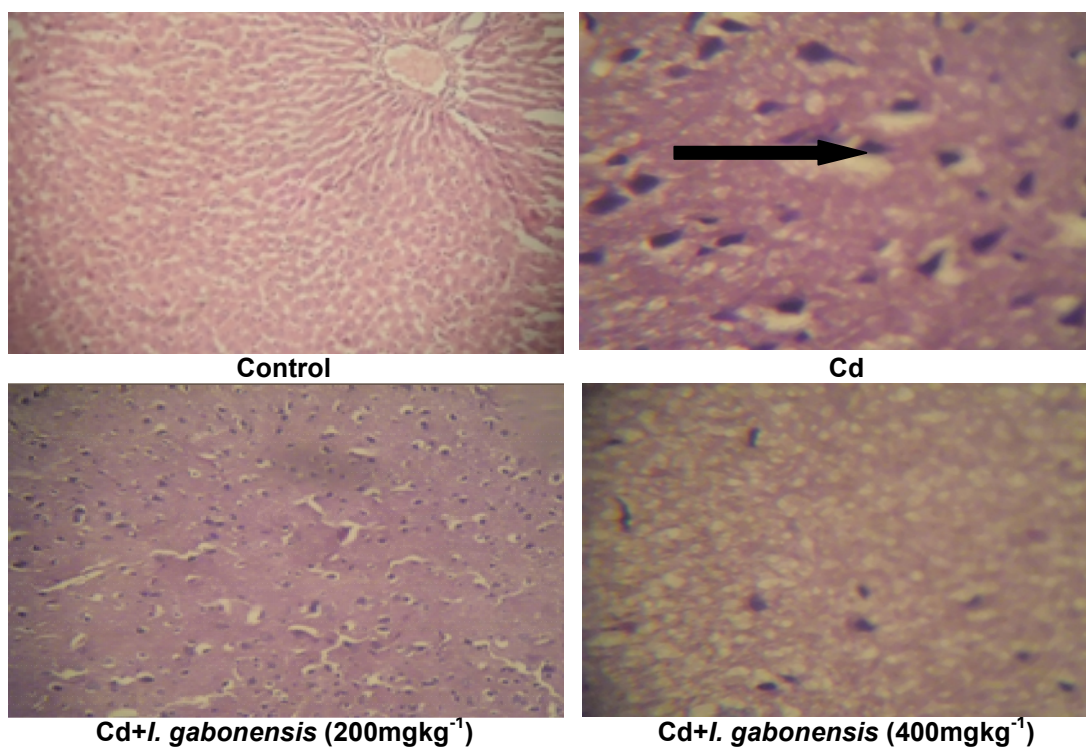
The histology of brain slide of cadmium untreated rats showed mild spongiosis, severe congestion and hemorrhage at the meninges (Fig. 1). Treatment with ethanolic extract of stem bark *Irvingia gabonensis* ( $200$  and  $400\text{mgkg}^{-1}$ ) confirmed the neuroprotective activity as a significant recovery of neuronal damage and decreased necrosis was evident against cadmium induced oxidative damage in the brain of the rats, which is similar to their control.

The histological results further corroborated the biochemical findings suggesting the useful effects of *Irvingia gabonensis* stem bark in cadmium-induced toxicity in rats.

**Table 5. Changes in the levels of serum antioxidant parameters in cadmium-induced rats treated with ethanolic extract of *Irvingia gabonensis***

Treatment	Serum			
	GSH	GPx(mg /gtissue)	SOD	CAT(U/mg protein)
Control	9.85±0.15	8.55±0.10	8.26±0.07	8.77±0.08
Cadmium untreated	2.23±0.11*	2.75±0.11*	2.53±0.28*	2.22±0.12*
Cadmium+200mgkg <sup>-1</sup>	5.33±0.14**	6.23±0.25**	5.88±0.02**	6.89±0.32**
Cadmium+400mgkg <sup>-1</sup>	8.21±0.25**	7.48±0.22**	7.34±0.18**	7.12±0.21**

Values are means±S.E.M. of 5 animals per group, cadmium Treated = *Irvingia gabonensis* at 200mgkg<sup>-1</sup>, cadmium treated=*Irvingia gabonensis* at 400mgkg<sup>-1</sup>, \*significantly different from control (P=0.05), \*\* significantly different from cadmium untreated (P=.05)



**Fig. 1. Changes in histology of brain samples of cadmium-induced oxidative stress in rats treated with *Irvingia gabonensis* ethanolic stem bark extract**

Black arrow: mild spongiosis, severe congestion and hemorrhage at the meninges

The phytochemical study of *Irvingia gabonensis* stem bark extracts revealed the presence of polyphenol-rich compounds. Polyphenols have been suggested to decrease the oxidative stress in human. Flavonoids found in the extract may inhibit the oxidative stress by scavenging free radicals by acting as reducing agent, hydrogen atom donating molecules or singlet oxygen quenchers; chelating metal ions and sparing other antioxidants (e.g. carotene, vitamin C and E) [27]. Literature reveals that, the carbonyl groups present in the



flavonoids and phenolic compounds were responsible for antioxidant activity [28]. This research revealed that the *Irvingia gabonensis* contain pharmacologically active substance (s) such as alkaloids, glycosides, saponins, tannins, flavonoids and phenolic compounds, which are responsible for the antioxidant activity.

**Table 6. Changes in the activities of serum and brain alanine and aspartate aminotransferases in cadmium-induced rats treated with ethanolic extract of *Irvingia gabonensis***

Treatments	Brain (U/L)		Serum (U/L)	
	AST	ALT	AST	ALT
Control	58.65±0.02	65.56±2.24	4.54±1.77	6.37±1.46
Cadmium untreated	22.42±2.38*	21.28±2.04*	8.39±0.56*	9.89±2.24*
Cadmium+200mgkg <sup>-1</sup>	41.02±1.25**	40.12±1.24**	4.13±1.50**	5.78±1.38**
Cadmium+400mgkg <sup>-1</sup>	49.02±1.45**	58.12±1.02**	4.01±1.42**	6.01±1.28**

Values are means±S.E.M. of 5 animals per group, DDVP=at 50mgkg<sup>-1</sup> cadmium Treated=*Irvingia gabonensis* at 200mgkg<sup>-1</sup>, cadmium treated = *Irvingia gabonensis* at 400mgkg<sup>-1</sup>, \*significantly different from control (P=.05), \*\* significantly different from cadmium untreated (P=.05)

The brain has a high rate of oxidative metabolism, consuming ~20% of the cardiac output. At the same time, the brain compared to lung, liver and other organs, contains relatively low levels of enzymatic and non-enzymatic antioxidants and high amounts of peroxidizable unsaturated lipids, rendering it more vulnerable to oxidative stress compared to other tissues [29]. Increasing evidences suggested that, excessive production free radicals in brain and the imbalance between oxidative species and antioxidant defenses are related to the pathogenesis of neurodegenerative diseases [30]. Recent studies have shown that cadmium produces ROS, resulting in an increased lipid peroxidation, depletion of sulfhydryl, altered calcium homeostasis, impairment of antioxidant defenses and finally DNA damage [31,32]. Cadmium induced rats treated with *Irvingia gabonensis*, the changed body weight and brain weight parameters recovered to near normal levels due to the antioxidant effects of found in *Irvingia gabonensis* stem bark. Cd induces oxidative damage by producing reactive oxygen species [30,33,34] and decreasing the biological activities of some antioxidant enzymes, such as vitamins C, E, GSH, superoxide dismutase, catalase and glutathione peroxidase [35-37] which play an important role in anti-oxidation and elimination of free radicals. Cd has also been reported to cause damage to lipids and by that to generate LPO [38,39]. Cd induced damage is associated with increased lipid peroxidation [40,41]. Cell membranes are phospholipid bilayers with extrinsic proteins and are the direct target of lipid peroxidation which leads to a number of deleterious effects such as increased membrane rigidity, osmotic fragility, cell membrane destruction and cell damage [42]. The observed increase in the level of serum LPO in Cd toxicity is generally thought to be the consequence of an increased production and liberation of tissue lipid peroxides into circulation due to the pathological changes in tissues [43]. Observed that the treatment with Cd increased the lipid peroxide concentration in blood. Increase in lipid peroxidation in serum and tissues have been implicated in cadmium-induced organ damage and dysfunction [14,44]. Treatment with *Irvingia gabonensis* significantly reversed the Cd induced peroxidative damage in serum which is evidenced from the lowered levels of LPO. This may be due to the anti-oxidative effect of *Irvingia gabonensis* which decrease the oxidative damage by blocking free radicals produce, and thus inhibited lipid peroxidation. It has been shown in many studies that Cd induces oxidative damage by producing ROS [30,34] and decreasing the biological activities of some antioxidant enzymes, such as SOD and CAT [36,37] which play an important role in antioxidant profile, and in scavenging of free radicals. GSH is known to play a major

role in the regulation of intracellular levels of reactive oxygen species by direct reaction, scavenging, or via the GSH peroxidase or GSH system. Thus, GSH is a major cellular antioxidant and protects cells against oxidative damage. The present study indicates that the Cd will reduce the levels of non-enzymatic antioxidants in serum [35]. Reported the depressed level of reduced glutathione, vitamin C and vitamin E in Cd-intoxicated rats which is in consonance with our findings. Treatment with *Irvingia gabonensis* in Cd-intoxicated rats protects the depletion of non-enzymatic antioxidants via its metal-chelating and antioxidant property [45] and may minimize the usage of these antioxidants, thus restoring their levels.

The detoxification of ROS in brain involves the cooperative action of the intracellular antioxidant enzymes, superoxide dismutase, catalase and glutathione peroxidase. The decreased activity of these antioxidant enzymes in the brain of cadmium intoxicated rats was also observed in the present study. The diminished activities of these antioxidant enzymes might be due to the binding of cadmium with sulfhydryl group of enzymes, replacement of essential metals from their active sites and oxidative modification of amino acid side chains, which alters the enzyme structure and leads to the inactivation or impaired activity of enzymes. Thus, the decreased activity of brain antioxidant system by cadmium leads to the accumulation of free radicals and increased the levels of LPO, which in turn increases the oxidative damage to the brain tissue. Well known biomarkers (ALT and AST) and histological changes were examined to evaluate the neuroprotective effects of *Irvingia gabonensis*. Consistent with previous studies, our study confirmed that acute cadmium exposure damaged the brain, as shown by elevation of the serum aminotransferase activities and morphological changes observed in the brain sections [35,46]. Interestingly, these adverse effects were significantly attenuated by *Irvingia gabonensis* in the treatment groups, which indicated a prominent neuroprotective effect of *Irvingia gabonensis* against cadmium toxicity. The increased levels of serum AST and ALT in cadmium-induced rats indicate an increased permeability and damage and neurosis of brain. In our study, we found that extract of *Irvingia gabonensis* at a dose of 400 mg kg<sup>-1</sup> caused a significant decrease in the activities of serum AST, ALT which further supports the beneficial effects of the extract of *Irvingia gabonensis* in cadmium-induced rats.

Histological examination of the brain tissue reveals that cadmium intoxication caused abnormal ultra-structural changes in the brain tissue including spongiform necrosis, nuclear vacuolization pycnosis and lymphocytic inflammatory changes. Regarding the histopathological observation, *Irvingia gabonensis* treated cadmium intoxicated rats the observed pathological impairments by cadmium have been recovered significantly which indicates that *Irvingia gabonensis* is capable of preventing the neuronal damage induced by cadmium. Therefore, it may be suggested that *Irvingia gabonensis* might inhibit Cd induced brain damage. However, further studies are necessary to find out the actual mechanism of action of phytochemicals and their doses in the presence of oxidative stress due to Cd intoxication.

#### 4. CONCLUSION

In conclusion, the results of the present study demonstrates that *Irvingia gabonensis* exhibited a significant protective action against cadmium induced neurotoxicity in rats via inhibiting the lipid peroxidation and increasing the endogenous antioxidant defense systems in serum and brain and subsequent restoration of the normal histo-architecture of the brain tissue. Furthermore, *Irvingia gabonensis* stem bark extract may play a very useful role in reduction of the neurotoxicological damage induced by cadmium. It eliminates the deleterious effects of toxic metabolites from cadmium when administered orally. Further

studies are required to identify the active component(s) and mechanism(s) underlying the beneficial effects of this plant.

## CONSENT

Not applicable.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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