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ZINGIBER METHANOLIC EXTRACT (ZME) OF DRY GINGER POWDER MITIGATES POTASSIUM DICHROMATE-NEUROTOXICITY IN RAT BRAIN

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ABSTRACT

The wide environmental distribution of Cr (VI) has increased the interest of its possible toxicity. Chromium (VI) the most toxic form, has been demonstrated to induce nephrotoxicity associated with oxidative stress in humans and animals. The present study was undertaken to evaluate the effect of *Zingiber* methanolic extract (ZME) on brain of normal as well as potassium dichromate (PDC) intoxicated adult male albino rats. Adult male rats were divided into seven groups: (I) served as control, (II) (KCr), received a single i.p. injection of PDC (15mg\ Kg body weight), (III) (ZME) Rats were administered *Zingiber* methanolic extract (ZME) dissolved in tween 20 (10%) (200mg\ Kgbody weight), (IV) rats were treated with ZME and PDC. Administration of ZME either alone or to $K_2Cr_2O_7$-treated rats revealed changes in the investigated biochemical parameters (malonaldehyde (MDA), catalase (CAT) and superoxide dismutase (SOD), glutathion (GSH), glutathione peroxidase (GPx), glutathione S-transferase (GSt) and nitric oxide (NO), in brains tissue homogenate of all groups. Other parameters namely; tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) contents were also recorded as a correlation between oxidative stress as well as pro-inflammatory cytokines. Histopathological investigations revealed that PDC injection caused neuron necrosis in cerebral cortex, hippocampus and striatum associated with neuronophagia. Meanwhile, ZME administration to KCr –treated rats was found to diminish necrosis observed in brain of KCr-treated rats and increase cellular density. It could be concluded from this study that ZME of dry ginger indicated its antioxidant anti-inflammatory property, as it seemed to affect brain oxidant-antioxidant status and ameliorate PDC neurotoxicity.

KEYWORDS: Ginger extract, Potassium dichromate, Neurotoxicity, Oxidative Stress, Necrosis.

Abbreviations: ZME, *Zingiber* methanolic extract; Cr, Chromium; $K_2Cr_2O_7$, PDC, potassium dichromate; bw, body weight; i.p., intraperitoneal; MDA, malonaldehyde; GSH, glutathion; CAT, catalase; SOD, superoxide dismutase; TNF-α, tumor necrosis factor-alpha; IL-6, interleukin-6; EDTA, ethylene diamine tetra acetic acid; DTNB, Dithio, bis nitrobenzoic; Nacl, sodium chloride; Na_2HPO_4, disodium hydrogen phosphate; NaH_2PO_4, sodium dihydrogen phosphate; TCA, tri-chloro-acetic acid; H_2O_2, hydrogen peroxide; NADH, nicotine amide dinucleotide hydrogen; NBT, nitroblue tetrazolium; PMS, phenazine methosulphate; $Na_4P_2O_7$, sodium pyrophosphate; GC-MS (GCM), gas chromatography mass spectrum; NIH, National Institutes of Health; HCl, hydrochloric acid; ELISA, Enzyme-Linked Immunosorbent Assay; HD, high definition; TBARS, Thiobarbituric acid reactive substances; H_2O_2, hydrogen peroxide; NF-kB, nuclear factor-kappa beta; COX-2, cyclooxegenase-2; IL-1, interleukin-1; IL-8, interleukin-8; DNA, deoxyribonucleic acid; ROS, reactive oxygen species; GPx, glutathion glutathione peroxidase; GSt, glutathioneS-transferase; NO, nitric oxide.

1. INTRODUCTION

Due to the widespread industrial use of chromium (Cr), large quantities of Cr compounds were discharged in the environment (Kotyzova´ et al., 2015). Cr exists mainly in two states, the trivalent (Cr III) and hexavalent (Cr VI) (Bagchi et al., 2002); both are used in various industrial activities such as steel works, metal finishing, petroleum refining, Cr electroplating, and leather tanning (Keane et al., 2015 and Pesch et al., 2015). Oral ingestion of contaminated water or direct dermal contact with products manufactured using Cr such as pressure-treated wood are potential modes of exposure (Park et al., 2004). Occupational exposure to Cr (VI) compounds is known to cause an increased incidence of cancers of the respiratory system (Salnikow and Zhitkovich, 2008). Cr (IV) also causes acute and chronic toxicity, neurotoxicity, dermatotoxicity, genotoxicity, carcinogenicity, and immunotoxicity (Von Burg and Liu, 1993).

Ginger (*Zingiber)*, member of the family of *Zingiberaceae*, and widely used as a spice. It is, also, used in Asian traditional medicine for various purposes including stomachache (Mascolo et al., 1989), nausea and diarrhea, and joint and muscle pain (Ojewole, 2006). In

addition, ginger extract also possesses antioxidant activity (Ahmed et al., 2005; Stoilova et al. 2007 and Nanjundaiah et al., 2009), neuroprotective effect (Waggas ,2009). Because ginger could scavenge free radicals, an important factor in producing brain damage, its extract might be able to protect brain against oxidative stress damage. The objective of this study is to determine the possible effects of (ZME) on oxidative stress and histopathological changes after $K_2Cr_2O_7$ – (PDC) induced toxicity.

The objective of the current study was to explore the neuroprotective effects of of ZME of dry ginger powder against PDC- neurotoxicity brain damage in adult male albino rats.. Therefore, this study was to evaluate the effect of ZME administration on brain oxidant-antioxidant parameters; MDA, CAT, SOD, NO, GSH, GPx and GSt, on brain tissue of PDC-intoxicated rats. Tumor necrosis factor (TNF-α) and Interleukin-6 (IL-6) which is produced by different brain cells and may signal in a complex manner. Neurons, astrocytes, microglia and endothelial cells the essential sources of IL-6 in the CNS, were also measured in brain homogenates of all treated rats groups, as two inflammatory cytokine mediators. The histopathological architecture of the brain was also investigated microscopically to evaluate the effect of ZME administration on rat brain neurotoxicity induced by PDC.

2. MATERIALS AND METHODS

2.1. Chemicals

Chemicals in the present study were products of Sigma (US), Merck (Germany), and BDH (England): PDC ($K_2Cr_2O_7$), methanol, Tris/ HCl, sucrose, sodium tungstate, Ethylene diamine tetra acetic acid (EDTA), Dithio,bis nitrobenzoic (DTNB), Sodium chloride Nacl, Disodium hydrogen phosphate (Na_2HPO_4), Sodium dihydrogen phosphate (NaH_2PO_4), Tri-chloro acetic acid (TCA), Thiobarbituric acid, Hydrochloric acid, Hydrogen peroxide(H_2O_2), Nicotine amide dinucleotide hydrogen (NADH), phenazine methosulphate, nitroblue tetrazolium (NBT) and sodium pyrophosphate.

2.2. Preparation of ZME

Ginger dry powder was purchased from a local large market in Giza, Egypt. Preparation of the extract was carried out as previously reported by (Mahmoud and Mohamed, 2018). A concentration of 200 mg/0.5 ml/Kg body weight was used in the present study.

2.3. Animals

Adult male albino rats (160 -200 g) were selected for this study. Food and Water were given *Ad libitum* to all animals. General condition of rats was observed, daily. The National

Institutes of Health (NIH) guidelines for animal health and accommodation (Gordon, 1993; Smith et al., 2004; NIH, 2006, 2007) were supervened.

2.4. Experimental Design

Twenty four male rats were used in the present Study. Animals were randomly divided into (4) groups (6 rats each). Group (I) Con, served as normal healthy control, those rats were giving distilled water, using an oral gavage, for 10 days. Group (II) KCr animals were pre-treated with distilled water, using an oral gavage, for seven days followed by a single injection of PDC (15mg\Kg body weight). Animals of group (III) were administered ZME dissolved in 10% tween 20 (ZME) (200mg\Kg body weight) for 10 days. Group (IV); were pretreated with ZME for seven days followed with a single intraperitoneal PDC injection (ZME +KCr), and continued ZMP administration till day 10. Animals of all groups were decapitated at the 11th day of treatment.

2.5. Sample Preparations

Brains of all treated rats groups were quickly removed, dissected on an ice cold plate into two halves, the left part of each brain was stored immediately at −20°C for biochemical analysis. Then each brain was homogenized (1 : 5 w/v) in 5 mmol/L cold Tris\Hcl-sucrose buffer PH 7.4 solution, centrifuged at 3000 rpm for 10 minutes, separated from the supernatant and stored at −20◦C for further antioxidant determinations. While the other half of the rat brain was fixed in 10% neutralized formalin for histology Tissue sample.

2.6. Biochemical Assays in Rats Brain Tissue Homogenate

2.6.1. Determination of MDA & GSH Contents and SOD & CAT Activities

Determination of MDA in rats brain was in the form of TBARS according to Ohkawa et al., (1982). GSH estimation was carried out according to Prins and Loose (1969). The antioxidant enzyme activities, CAT was assayed by the method of Aebi (1984). Enzymatic reaction was initiated by adding an aliquot of 20: l of the homogenized brain tissue. CAT activity was calculated in terms of µmole H_2O_2 consumed/ min/mg of protein. The difference in the absorbance per unit time is the measure of CAT activity. While SOD activity was assayed in brain homogenate using a method by Nishikimi et al., (1972). The oxidation of NADH in the reaction was mediated by superoxide radical and the following increase in absorbance was measured at 560nm.

2.6.2. Determination of NO Content And Both GPx & GST Activity

NO content was estimated according to Montgomery and Dymock (1961), while GPx and GSt activities were assayed in brain tissue homogenate according to Paglia and Valentine (1967) and Habig et al., (1974), respectively. All experiments were carried out according to manufacturer procedures, using local kits from Biodiagnostic, Egypt.

2.7. Determination of Renal cytokines, IL-6 and TNF-α Contents

Rats brain of all treated groups were homogenized in 5 mmol/L Tris–HCl / Sucrose buffered solution (pH=7.4) and centrifuged at 9 000 rpm for 20 min at 4°C. at 4°C. The resultant supernatant was used for cytokine determination, TNF-α and IL-6, using (Enzyme-Linked Immunosorbent Assay) ELISA kits purchased from Wkea Med Supplies Corp. Changchun130012, China. Performance of all analyses were accordance to the instructions provided by the manufacturers. Using (ELISA) EL*808TM, from Biotek Instruments, inc. Highland Park P.O. Box 998, Winooski, Vermont, USA.

2.8. Histopathological examination

Formalin-fixed brain specimens were routinely processed using paraffin embedding technique. Sections of about 4–5 μm were stained with H&E (Bancroft & Gamble, 2008).

3. RESULTS

3.1. Effect of ZME Administration on Oxidative Stress Markers in Brain of Normal and PDC Intoxicated Adult Male Rats

As documented by previously, a single injection of 15 mg/kg PDC to adult male albino rats, was reported to cause kidney injury and liver intoxication (Fatima and Mahmood, 2007; Yam-Canul et al., 2008 and Pedraza-Chaverri et al., 2008, Parveen et al. 2009 and Mahmoud & Mohamed, 2018). The present study investigates the effect of ZME on PDC induced neurotoxicity in adult male albino rats. Markers of oxidant-antioxidant status were evaluated by measuring MDA, GSH contents as well as, CAT, SOD also, GPx and GSt, activities in rat brain tissue homogenates of ZME against normal control, KCr and ZME+KCr groups.

As depicted from Figure (1), results of the present study of indicated that $K_2Cr_2O_7$ injection provoked an increase in MDA content of brain of KCr treated rats group at a significant change at $p < 0.05$, with a percentage difference of (12.20 %), if compared to control treated rats. ZME administration (200mg in 10%Tween20 /kg/day) for 10 days did not affect brain MDA content of ZME treated rats group compared to control group. Administration of ZME to KCr treated rats increased MDA content, if compared to control rats at a significant change

at ($p< 0.05$) with a percentage difference of (6.51 %), but was found to ameliorate the MDA content in brains tissue homogenate of ZME+KCr treated rats, to almost normal values, if compared to KCr treated rats group values.

As illustrated in Figure (1), either PDC injection or ZME administration to adult male rats was found to inhibit brain GSH content of normal rats at a significant change at ($p< 0.05$), with a percentage difference of (- 22.53 %) and (- 48.20 %), respectively, with respect to control values . Likely, ZME+KCr treated rats group resulted in a sharp decrease in brain content of GSH, indicating the inability of ZME to restore normal GSH content, as noticed in Figure (2), being of a significant change at ($p< 0.05$) with a percentage difference of (-81.54%), when compared to control rats group. Regarding the GSt content, KCr-treatment revealed a sharp dramatic decrease in GSt level in brain tissue of rats being of a significant change at ($p < 0.05$) with a percentage difference of (-89.27%), while ZME administration increased its level with a significant change at ($p < 0.05$) being of a percentage difference of (51.90%) in brain tissue homogenate of ZME-treated rats group. Meanwhile, administration of ZME to KCr treated rats revealed the ability of ZME to increase GSt level in rats brain during $K_2Cr_2O_7$ intoxication above the normal GSt content with a percentage difference of (50.00 %) if compared to control values.

GPx is a GSH using enzyme and plays an important role in maintaining GSH homeostasis and tissue detoxification as well. The present data indicate that GPx level was enhanced by KCr treatment while inhibited with ZME treatment which may be due to the depleted level of GSH, these changes were of significant change at ($p < 0.05$) with a percentage difference of (29.95 %) in KCr treated rats group, and with a percentage difference of (-64.86 %) in ZME treated rats group, if compared with control values. Results of GPx level in brain tissue of ZME+KCr treated rats group indicated an increase of a significant change at ($p < 0.05$) with a percentage difference of (52.39 %),if compared with control rats group values, indicating the increased oxidation of GSH in brain homogenates of ZME+KCr treated rats.

KCr treatment in the present study, as shown in Figure (1), decreased SOD and CAT levels in brain tissue homogenate of KCr- treated rats, the decrease was sharp and of a significant change at ($p< 0.05$) with a percentage difference of (-60.91 %),and (-31.83%) in SOD and CAT levels with respect to control values. On the other hand, ZME treatment increased both SOD and CAT levels, with a significant change at ($p< 0.05$), with a percentage difference of (60.41 %) and (51.90 %), respectively, with respect to control values. ZME administration to

PDC intoxicated rats did not ameliorate SOD and inhibition persists brains of ZME+KCr treated rats group SOD level with a significant change at ($p< 0.05$),of a percentage difference of (-59.90 %) with respect to control values, while CAT level showed a ameliorated partial recovery in activity with a percentage difference of (-16.00 %) but of a non significant change, if compared to control values.

3.2. Effect of ZME Administration on NO, IL-6 and TNF-α Contents In Brain Tissue Homogenate of Normal and PDC Intoxicated Adult Male Rats.

As illustrated in **Figure (2)**, results of the present study indicated increased NO content in brain tissue homogenate of both KCr and ZME treated rats groups, as shown in Fig. (2). The increase was of a significant change at ($p< 0.05$), in the KCr treated rats group, with a percentage change of (140.0 %), but with a percentage change of (80.48 %)in the ZME treated rats group, when both groups were compared to control group. ZME treatment to $K_2Cr_2O_7$ intoxicated rats was found to ameliorate the elevated brain NO level in ZME+KCr treated rats group with a percentage difference of (69.85%), though, of nonsignificant change, if compared to KCr treated rats.

As also depicted in **Figure (2)**, results of the present study shed light on IL-6 and TNF-α as two pro-inflammatory cytokines in rats brain tissue homogenate after 10 days of $K_2Cr_2O_7$ and\or ZME administration. KCr-treated Rats indicated an increase in their brain content of IL-6, though of a nonsignificant change. While, ZME treatment to KCr treated rats did decreased it if compared to KCr treated rats group. Moreover, brain tissue homogenate of rats of all treated groups revealed non-significant changes in their TNF-α content. ZME administration to KCr treated rats was found to decrease, brain IL- 6 content towards normal level, though of nonsignificant change, if compared to KCr treated rats group values.

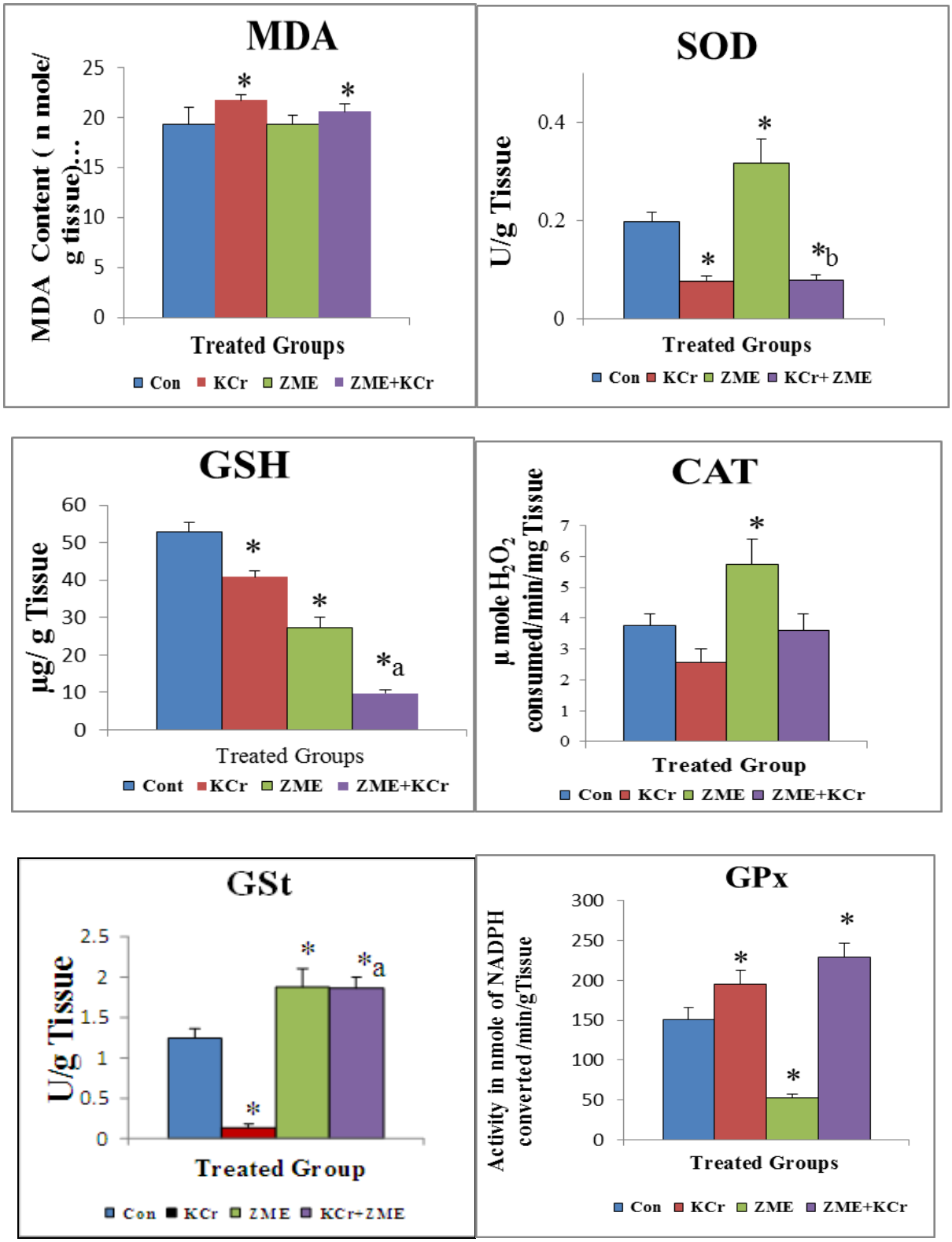

Figure (1): Effect of ZME Administration (200mg/ Kg, bw.) And $K_2Cr_2O_7$ (15mg/Kg, b w., i.p) On Oxidative. Stress Markers; Malonaldehyde (MDA) and Glutathione (GSH) Contents, Catalase (CAT), Superoxide. Dismutase (SOD), Glutathione S-Transferase (GST) and Glutathione Peroxidase (GPx) Activities, In Brain of Treated Rats Groups.

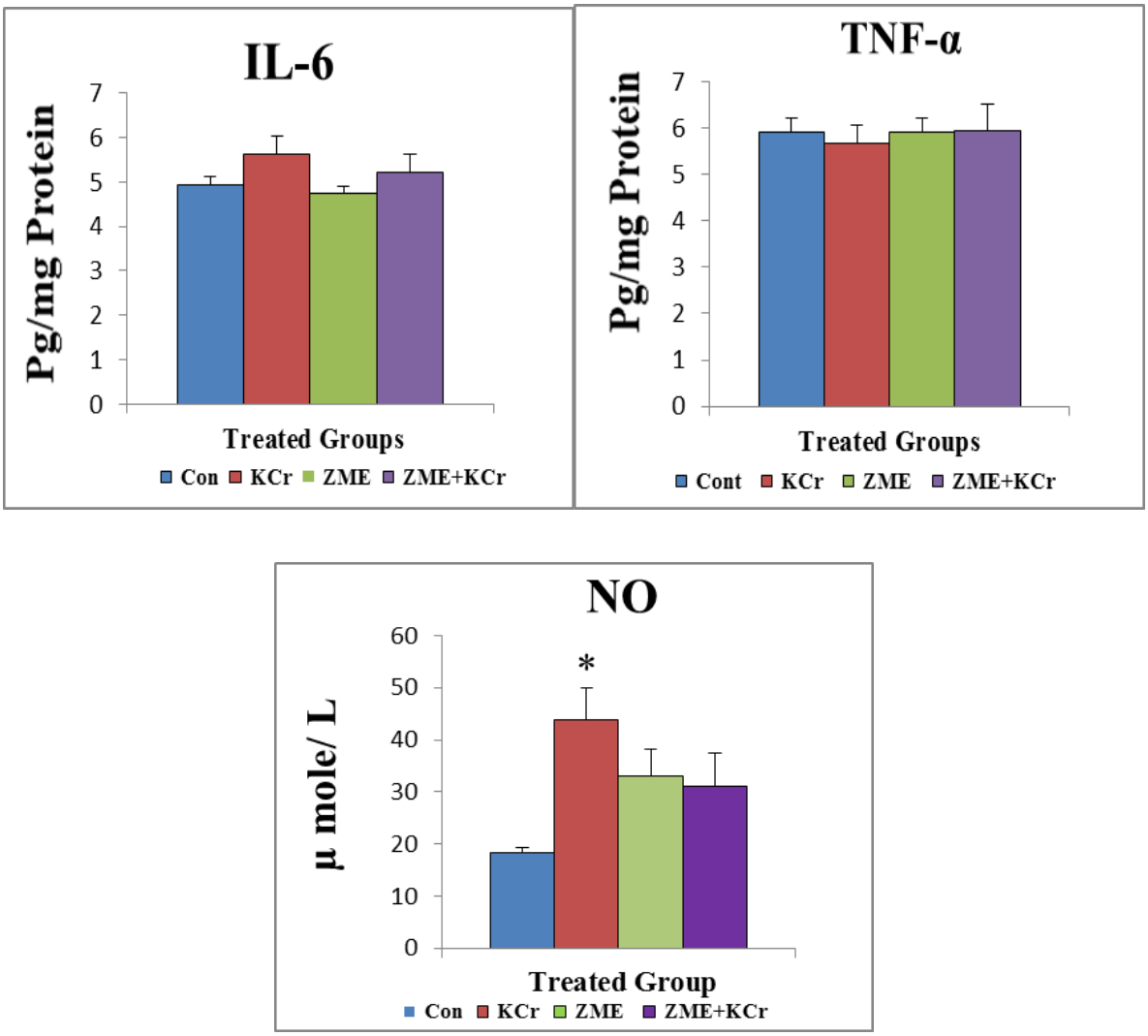

Figure (2): Effect of either ZME Administration (200mg/Kg, bw) And/or $K_2Cr_2O_7$ (15mg/ Kg .bw, i.p.,). On Nitric Oxide (NO) Interleukin-6 (IL-6) And Tumor Necrosis Factor-alpha (TNF-α) Contents, In Brain of Treated Rats Groups.

3.3. Histopathological Findings

The microscopic examination of both control untreated and ZME treated groups revealed normal histological structure of cerebral cortex, hippocampus and striatum (Fig.3). While histopathological alterations were detected in KCr-treated rats group, concerning brain neuronal necrosis and neuronophagia were observed in all layer of cerebral grey matter started from external granular layer that showed necrosis of small pyramidal cells (Fig.4a) and extended into multiform layer (Fig.4b) the necrosis was rarely detected in ZME+KCr treated rats as individual neuronal cytopathology was detected in cerebral cortex especially in external granular layer (Fig.3c) but no reaction was detected in neurons of multiform layer (Fig.4d). No histopathological changes were detected in the cerebellum and cerebral white

matter in KCr or ZME+KCr. Concerning the hippocampus there was a massive reduction in cellular density of all areas of hippocampus and more pronounced in CA3 area (Fig.4e). While there was an amelioration of cellular reduction of pyramidal neurons comprising the all sectors of the hippocampus and the reduction and loss of pyramidal neurons became less (Fig.4f).The neuronal necrosis with neuronophagia associated with glia cell proliferation become more pronounced in striatum than cerebral cortex in KCr-treated group (Figs.5a&b) while in KCr+ZME treated group the striatum showed scarce changes in neurons with individual neuronal necrosis (Figs.5 b&c).

3.4. Statistical Analysis

All data were expressed as mean ±SE of five rats in each group. Statistical analysis was carried out by one-way analysis of variance (equal cell size) using PC-stat, version 1A, copy right 1985, the University of Georgia. Level of statistical significance was taken at $p<0.05$.

$$\% \text{ change} = \frac{\text{Mean of control} - \text{Mean of treated}}{\text{Mean of control}} \times 100$$

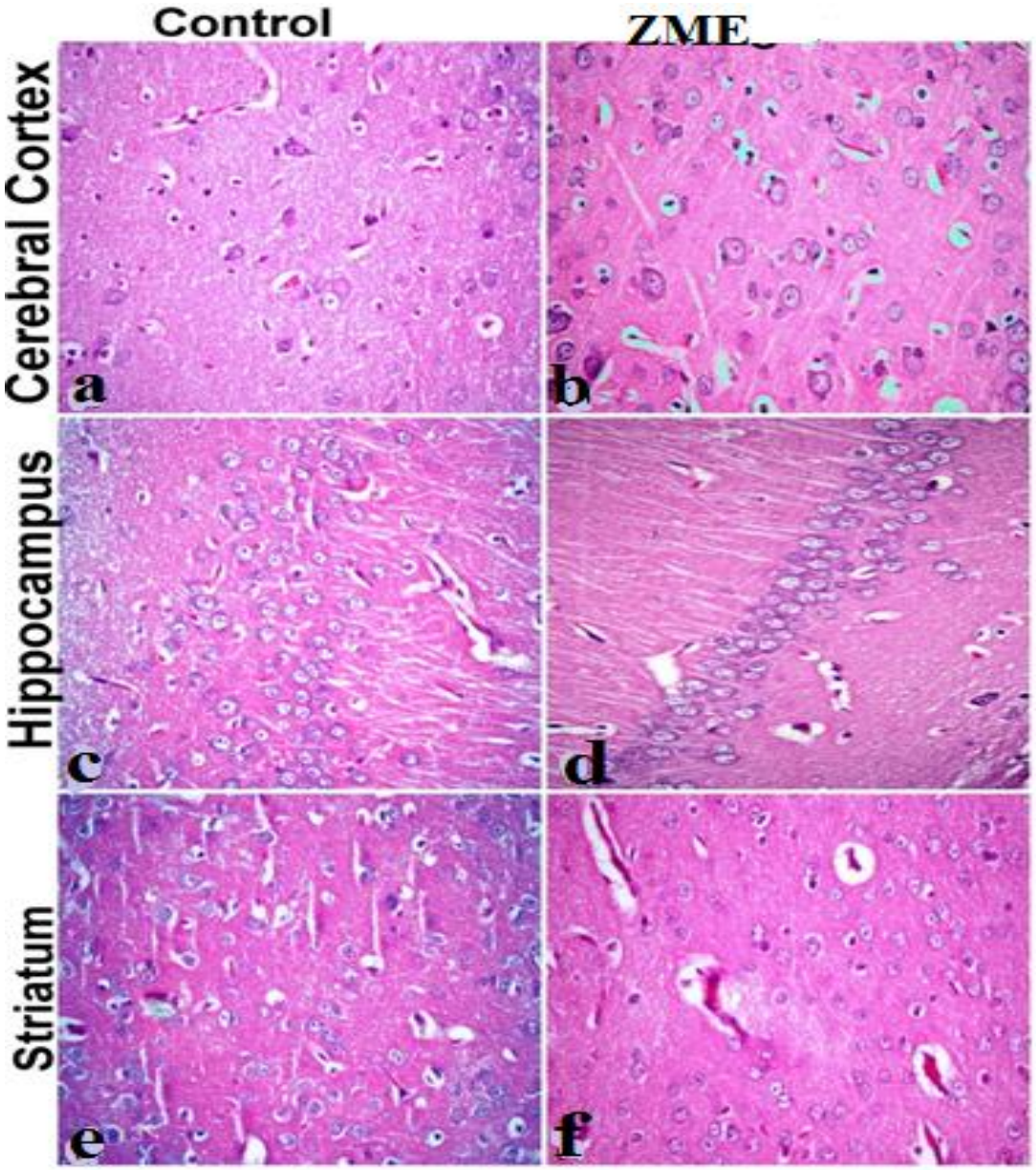

Fig. (3): Histological section of cerebrum from different treated groups (H&E,X400.) a) Cerebral cortex grey matter of control untreated rat showing normal histological structure of small pyramidal neurons and granular nerve cells. b) Cerebral grey matter of ZME treated rat showing normal histological structure of large pyramidal neurons of inner pyramidal layer with normal distribution of glial cells.c) Hippocampus of control untreated rat showing normal histological structure of pyramidal neurons comprising the CA1 region d) Hippocampus of ginger treated rat showing normal histological structure of pyramidal neurons comprising the CA2 region. e) & f) Striatum of control untreated, & ZME treated rats showing normal histological structure of neurons and neuropil.

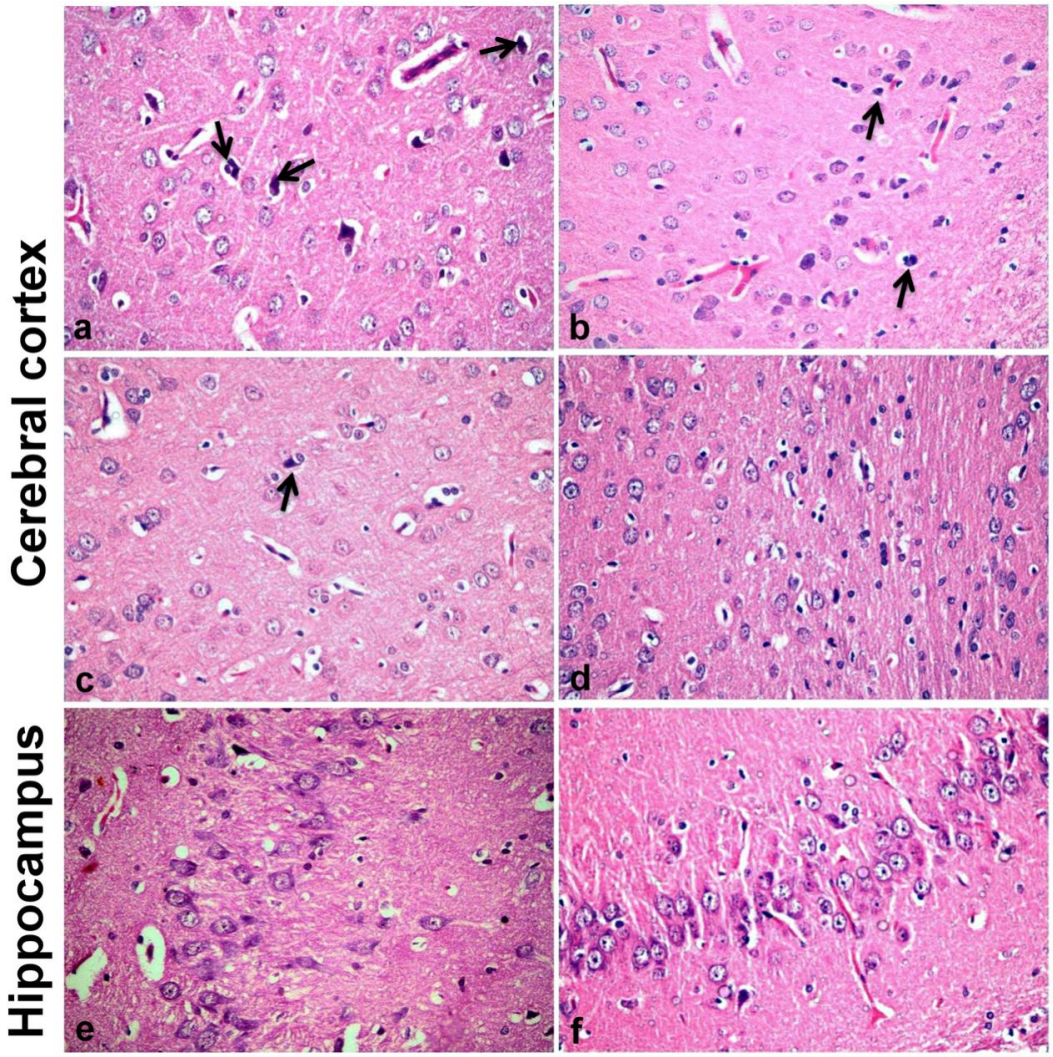

Fig. (4): Histological section of the cerebral cortex of KCr treated rat (a-b) and KCr+ZME treated rat (c-d): a) External granular layer of cerebral grey cortical matter showing necrosis of small pyramidal cells that was surrounded by glia cells (arrow).b)Internal pyramidal and multiform layer showed necrosis of neurons (arrow) .c)Internal granular layer of cerebral cortical grey matter showing individual necrosis of granular neurons (arrow).e) Hippocampus of KCr treated rat showing marked reduction of cellular density with loss of pyramidal neurons .f) Hippocampus of KCr+ZME- treated rat showing mild reduction and loss of pyramidal neurons (H&E, X400).

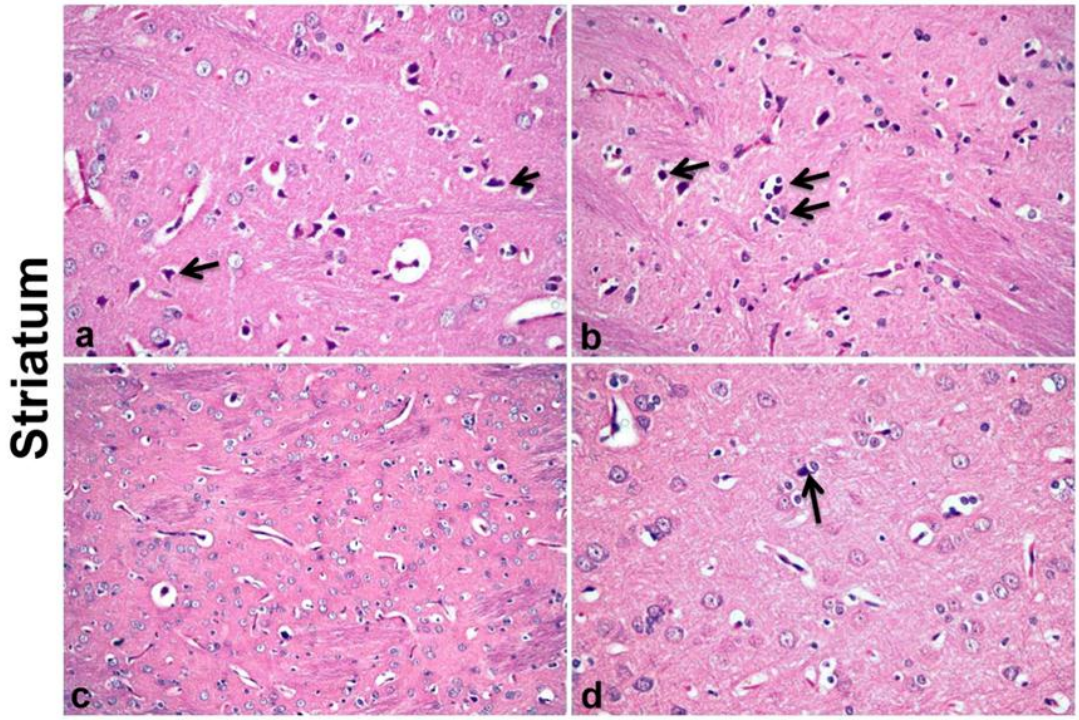

Fig. (5): Histological section of striatum of KCr-treated rat (a-b) and KCr+ZME - treated rat (c-d): a) Striatum showing necrosis of neurons (arrow) (H&E,X400). b) striatum showing neuronophagia (arrow) with glia cell proliferation (H&E,X400) c) Striatum showed minor histological alteration with individual neuronal necrosis and histologically normal of the reminder neurons involved in this field (H&E,X200). d) Higher magnification of the previous showed individual necrosis of neurons (arrow) (H&E,X400).

4. DISCUSSION

Occupational exposure to Cr (VI) compounds gained concern in many Cr-related industries and their surrounding environment (Pellerin and Booker, 2000). Hexavalent form (Cr(VI)) is usually linked with oxygen (chromate CrO_4^{2-}; dichromate $Cr_2O_7^{2-}$) and is a proven toxic and carcinogenic (Bagchi et al., 2001 and Costa, 1997). The Cr (VI) compounds are easily absorbed, therefore, can diffuse across cell membranes, and have strong oxidative potential (Alexander and Aaseth, 1995). Induction of oxidative stress by Cr (VI) was documented earlier, (Blasiak and Kowalik, 2000 and Park et al., 2004). In the present work, three days following a single i.p. injection of PDC led to Cr neurotoxicity, eliciting a significant increase in brain MDA level, with a significant decrease in GSH content, as well as, inhibition of SOD and catalase activity when compared to their normal rat brain content of KCr treated rats group, these finding agree with Soudani et al., (2012). Central nervous system is very

susceptible to any damage caused by external agents. The brain is particularly susceptible to oxidative stress due to its high lipid content (Sun et al., 2002), as containing large amounts of polyunsaturated fatty acids and aerobic metabolic enzymes activities in the nervous tissues, its lower antioxidant having a relatively poor antioxidant defense system levels as compared to other organs, also, a major site of oxygen consumption as it consumes about 20% of the body‟s oxygen Bagchi et al. (1997). Toxic effects elicited by PDC in the brain of adult rat may be due to its accumulation in the rat hypothalamus and anterior pituitary (Quinteros et al., 2007), increased lipid peroxidation in cerebrum and cerebellum of chromium-treated rats (Soudani et al., 2012 and Salama et al., 2016). ROS production causes the imbalance between antioxidant and oxidative stress, that will lead to neuronal cell damage (Halliwel, 2006).

Protection against oxidative cellular damage can be achieved through non-enzymatic and enzymatic antioxidant system. Glutathione is the major soluble intracellular thiol-based antioxidant in all living aerobic cells as it serves as an antioxidant by reacting directly with free radicals or by providing a substrate for the GSH-related enzyme GPx. In the present study, brains of PDC treated rats showed a significant decrease in GSH level, accompanied with a significant decrease in GSt activity and a significant increase in GPx activity indicating the greatly impaired GSH synthesis, as indicated by results of brain of KCr treated group, similar finding were reported in cerebrum and cerebellum by Soudani et al., (2012). This decrease was attributed to GSH consumption through oxidative stress as GSH may act as an electron donor as Cr (VI) is reduced inside cells to the reactive intermediates Cr (V) and (IV) and ultimately to the more stable Cr (III) by cellular reductant (Hojo and Satomi 1991 and Liu and Shi, 2001), and the high affinity for metals found in the sulfhydryl group of cysteine moiety of glutathione leading to formation of thermodynamically stable mercaptide complexes with several metals (El-Sharaky et al.,2007). Inhibition of both GSH and glutathione reductase as reported earlier is required to reduce Cr(VI) to Cr(III) (Raghunathan et al., 2009). Furthermore, GSH plays a critical role in vitamin C regeneration from its oxidized by-products which was, also, reported to decrease (Soudani et al., 2012) leading to increased susceptibility of the brain tissue to free radical damage.

Antioxidant enzymes are also considered the body’s primary defense, enriched in astrocytes, and they may function as better scavengers for free radicals (Ransom et al., 2003; Li et al., 2008) preventing biological macromolecules from oxidative injury and removing peroxides, free radicals, and superoxide anion generated within the cell. The antioxidant defense system

includes SOD and CAT (Gul et al., 2000). SOD is an antioxidant enzyme that catalyzes the conversion of anion superoxide ($O2^{\bullet -}$) to oxygen (O2) and hydrogen peroxide (H2O2). In the present study, sharp reduction of SOD activity in brain of KCr - treated rats group induced by PDC treatment may be due to the generation of excessive anion, ($O2^{\bullet -}$) that causes this enzyme to become inactivated. CAT enzyme involves in the conversion of H2O2 to H2O and O2. The current study showed that KCr decreased CAT activity in the brain, this suggest that the decreased in the CAT activity may be due to the loss of nicotinamide adenine dinucleotide phosphate (NADPH), anion ($O2^{\bullet -}$) production and lipid peroxidation (Das et al., 2008). Neuronal degeneration can be enhanced by dysfunctional and reduced astrocytes as reported by Takuma et al., (2004). Moreover, Wang et al., (2009) indicated increased resistance of astrocytes to Cr(VI) more than neurons via diminishing the secretion of trophic factors needed for the survival support for neurons. The increase in enzymes activity found in the present study after ZME treatment may be due to their activation and/or their new synthesis (Travacio and Lesuy, 1996). This response follows the decrease of enzyme activities immediately after oxidative stress.

GPx enzyme is responsible for detoxifying H_2O_2 by converting it to H_2O in which GSH acts as an electron donor in this reaction (Aoyama et al., 2008). Both GPx and CAT are the major enzymes that remove hydrogen peroxide generated by SOD in the cytosol and mitochondria by oxidizing GSH to GSSG (Park et al., 1994). In the present study, 3 days following PDC injection, a significant increase of GPx, was observed. An inhibition of GPx activity in rat liver and kidneys 12 hrs, was also reported by Anand (2005a, 2005b), after oral administration of PDC at a highly toxic dose of 127 mg/kg. Kotyova et al., (2013) documented that Cr (VI) affects GPx activity, differently, with the organ examined. There is evidence that transition metals act as catalysts in the oxidative deterioration of biological molecules, therefore, their toxicity is associated with oxidative damage (Kasprzak, 2002). Furthermore, lack of antioxidant activities (CAT, SOD and GPx) in the brain also make it more susceptible to damage caused by ROS. Oxidative stress is caused by an imbalance of redox state in the cell, either excess ROS or defect in the antioxidant system (Gandhi, Abramov, 2012). Moreover, administration of Cr (VI) was found to cause inhibition of thio-redoxin reductase (TrxR) as reported by Kotyzova´et al.,(2013) suggesting its critical role for cell survival (Nordberg and Arner, 2001), by enhancing susceptibility of cells to oxidants and favoring apoptosis by a direct redox interactions between Cr(VI) and Trx (Myers et al., 2008 and Myers & Myers, 2009) which could contribute to its cytotoxic effects. So, inhibition of

enzymes may take place through the interaction between Cr(VI) , and the selenoenzymes and glutathione peroxidase (GPx) which are important components of the enzymatic antioxidant system (Kotyzova´et al.,2013).

For thousands of years, several spices have been widely used as food flavorings and in folk medicine as well. Ginger (*Zingiber*) is widely used as a common spice in foods and beverages worldwide, being a member of the *Zingiberaceae* family, and also used in traditional Asian medicine for digestive disorders, common cold, and rheumatism (Mascolo et al. 1989 and Ojewole, 2006). As the ingredients of these plants may contain many bioactive compounds giving them the antioxidant, anti-inflammatory, antifungal, anti-mycobacterial, and anti-carcinogenic properties thus having lot of beneficial health effects, either therapeutic or preventive effects for several ailments ranging from common cold to cancer ginger was also found to treat various diseases (Zheng et al., 2016, Prasad and Tyagi, 2015, Haniadka et al., 2012 and Pereira et al., 2011]. Since the concentration of ginger phenolics is high in fresh ginger, whereas shogaols are abundant in the dried form as reported by (Gan et al., 2011); gingerols and shogaol are documented as markers for the fresh and the dried conditions, respectively (Mukkavilli et al., 2018).

The present data indicate that treatment with ZME prepared from dry ginger did not increase GSH content but decreased it with a significant change and keeping MDA content about normal value. ZME administration decreased oxidative stress by increasing the activity of SOD, CAT, and GSt but decreased MDA, GSH contents and GPx activity in the whole rat brain. A similar increase in antioxidant enzyme activity was recorded with decreased lipid peroxidation level in all brain areas (Wattanathorn et al., 2011). It was also reported that *Z. officinale* at the dose of 200 mg/kg body weight could protect the brain against ischemic brain damage and reduced cognitive deficits in a rat model of focal cerebral ischemia (Maralla and Reddy, 2014). Furthermore, Hamid et al., (2018) suggested that *Z. zerumbet* decreased lipid and protein oxidation and increased antioxidant activities thus having a potential effect as a neuroprotective agent against ethanol-induced brain damage in Wistar rats.

The present data concerning NO content revealed a significant increase in NO of KCr treated rats brain. Also, rats brain homogenate indicated an increase in IL-6 content being of non-significant change, accompanied with a decrease in TNF-α content of the same treated group of non significant change. ZME treated rats group recorded non significant effects on IL-6

and TNF-α contents in rat brain. The present results from ZME+KCr treated rats group revealed the ability of ZME to decrease the elevated non significant increase of IL-6 resulting from PDC intoxication. IL-6 is a major cytokine in the central nervous system, besides its role in controlling other immune cells such as T cells, IL-6 also important in the regulation of hepatocytes, hematopoietic progenitor cells, the skeleton, the cardiovascular system, the placenta and the endocrine system (Kishimoto et al., 1995). CNS IL-6 is up-regulated whenever neuroinflammation is expected, such as following CNS infection or injury or in a number of CNS diseases (Erta et al., 2012). Many of its effects are caused by trans-signaling, while others are mediated by the membrane receptor; both can be essentially considered an integrated, unique system. The relevance of trans-signaling in vivo in a number of peripheral and CNS diseases is recognized (Jones, 2005 and Lemmers et al., 2009). In the late phase of this response, IL-6 orchestrates the transition between innate and adaptive immune response, not only inhibiting neutrophils but recruiting monocytes and T-cells for a late inflammatory response. Besides, it induces astrogliosis and angiogenesis needed for the tissue re-modelation and recovering (Erta et al., 2012). IL-6 participates in neurogenesis (influencing both neurons and glial cells), and in the response of mature neurons and glial cells in normal conditions and of injury models. In many respects, IL-6 behaves in a neurotrophin-like fashion (Erta et al., 2012)

Excessive production of inflammatory mediators such as NO, and pro-inflammatory cytokines, including TNF-α from activated microglia were found to contribute to uncontrolled inflammation in neurodegenerative diseases (Kim et al., 2006 and Jung et al., 2009). The immediate acute inflammatory reaction in brain tissue, was elicited by increased TNF- α and IL-1β levels which are main proinflammatory cytokines, (Kim et al., 2006) that is produced by activated microglia during CNS inflammation content (Soudani et al.,2012) and they attributed this to its up-regulation, 24 hours following a single PDC injection which lead to neutrophil degranulation and tissue destruction by means of metalloproteinase (MMP) and TGF-β, while IL-6 inhibits TNF-α and neutrophils' diapedesis. Activated microglia can promote neuronal injury through the release of proinflammatory and cytotoxic factors, including TNF-α, NO, inducible nitric oxide synthase (iNOS), and cyclooxygenase-2 (COX-2) (Przedborski, 2007).

Ginger and its constituents are believed to have anti-inflammatory properties in macrophage activation and function with suppressing inflammatory mediator productions. Suppression of

proinflammatory cytokines genes expression in microglia was correlated with their inhibited release (TNF-α and IL-1β), as indicated by Jung et al., (2009). The increase in NO content may be attributed to increased iNOS expression or reactive oxygen intermediates such as TNF-α and IL-1β, which are attenuated by the inhibition of either NF-jB and p38 MAPK (Kim et al., 2006). Moreover, Wang et al., (2009) indicated that NF-*kB* activation might be an early event of Cr(VI)-induced oxidative stress as NF-*kB* activation might be involved in the astrocytic defense mechanism against Cr(VI)-oxidative damage or via attenuation of NF-*jB*-mediated iNOS gene expression in mouse macrophages (Aktan et al., 2006).

The present data demonstrate that ZME of dry ginger has an anti-inflammatory property in inhibiting inflammation, though ZME in the present study, lacks gingerols and shogaol, as previously reported (Mahmoud and Mohamed, 2018). ZME was rich in Zingerone, beta-Farnesene, Zingiberene, α-curcumene, β-bisabolene, 6-Paradol and cineol as documented by Mahmoud and Mohamed (2018), to which the effect of dry ginger in this study is attributed to, thus exerting its anti-inflammatory properties via NO suppression when ZME was administered with PDC which could be associated with gene expression of (NO, TNF-α and IL-6), agreeing with Jung et al.,(2009) and that may occur by blocking and attenuating NF-*jβ* pathway. Although ZME did not completely block the production of inflammatory mediators, The present study suggests that ZME affects and modulates TNF-α and IL-6 levels release after microglial activation which could be via their gene expression(Jung et al., 2009) thus controlling their release.

Histopathological studies also provided an important evidence for the biochemical analysis and confirms the present results. The cerebral cortex consists of three levels; molecular layer, granular layer and a layer of Purkinje, and contains billion of neurons that play a role in memory, intelligence and muscle movement (Tortora et al.2005). The hippocampus is one of the rare areas of the brain that exhibits neurogenesis. These new neurons created by the hippocampus are important for memory and learning and require brain-derived neurotrophic factor. That is why cerebral cortex, hippocampus and striatum the main target to investigate their histopathology in this study. Under microscopic examination, distortions in cellular architecture were observed. Chromium treatment provoked neuronal degeneration of the cerebral cortex, Necrosis was observed in neuron cells (cerebral cortex) of KCr-treated group with pyknotic characteristic such as darken nucleus and loss of its normal shape under light microscope. As all layer of cerebral grey matter started from external granular layer that

showed necrosis of small pyramidal cells indicated neuronal necrosis and neuronophagia. Neuronal necrosis with neuronophagia were associated with glia cell proliferation was more pronounced in striatum than cerebral cortex in KCr-treated group while in the hippocampus there was a massive reduction in cellular density of all areas which was more pronounced in CA3 area. This suggests that PDC interrupts cerebral cortex functions (memory and muscle movement), also, the hippocampal functions and decrease neurogenesis. In contrast, concurrent treatment of KCr-treated rats with ZME, was found to protect cerebral cortex, hippocampus and striatum against PDC-induced brain damage as evidenced, though showing scarce changes in striatal neurons in ZME+KCr treated rats as individual neuronal cytopathology, thus eliciting ZME ameliorative role on the brain in regenerating neuronal cells. The possible explanation may be due to differences in the distribution of signal molecules and growth factors that play important roles in cell survival and increasing neurogenesis(Alonso et al., 2004), thus indicating the ameliorating antioxidant, anti-inflammatory effect of ZME dry ginger extract against PDC-induced neurotoxicity.

CONCLUSION

The present study indicated that ZME of dry ginger may exert antioxidant and anti-inflammatory effects, thereby improving the detrimental state of brain cells, which unravels its use in the treatment of chromium neurotoxicity. Anti-inflammatory agents, including plants used in oriental medicine, may be suggested to delay the progression of neurodegeneration resulting from PDC intoxication through the inhibition of microglial activation (Jung et al., 2009). A neuroprotective effect of ZME of dry ginger powder was more evident in the cerebral cortex, hippocampus and also in the striatum of brain of ZME+KCr treated rats, reflecting its minimizing effect on PDC neurotoxicity by showing mild reduction and loss of pyramidal neurons by decreasing oxidative stress through decreasing lipid peroxidation level, increasing antioxidant enzyme activity, decreasing IL-6 and NO production if compared with KCr- treated rats group.

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CONFLICT OF INTERESTS

There is no conflict of interests between this work and any work.

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