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Full length Research Article

Effect of Aqueous Extract of *Hibiscus Sabdariffa* on Cadmium Chloride-Induced Neurotoxicity in Male Wistar Rats

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Summary: This study investigated the neurologic effect of aqueous extract of *Hibiscus Sabdariffa* on cadmium chloride-induced neurotoxicity in Wistar rats. Thirty male Wistar rats were grouped accordingly; Group A: control, Group B-F: CdCl2 0.3ml; *H. Sabdariffa* 1.5ml; CdCl2 + *H. Sabdariffa* 1.0ml; CdCl2 + *H. Sabdariffa* 1.5ml; CdCl2 + *H. Sabdariffa* 2ml, respectively. Gas chromatography coupled to flame ionization detector (GC-FID), total antioxidant capacity (TAC) and mineral analysis of *H. Sabdariffa* was done to reveal the bioactive agents in the plant sample. Oxidative stress, muscle function markers and plasma electrolytes were assayed. Phytochemical screening revealed alkaloids, phytate, anthraquinone and flavonoids. The mineral analysis revealed predominantly, Mn, Ca, K and Fe. The biochemical results were in comparison with control and statistically significant at 95% confidence interval. There was an increase in SOD, CAT, GSH, GR and GPx in groups C-F, MDA decreased in group C, E and F while PC decreased in group C and F. There was a decrease in plasma creatine kinase in groups C to F and increase in AChE in group C. Ca and K levels had no significant change ($P<0.05$). Nitric oxide level had no significant change in group C, E and F, but increased in group D. This study demonstrated that *H. Sabdariffa* has the potential to ameliorate the neurotoxicity caused by CdCl2 in Wistar rats.

Keywords*: Neurotoxicity; Hibiscus sabdariffa; cadmium; neuromuscular function; phytochemicals*

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INTRODUCTION

Cadmium (Cd) is a toxic, nonessential transition metal that is classified as a human carcinogen (Wang and Du 2013; Viaene, 2000). Over the years, many different forms of exposure to cadmium have been identified, with cadmium being present in the environment as a result of many human exploratory activities (Mehrdad *et al*., 2017). The constant sources of cadmium contamination are related to its industrial application as a corrosive reagent, as well as its use as a stabilizer in polyvinyl chloride (PVC) products, color pigments, and Ni-Cd batteries (Genchi, *et al*., 2020). In areas with contaminated soils, house dust is a potential route for cadmium exposure (Janneke, *et al*., 2007). Cadmium produces neuropathological and neurochemical alterations in central nervous system (CNS), leading to cognitive dysfunction (Sola, *et al*., 2022). Over the years, interest in food diversity for improved health and nutrition has increased in favor of plant-based natural food products with high bioactive compounds. *Hibiscus Sabdariffa* is a plant of the Malvaceae family, commonly known as roselle (Salem, *et al*., 2021; Lucresse, *et al*., 2019). Also called karkade, bissap, sobolo or zobo, depending on the region, *H. Sabdariffa* is prepared as a traditional drink in many African countries. The flowers are mainly used, reputed for their thirst-quenching and relaxing properties (Lucresse, *et al*., 2019). *H. Sabdariffa* has been used as a traditional medicine for various ailments, such as hypertension (Ellis, *et al*., 2022; Koch, *et al*., 2019), liver disorders (Ellis, *et al*., 2022), inflammation (Alshami and Ahmed, 2014), diabetes (Ahmed and Abozed, 2015) and cancer (Koch, *et al*., 2019). Due to an increase in prevalence of neurological disorders, there is an urgent need to discover complementary source of medicines to reduce the challenges in availability or cost related to conventional medicines. The aim of this study is to investigate the neurologic effects of aqueous extract of *Hibiscus Sabdariffa* on cadmium chloride-induce neurotoxicity in male Wister rats

MATERIALS AND METHODS

Ethical approval: This study was approved by the Ethical Considerations Committee, Directorate of Research and Human Development, Madonna University, Elele, Rivers State, Nigeria. The reference Number is MUECC/20230148.

Apparatus and Equipment: Surgical latex gloves, oral gavage/cannula, syringe, lithium heparin bottles, cotton wool, micro hematocrit tube. Aluminium cages and bedding, feeding troughs, orogastric tubes, plain sample bottles, test tubes of various sizes, dissecting kit, dissecting board, water bath, medicated soap, desiccator bottle, slides and cover slips and electronic weighing scale.

Chemical agents: Cadmium chloride (CdCl2), normal saline, distilled water, Diethyl ether, Methylated Spirit, Heparin. The concentration of CdCl2 was 2mg/ml.

Feed: The feed used was the pelleted Top Feed®

Plant collection: The calyx of *H. Sabdariffa* used for this study was obtained from the herbarium of Department of Pharmacognosy, Faculty of Pharmacy, Madonna University, Elele, Rivers State, Nigeria.

Extraction Procedure: Dried *Hibiscus Sabdariffa* calyces (flowers) were obtained from Madonna University Botanical Garden. A measure quantity of 100g of the calyces was grinded into smaller pieces in order to facilitate extraction and was added to 70cl of distilled water in a ratio of 1:7 and was allowed to boil for 35 minutes. After heating, the mixture was allowed to cool to room temperature and then strained using filter paper to separate the liquid extract from the solid plant material which was then stored in a refrigerator daily after administration.

Phytochemical Screening: The Phytochemical Screening to determine the different bioactive constituents of *H. Sabdariffa* was done by Gas Chromatography Coupled to flame ionization detector (GCFID) using standard procedure by Lee and Harnly (Lee and Harnly, 2005), Total Antioxidant Capacity (TAC) using standard procedure by Narendhirakannan and Rajeswari (Narendhirakannan and Rajeswari, 2010), and mineral analysis was performed by Atomic Absorption Spectroscopy (AAS).

Table 1:
The design of this study

Groups	Treatments
A	Feed and water only
B	$CdCl_2$ 0.3ml
C	*H. Sabdariffa* 1.5ml
D	$CdCl_2$ + *H. Sabdariffa* 1.0ml
E	$CdCl_2$ + *H. Sabdariffa* 1.5ml
F	$CdCl_2$ + *H. Sabdariffa* 2.0ml

Animal protocol: A total of thirty (30) male Wistar rats weighing 150g to 170g were sampled. They were purchased and housed in Madonna University Biomedical Research Animal House, Faculty of Basic Medical Sciences; in aluminum cages and were allowed to acclimatize for 2 weeks, exposed to 12/12 hours light/dark cycle. Feed and water were provided ad libitum. The rats were randomly grouped into 6, with each cage housing five rats. After 2 weeks of acclimatization, administration $CdCl_2$ and *H. Sabdariffa* commenced.

Study duration: After 2 weeks (14 days) of acclimatization, this study lasted for 4 weeks (28 days).

Sample collection: The animals were anaesthetized with diethyl ether before an incision was made in the cranial region, an incision was made in the scalp and the skull was opened after which the brain was carefully removed and placed on ice before homogenization.

Brain Tissue Homogenization: All animals were anaesthetized with chloroform (0.5 ml intraperitoneally) and transcardially perfused with normal Saline (0.9% NaCl) followed by 4% Paraformaldehyde (PFA) in phosphate buffer (PB; 0.1 M pH 7.4). The brains were removed from the skull and placed in normal saline for biochemical analysis. Oxidative stress markers and electrolytes were assayed.

Biochemical analysis

Assay for oxidative stress markers: The oxidative stress markers tested for include superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), reduced glutathione (GSH), protein carbonyl (PC) and malondialdehyde (MDA).

Assay for Superoxide dismutase: Using method by Rani, *et al*., 2013. The SOD assay measures the activity of superoxide dismutase (SOD) by inhibiting the autoxidation of adrenaline. Superoxide anions produced during adrenaline oxidation can turn it into adrenochrome, but SOD prevents this reaction by converting superoxide anions into hydrogen peroxide. The amount of adrenochrome formed is directly proportional to the SOD activity (Rani, *et al*., 2013).

Assay for Catalase: Using the method by Chin-Chan, *et al*., 2015. The assay of Catalase (CAT) activity is based on the measurement of the decomposition of hydrogen peroxide (H2O2) by CAT. CAT catalyzes the breakdown of H2O2 into water and molecular oxygen. The rate of decomposition of H2O2 can be measured spectrophotometrically by monitoring the decrease in absorbance at a suitable wavelength, directly indicating the CAT activity (Chin-Chan, *et al*., 2015).

Assay for Glutathione Peroxidase: Using the method by Saez and Nuria Están-Capell, 2014. The assay for Glutathione Peroxidase (GPx) activity is based on the measurement of the rate of glutathione (GSH) oxidation by hydrogen peroxide (H2O2) in the presence of GPx. GPx catalyzes the reduction of H2O2 and organic hydroperoxides using GSH as a cofactor, regenerating GSH in the process. The decrease in absorbance resulting from the oxidation of GSH can be measured spectrophotometrically and is directly related to GPx activity (Saez and Nuria Están-Capell, 2014).

Assay for Glutathione reductase: This was based on the method by Chatterjee, *et al*. 2009. Glutathione reductase together with its co-factor, NADPH, catalyzes the reduction of oxidized glutathione (glutathione disulfide, GSSG) to glutathione wherein the oxidation of NADPH to NADP+ is monitored as a decrease in absorbance at 340 nm. This rate of decrease in absorbance is directly proportional to the

glutathione reductase activity in the sample because the enzyme is present at rate limiting concentrations (Chatterjee, *et al*. 2009).

Assay for Reduced Glutathione: Using Satish and Pal, 2015 method. The reduced form of glutathione comprised in most instances the bulk of cellular non-protein sulfhydryl groups. This method is therefore based upon the development of a relatively stable yellow colour when 5,5 dithiobis-2-nitrobenzoic acid (Ellman's reagent) is added to sulfhydryl- compounds. The chromorphoric product resulting from the reaction of Ellman's reagent with the reduced GSH is measured at 412nm (Satish and Pal, 2015).

Assay for Malondialdehyde: This protocol is based on Esterbauer and Cheeseman, 1990 method and can be adapted depending on specific requirements and sample types. The assay of Malondialdehyde (MDA) is based on the reaction of MDA with thiobarbituric acid (TBA) under acidic conditions to form a chromophore that can be measured spectrophotometrically. MDA is a reactive carbonyl compound and a product of lipid peroxidation, making it a commonly used marker for oxidative stress and lipid damage (Esterbauer and Cheeseman, 1990).

Assay for Protein carbonyl: This protocol is based on Chong, *et al*. 2017 method. The assay for protein carbonyl content involves the detection of carbonyl groups that are formed on proteins through oxidative damage. Carbonyl groups are reacted with a suitable reagent, such as 2,4-dinitrophenylhydrazine (DNPH), to form stable protein hydrazones. The hydrazones are then measured spectrophotometrically, and the amount of protein carbonyl content is quantified (Chong, *et al*., 2017).

Assay for Muscle function markers

Assay for Creatine Kinase (CK): Using Shackebaei, *et al*. 2010 method. Creatine kinase (CK), present in the sample, catalyses the transfer of a high energy phosphate group from creatine phosphate to ADP. The ATP produced in this reaction is subsequently used to phosphorylate glucose to produce glucose-6-phosphate (G-6-P) in the presence of hexokinase. G-6-P is then oxidized by glucose-6-phosphate dehydrogenase (G-6-PDH) with the concomitant reduction of nicotinamide adenine dinucleotide phosphate (NADP) to nicotinamide adenine dinucleotide phosphate reduced (NADPH). The rate of formation of NADPH is monitored at 340 nm and is proportional to the activity of CK in the sample (Shackebaei, *et al*. 2010).

Assay for Acetylcholinesterase: Using Méndez-Armenta and Rios 2007 method. It is based on the reaction between thiols and chromogenic 5,5'- dithiobis-2-nitrobenzoic acid (DTNB) as it measures the formation of the yellow ion of 5-thio-2-nitrobenzoic acid (TNB) (Méndez-Armenta and Rios, 2007).

Assay for Nitric oxide: Using the method by Moncada and Higgs, 1993. The assay is a widely used colorimetric method to indirectly measure nitric oxide levels by detecting its stable reaction product, nitrite (NO2-) (Moncada and Higgs, 1993).

Electrolyte Assay: Calcium is measured using a Potentiometer. This method determines the potential difference that develops between the inner and outer phases of an ion selective electrode. The electrode is made of a selectively permeable material to calcium ion. The potential is measured by comparing it to the potential of reference electrode. Since the reference electrode has a constant potential, the voltage difference between the two electrodes is attributed to the concentration of ionized calcium in the sample 25. Determination of Serum Potassium Concentration were measured using Centronic GmbH kit via turbidimetric determination method by Hillmann et. al., 1967 and Tietz, 1976.

Statistical analysis: Data was expressed as mean ± SEM and ($P<0.05$) was considered statistically significant. Data collected from this study was analyzed using One-Way analysis of variance (ANOVA) and Post Hoc analysis with the aid of IBM®SPSS Version 21.0.

RESULTS

Phytochemical Analysis of *H. Sabdariffa:* Table 2 shows the results of phytochemical analysis using GC-FID. The concentrations of alkaloid, phytate, anthraquinone and flavonoids in *H. sabdariffa* are 140.512 ug/ml, 231.13 ug/ml, 191.230 ug/ml and 240.124 ppm. The results indicate that flavonoid is the most abundant phytochemical in *Hibiscus sabdariffa*.

Table 2:
Gas chromatography coupled to flame ionization detector (GC-FID) screening of *H. Sabdariffa*

Component	Concentration	Unit
Anthocyanins	132.101	ug/ml
Polyphenols	127.011	ppm
Tannins	3.421	ug/ml
Alkaloids	140.512*	ug/ml
Oxalates	28.120	ug/ml
Phytate	231.13*	ug/ml
Terpenoids	2.4540	ug/ml
Anthraquinone	191.230*	ug/ml
Flavonoids	240.124*	ppm
Phenols	134.245	ppm
Saponins	10.712	ug/ml
Glycosides	11.261	ug/ml
Steroids	12.120	ppm
Citric acid	12.781	ug/ml
Beta-carotene	15.3470	ug/ml
Malic acid	10.241	ug/ml
Flavonols	7.8931	ppm
Thiamine	2.252	ug/ml
Glycosides	6.452	ug/ml
Ribalinidine	12.4131	ug/ml

Key - * Major phytonutrients in GC-FID analysis of *H. Sabdariffa*

Total Antioxidant Capacity (TAC) of *H. sabdariff* : At 20mg/ml and 40mg/ml the absorbance of *H. Sabdariffa* was 0.58 and 0.71 and that of ascorbic acid, a well-known antioxidant which was used as a standard, was 0.123 and 0.245 respectively. Consequently, an increased concentration of *H. Sabdariffa* (100mg/ml) showed an absorbance of 0.150 indicating a positive correlation in the absorbance of *H. Sabdariffa* and Ascorbic acid. This

indicates that *H. Sabdariffa* has a potential antioxidant activity to ascorbic acid.

Mineral analysis of *H. Sabdariffa*: The results of mineral analysis of *H. Sabdariffa* (Table 4) show that there are high levels of Fe, K, Ca, Mn present in the extract.

Table 3:
Total Antioxidant Capacity of *H. Sabdariffa*

Concentration	Abs. of ascorbic acid	Abs. of *H. Sabdariffa*
20mg/ml	0.123	0.58
40mg/ml	0.245	0.71
60mg/ml	-	0.90
80mg/ml	-	0.120
100mg/ml	-	0.150

Table 4:
Mineral analysis of *H. Sabdariffa*

Mineral	Concentration (mg/kg)
Fe	88.12
K	91.20
Cu	0.10
Ca	102.40
Mg	21.23
Mn	110.61
Zn	0.10

Results from table 5, showed a significant increase (P≤0.05) in SOD in group C, D and F when compared to control and $CdCl_2$ group. There was also a significant decrease (P≤0.05) in SOD levels in Group B when compared to control. In comparison with CdCl group, there was a significant increase in group E. In comparison with group C, SOD levels in group B, D, E and F were significantly decreased. There was a significant increase (P≤0.05) in CAT levels in group C, E and F when compared to the control and $CdCl_2$ group. Also, there was a significant decrease (P≤0.05) in CAT levels in Group B and group D when compared to control. In comparison with Hibiscus group CAT levels in group B, D, E and F were significantly decreased. There was a significant increase (P≤0.05) in GPx levels in group C, E and F when compared to the control and $CdCl_2$ group and also a significant decrease (P≤0.05) in GPx levels in Group B ($CdCl_2$ 0.3ml) and group D when compared to control. In comparison with group C, GPx levels in group B, D, E and F were significantly decreased. The results also showed a significant increase (P≤0.05) in GR levels in group C, D, E and F when compared to the control and $CdCl_2$ group and a significant decrease (P≤0.05) in GR levels in Group B when compared to control. In comparison with group C, GR levels in group B, D, E and F were significantly decreased (P≤0.05).

Table 5 also showed a significant increase (P≤0.05) in GSH levels in group C, E and F when compared to control and $CdCl_2$ group and also a significant decrease (P≤0.05) in GSH levels in Group B and Group D when compared to control. In comparison with Hibiscus group, GSH levels in group B, D, E and F were significantly decreased(P≤0.05). There was a significant decrease (P≤0.05) in MDA levels in group C, E and F when compared to control and $CdCl_2$ group and also a significant increase in MDA level was also seen in group D when compared to control and a significant increase (P≤0.05) in MDA levels in Group B when compared to control. In comparison with Hibiscus group, MDA levels in group B, D, E and F were significantly increased (P≤0.05). Results showed a significant decrease (P≤0.05) in PC levels in group C and F when compared to the control and $CdCl_2$ group. There was also a significant decrease in PC level as seen in group D and E when compared to the $CdCl_2$ group and a significant increase (P≤0.05) in PC levels in Group B and group D when compared to control. In comparison with Hibiscus group, PC levels in group B, D, E and F were significantly increased (P≤0.05).

Results from Table 6 showed a significant decrease (P≤0.05) in Ca^{2+} levels in group B ($CdCl_2$ 0.3ml) when compared to control and Hibiscus group. In comparison with $CdCl_2$ group, Ca^{2+} levels in group C, D, E and F were significantly increased (P≤0.05). The results also showed a significant decrease (P≤0.05) in K^+ levels in group B ($CdCl_2$ 0.3ml) when compared to the control and Hibiscus group. In comparison with $CdCl_2$ group, K^+ levels in group C, D, E and F were significantly increased (P≤0.05). There was a significant increase (P≤0.05) in NO levels in group B ($CdCl_2$ 0.3ml) when compared to the control and Hibiscus group. There was a significant increase (P≤0.05) in NO levels in Group D when compared to control and Hibiscus group. In comparison with $CdCl_2$ group, NO levels in group C, D, E and F were significantly decreased (P≤0.05).

Table 5:
Changes in brain stress markers in response to treatments

Groups	SOD (U/g)	CAT (U/g)	GPx (U/g)	GR (U/g)	GSH (U/g)	MDA (mmol/g)	PC (mmol/g)
Control	12.00 ± 0.46^{bc}	2.86 ± 0.08^{bc}	2.26 ± 0.06^{bc}	2.20 ± 0.10^{bc}	1.40 ± 0.08^{bc}	1.16 ± 0.05^{b}	0.40 ± 0.05^{bc}
$CdCl_2$ 0.3ml	1.40 ± 0.10^{ac}	0.18 ± 0.06^{ac}	0.18 ± 0.04^{ac}	0.34 ± 0.12^{ac}	0.14 ± 0.02^{ac}	12.40 ± 1.77^{ac}	5.36 ± 0.09^{ac}
H. sabdariffa 1.5ml	15.14 ± 0.59^{ab}	5.70 ± 0.04^{ab}	8.26 ± 0.17^{ab}	5.82 ± 0.07^{ab}	5.46 ± 0.12^{ab}	0.09 ± 0.03^{b}	0.18 ± 0.09^{ab}
$CdCl_2$ + *H. sabdariffa* 1.0ml	8.46 ± 0.42^{abc}	2.78 ± 0.19^{bc}	1.74 ± 0.02^{abc}	1.34 ± 0.10^{abc}	1.22 ± 0.09^{bc}	1.80 ± 0.35^{b}	0.52 ± 0.06^{bc}
$CdCl_2$ + *H. sabdariffa* 1.5ml	12.34 ± 0.19^{bc}	4.48 ± 0.10^{abc}	3.20 ± 0.18^{abc}	2.62 ± 0.13^{abc}	3.78 ± 0.07^{abc}	0.35 ± 0.21^{b}	0.26 ± 0.04^{bc}
$CdCl_2$ + *H. sabdariffa* 2.0ml	13.94 ± 0.13^{abc}	4.72 ± 0.04^{abc}	4.48 ± 0.12^{abc}	$4.48\pm.18^{abc}$	4.54 ± 0.12^{abc}	0.12 ± 0.02^{b}	0.14 ± 0.02^{abc}
Total	63.28	20.72	20.12	16.80	**16.54**	**15.92**	**6.86**
Average	10.55	3.453	3.353	2.80	**2.76**	**2.65**	**1.14**

Key: [a](P≤0.05)-Significantly different compared to control group, [b] (P<0.05)-Significantly different compared to $CdCl_2$ group [c](P≤0.05) - Significantly different compared to *Hibiscus* group. Data represented as Mean ±SEM. P≤0.05 was considered significantly different.
SOD- superoxide dismutase ; CAT-catalase; GPx-glutathione peroxidase ; GR-glutathione reductase; GSH-reduced glutathione; MDA-malondialdehyde; PC-protein carbonyl

Table 6:
Changes in muscle function markers in response to treatments

Groups	CK-MM(U/L)	AChE (U/L)
Control	22.04±0.57[bc]	32.52±0.54[bc]
$CdCl_2$ 0.3ml	44.04±0.95[ac]	11.90±2.33[ac]
H. Sabdariffa 1.5ml	11.30±0.32[ab]	46.60±0.58[ab]
CdCl2 + *H. Sabdariffa* 1.0ml	19.82±0.45[abc]	10.88±0.69[ac]
CdCl2 + *H. Sabdariffa* 1.5ml	13.74±0.27[abc]	36.38±1.54[bc]
CdCl2 + *H. Sabdariffa* 2.0ml	12.34±0.39[ab]	51.14±1.65[abc]
Total	123.28	189.42
Average	20.55	31.57

Key: [a](P≤0.05)-Significantly different compared to control group, [b] (P<0.05)-Significantly different compared to $CdCl_2$ group [c] (P≤0.05) -Significantly different compared to *Hibiscus* group. Data represented as Mean ±SEM. P≤0.05 was considered significantly different.

Table 7:
Changes in electrolytes in response to treatments

Groups	Ca (mg/dl)	K (meq/L)	NO (µmol/L)
Control	5.06±0.07[b]	4.46±0.09[b]	0.21±0.15[b]
$CdCl_2$ 0.3ml	2.26±0.07[ac]	2.22±0.40[ac]	2.48±0.10[ac]
H. Sabdariffa 1.5ml	5.08±0.12[b]	4.38±0.06[b]	0.02±0.00[b]
$CdCl_2$ + *H. Sabdariffa* 1.0ml	5.14±0.15[b]	4.38±0.11[b]	1.18±0.16[abc]
$CdCl_2$ + *H. Sabdariffa* 1.5ml	5.10±0.08[b]	4.44±0.10[b]	0.0±0.00[b]
$CdCl_2$ + *H. Sabdariffa* 2.0ml	4.92±0.06[b]	4.62±0.06[b]	0.02±00[b]
Total	27.56	24.5	3.91
Average	4.59	4.08	0.65

Key: [a](P≤0.05)-Significantly different compared to control group, [b] (P<0.05)-Significantly different compared to $CdCl_2$ group [c] (P≤0.05) -Significantly different compared to *Hibiscus* group. Data represented as Mean ±SEM. P≤0.05 was considered significantly different.

DISCUSSION

The outcome of this study showed an increase in antioxidant levels, as indicated by the positive changes in biomarkers such as SOD, CAT, GPx, GSH, and GR which lead to a decrease in lipid peroxidation and subsequently lower MDA levels. This suggests that the aqueous extract of *H. Sabdariffa* may have a protective effect against cadmium chloride-induced oxidative stress modifications in the brain (Olasehinde, *et al*., 2022). This effect of *H. Sabdariffa* could be attributed to several antioxidant compounds it contains.

Previous studies have shown that the extracts of the *H. Sabdariffa* plant have lipid-lowering and antidiabetic effects due to their high levels of bioactive compounds (Da-Costa-Rocha, 2014). The effect of *H. Sabdariffa* in the present study may add to the list of its potential therapeutic applications in complementary and alternative medicine. Calcium is important in the contraction and expansion of blood vessels, nerve impulse transmission, muscle contraction and hormone production (Kamel, 2017). In muscle cells, nerve endings release calcium ions, which bind to activator proteins. The activator proteins initiate the complex process of muscle contraction and allow your muscles to move (Kamel, 2017; Zheng, *et al*., 1991). Calcium channels are proteins that allow calcium ions to enter or leave nerve cells. Calcium channels are involved in various types of neurotransmissions, such as excitatory, inhibitory, modulatory, and synaptic plasticity (Brown, *et al*., 2019). The Group administered (cadmium only) CdCl2 show a significant decrease in calcium levels which can cause increased risk of calcium deficiency, which in turn reduces calcium release from bone tissues and increases the risk of osteoporosis (Mahdi, *et al*., 2021; Nordberg, *et al*., 2018). *H. Sabdariffa* treated groups showed no significant change in calcium levels compared to control, by reducing oxidative stress, the extract might have helped maintain calcium balance. *H. Sabdariffa* contains various phytochemicals that can act as metal chelators, such as anthocyanins, flavonoids, phenolic acids, and organic acids (Farag, *et al*., 2015), The extract's chelating properties might help sequester cadmium, preventing its interaction with calcium and maintaining calcium levels. Potassium is important in maintaining fluid balance, regulating nerve and muscle activity, and supporting cardiovascular health, neuronal excitability, and synaptic transmission (Kamel, 2017). The Group administered (cadmium only) CdCl2 showed a significant decrease, which can reduce the uptake of potassium by the cells, leading to lower intracellular potassium concentration and higher extracellular potassium concentration. This can affect the membrane potential of the cells (Christer, 2019) and can impair the function of sodium-potassium pumps, which are proteins that transport sodium and potassium ions across the cell membrane to maintain the membrane potential (Kamel, 2017). Acetylcholinesterase is an enzyme that breaks down a chemical called acetylcholine, which is a neurotransmitter that carries signals between nerve cells and muscle cells. Acetylcholinesterase is important for regulating the activity of acetylcholine and preventing its accumulation in the synapses, which are the gaps between nerve cells (Trang, 2023). Acetylcholinesterase is found in various parts of the body, such as the blood, the brain, the neuromuscular junctions, and other organs. Acetylcholinesterase has various functions, such as controlling muscle contraction and relaxation, modulating learning and memory, and influencing sleep and arousal (Trang, 2023; Ramesh, *et al*., 2013). The Group administered (cadmium only) CdCl2 show a significant decrease in AChE levels, in a similar study cadmium exposure caused a decrease in acetylcholinesterase activity in the blood, brain, and heart (Nunes *et al*., 2014). These effects may be related to oxidative stress and inflammation induced by cadmium. *H. Sabdariffa* treated groups showed significant increase in AChE levels compared to control, Cadmium-induced oxidative stress can negatively impact acetylcholine receptors and neurotransmission (Nunes, *et al*., 2014; Pretto, *et al*., 2009). The antioxidant properties of the extract might have mitigated this oxidative damage, resulting in the preservation of AChE levels. CK-MM is a type of creatine kinase (CK) enzyme that is mostly found in the skeletal muscle. CK is a protein that helps your body's cells do their jobs. CK-MM is the most common form of CK in the body, and it is involved in muscle contraction and relaxation. CK-MM levels can rise when you have muscle damage or disease, such as muscular dystrophy or rhabdomyolis (Salvatore, *et al*., 2016; Washington and Gerald, 2012). The Group administered (cadmium only) CdCl2 show a significant increase in CK-MM levels, in a similar study cadmium exposure caused an increase in CK-MM activity

in the serum and skeletal muscle, indicating muscle damage and dysfunction (Yang, 2015). These effects may be related to oxidative stress and inflammation induced by cadmium. *H. Sabdariffa* treated groups showed significant decrease in CK-MM levels, The *H. Sabdariffa* extract might possess antioxidant and anti-inflammatory properties that mitigate oxidative stress and inflammation induced by cadmium exposure, The extract might have metal-chelating properties that reduce cadmium-induced damage to muscle cells, thereby preventing CK-MM release (Da-Costa-Rocha, 2014; Bizzozero, 2009). Protein carbonyl is a biomarker of oxidative stress (Fernando, *et al*., 2016). Carbonylation is the most common protein modification that takes place as consequence of severe oxidative stress 50. The Group administered (cadmium only) CdCl2 show a significant increase in PC levels, this may be due to oxidative stress resulting to oxidation of proteins (Fernando, *et al*., 2016). *H. Sabdariffa* treated groups showed significant decrease in PC levels, The *H. Sabdariffa* extract might contain antioxidants that can scavenge reactive oxygen species (ROS) generated by cadmium exposure. This would prevent the oxidation of proteins and the formation of protein carbonyls (Farag, 2015; Pretto, *et al*., 2009). The extract could have metal-chelating properties that sequester cadmium ions. By reducing cadmium levels, the extract might indirectly prevent protein oxidation. Nitric oxide is a colorless gas that has many important roles in the human body. It is produced by various cells and tissues, such as the endothelial cells that line the blood vessels, the nerve cells that transmit signals, and the immune cells that fight infections. Nitric oxide helps to regulate blood pressure, blood flow, nerve transmission, and inflammation. It also has some effects on memory, learning, and sleep. Nitric oxide is a free radical, which means it has an unpaired electron that makes it very reactive. This can be beneficial or harmful, depending on the amount and location of nitric oxide in the body (Nui, *et al*., 2023; Singh, *et al*., 2019). The Group administered (cadmium only) CdCl2 show a significant increase in NO levels, by increasing the degradation of nitric oxide by enhancing the activity of reactive oxygen species (ROS), which are molecules that can cause cellular damage by reacting with lipids, proteins, and DNA. ROS can react with nitric oxide and form peroxynitrite, which is a highly toxic compound that can damage cellular components (Nui, *et al*., 2023; Singh, *et al*., 2019; Robbins and Grisham, 1997). *H. Sabdariffa* treated groups showed no significant change in NO levels compared to control, The extract contains antioxidants (Da-Costa-Rocha, 2014) that scavenge reactive oxygen species (ROS) generated by cadmium exposure. Reduced ROS levels would lead to lower peroxynitrite formation.

In conclusion, the findings of the study indicate that cadmium chloride triggers neurotoxic manifestations by disrupting balance in stress, muscle function markers and electrolyte balance. However, rats' treatment with aqueous extract of *Hibiscus Sabdariffa* may ameliorate this effect. This suggests that *H. Sabdariffa* can effectively act as a natural remedy to against neurotoxicity.

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