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Evaluation of Oral administration of Methanolic Extract of Zingiber Dry Powder on Liver of Normal and Potassium Dichromate-Induced Hepatotoxic Rats

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Abstract
Background: *Human exposure to Cr(VI) induces several adverse health effects,including liver damage.Ginger is a plant whose rhizome is used as a spice or folk medicine.The present study aimed to evaluate the effect of methanolic extract of Zingiber dry powder (ZME) administration on normal rat liver as well as potassium dichromate (PDC)-induced hepatotoxicity.*
Materials and Methods*:24 rats were allocated in 4 groups (6 rats each): (I) control, (II)KCr group, injected with a single intraperitoneal injection of PDC(15mg\Kg bw), (III)ZME group dissolved in 10% tween20 (200mg\Kg bw) orally (VI)ZME+KCr group; Rats received ZME(200mg\Kg bw) for 7 days+ single injection with (15mg\Kg bw)then continuing ZME administration till day 10 and all animals from all groups were decapitated at 11th day. Liver function indices in serum, oxidant/antioxidant parameters and inflammatory markers were determined in rat liver homogenate.*
Results: *PDC increased AST and ALP liver functions with a significant change at p<0.05. ZME alone or with PDC ameliorated most elevated liver function parameters specially, AST and bilirubin at p<0.05, compared with control group. Livers homogenate of ZME treated rats showed altered oxidant/antioxidant status in a similar manner observed with KCr group. Administration of ZME to PDC injected ratsdecreased malonaldehydenonsignificantlyandameliorated SOD activitynonsignificantly versus control,while increased CAT activity at p<0.05 if compared to control, KCr and ZME groups. ZME aloneshowed signifigcant increase in IL-6 levels at p<0.05 and indicated a significant decrease at p<0.05 in the ZME+KCr if compared to ZME group and decreased TNF-α level with no significant changes in theircorresponding groups, when compared to control group. Histopathological examination of livers of ZME treated rats indicated edema, congestion, activated kupffer cells and necrotic hepatocytes.* ***Conclusion****: ginger powder should be used cautiously to normal and chromium-exposed individuals.*
Key Words*: Ginger- Potassium dichromate- Hepatotoxicity-Oxidative Stress- inflammation.*

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I. Introduction

Chromium (Cr) compoundshave gained much interest in the field of toxicology research. Chromium (Cr), a naturally occurring heavy metal, found in the environment as Cr (III) and Cr (VI). Cr (III) is predominantly present in salts used as micronutrients and dietary supplements[1], while Cr(VI) salts such as potassium dichromate ($K_2Cr_2O_7$; PDC) is widely used in leather tanning, chrome-plating, and dye-producing industries and considered to be the toxic form[2]. Studies have documented serious damaging effects in man and animals after Cr(VI) exposure as it induced several adverse health effects, such as nephrotoxicity[3,4], and hepatotoxicity[5], also genotoxicity and carcinogenicity, in other vital organs[6,7]. Cr (VI) was reported to penetrate anionic channels in cellular membranes[8] and inside cells, it is reduced by glutathione and cysteine to Cr (III) by cellular reductants[9]. The redox forms of Cr showed to generate reactive oxygen species (ROS) and leading to genomic DNA damage and oxidative deterioration of lipids and proteins[10]. Liver is an organ capable of being injured by Cr (VI), and studies demonstrated that the exposition to PDC-induced hepatotoxicity associated with increased ROS levels[11], lipid peroxidation[12], inhibition ofantioxidantenzymes[13], structural tissue injury[14], and mitochondrial damage[15] including impaired mitochondrial bioenergetics[16].

Natural antioxidants have been reported to ameliorate or prevent PDC-induced hepatotoxicity[13]. Studies indicated the uniqueness of fresh ginger as one of the most widely used culinary spices, rhizomes of the plant *Zingiber*(Family:*Zingiberaceae*)[17].Ginger was documented to possess medicinal properties and myriad health benefits as it was used to treat different types of ailments, since antiquity, in various alternative and folk systems of medicine[18]. Ginger extract was found to possess antioxidant activity[19, 20] and neuroprotective effect[21].

This study aimed to evaluate the effect of methanolic extract of *Zingiber* dry powder (ZME) on liver function parameters in sera and liver oxidant status by measuring malondialdehyde (MDA) content and the antioxidant status by determining glutathione (GSH) content, also catalase (CAT) and superoxide dismutase (SOD) activities. Moreover, tumor necrosis factor (TNF-α), and interleukin-6 (IL-6) as two inflammatory mediators, were also estimated in liver tissues homogenate of normal and PDC-intoxicated adult male rats. Histopathological investigations were carried out to monitor the changes in liver architecture after PDC, or ZME administration either alone or in combination.

II. Materials and Methods

Chemicals

Chemicals were products of Sigma (US), Merck (Germany), and BDH (England): PDC ($K_2Cr_2O_7$), methanol, Tris/ HCl, sucrose, sodium tungstate, Ethylene diaminetetraacetic acid (EDTA), Dithio,bisnitrobenzoic (DTNB), Sodium chloride NaCl, Disodium hydrogen phosphate (Na_2HPO_4), Sodium dihydrogen phosphate (NaH_2PO_4), Trichloroacetic acid (TCA), Thiobarbituric acid, Hydrochloric acid, Hydrogen peroxide(H_2O_2) , Nicotine amide dinucleotide hydrogen (NADH), phenazinemethosulphate, nitrobluetetrazolium(NBT) and sodium pyrophosphate.

Preparationof the methanolic extract of Zingiberdry powder

Dry powder of ginger was purchased from a local large market in Giza, Egypt. Preparation of the extract was carried out as previously reported by[4].ZME was dissolved in 10% Tween 20, and administered as 200 mg/0.5 ml/Kg body weight[21].

Animals

Adult male albino rats (160 -200 g) were selected for this study. Food and water were given *Ad libitum* to all animals. The general condition of rats was observed, daily. The guidelines for animal health and accommodation of the National Institutes of Health[22]were supervened.

Experimental design

Twenty four male rats were used in the present study. Animals were randomly divided into six groups (6 rats in each):

Group (I) Con, served as normal healthy control, rats were given distilled water, using oral gavage, for 10 days.

Group (II)KCr, animals were pre-treated with distilled water, using oral gavage, for seven days followed by a single injection of PDC (15mg\Kg body weight).

Group (III): animals received ZME dissolved in 10% tween 20 (ZME) (200mg\Kg body weight) for10 days.

Group (IV) animals were pretreated with ZME for seven days followed with a single i.p. injection (ZME +KCr), and continued ZME administration till day 10.Animals of all groups were decapitated on the 11th day of treatment.

Sample preparations

Livers from all treated rats were quickly removed, rinsed with ice cold saline, plotted with filter papers and dissected then stored immediately at −20°C for biochemical analysis. Liver tissue samples were weighed and homogenized (1: 10 w/v) in 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. Samples of rats' liver tissue were taken and fixed in 10% neutralized formalin for further histopathological analysis.

Assay of liver functions in sera of normal and treated rats groups

Liver function parameters were determined in sera of all treated groups. The level of Serum transaminases ALT and AST were estimated[23], alkaline phosphatase (ALP) was recorded [24] and bilirubin were determined [25], while estimation of albumin and the total protein was carried out [26,27] and, respectively, using commercial kits from Bio-diagnostics, Egypt and according to the manufacturer procedures.

Biochemical assays in livers of normal and treated rats

Determination of MDA in the liver of rats was in the form of TBARS[28].GSH estimation was carried out [29]. CAT, as an antioxidant enzyme, its activity was assayed [30]. The enzymatic reaction was initiated by adding

an aliquot of 20: l of the homogenized brain tissue. The 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.SOD activity in rats' liver homogenate was, also, assayed[31]. The oxidation of NADH in the reaction was mediated by superoxide radical and the following increase in absorbance was measured at 560nm.

Determination IL-6and TNF-α contents in liver of normal and treated rats groups

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

Histopathological examination

Liver specimens were fixed in formalin and routinely processed using paraffin embedding technique. Sections of about 5 µm were stained with H&E[32]. Five fields counting per section for each histological alteration were performed for all treated group except the control 4 field counting was done per section using a computerized microscopic attached for full HD microscopic camera (Leica microsystems, Germany).

III. Results

Effect of ZME on liver function indices of normal and PDC hepatotoxicrats.

The effect of ZME on normal and PDC-induced hepatotoxicity in adult male albino rats was investigated by measuring liver function indices in sera as well as biochemical analysis of liver and histopathologicalinvestigation .As depicted from **Table (1)**, results indicated that ZME administration (200mg/ 0.5 ml TW /Kg body weight) for 10 days, affected rats liver by as it increased ALT level, total protein and albumin contents, being of significant change (at $p< 0.05$) in albumin content with a percentage difference of (50.15%) and decreased AST with a significant change (at $p< 0.05$) being of a percent difference of (-25.92%) and showed a normal bilirubin level if compared with control group values.

Results illustrated in **Table (1)** indicated that a single injection of PDC to adult male albino rats at a dose 15 mg/kg, decreased bilirubin content with a significant change (at $p< 0.05$) and increased liver enzymes level and total protein content in sera of adult rat being of significant change (at $p< 0.05$) in AST and ALP levels.

Data from ZME +KCr treated rats group showed decreased levels of AST, ALP, bilirubin and albumin levels in sera; these values were of significant changes at ($p< 0.05$) if compared to KCr-treated group values and with percentage difference of (-30.68%) and (-48.15%) in the AST and bilirubin levelsif compared to control values.

Effect of ZME on liver oxidant/ antioxidant markers of normal and PDC -Hepatotoxic rats

Data represented in **Figure (1)** from the ZME-treated rats group showed decreases in GSH being of significant changes at ($p< 0.05$) while hepatic GSH content showed no elevation its hepatic level in the ZME+KCr treated rats groups.

Results obtained PDC treated gtoup showed increased MDA content of liver of KCr-treated rats, but ZME -treatment decreased MDA content in rat liver homogenates, though of non-significant change when administered alone or with PDC**Figure (1)**.revealed that PDC suppressed hepatic GSH content and markedly reduced hepatic CAT activity of KCr-treated rats group, with a significant change at ($p< 0.05$), versus control group. Meanwhile, hepatic SOD activity was elevated in the same group with no significant change, when compared to control group values.

Table (1): Effect of themethanolic extract of *Zingiber* dry powder (ZME) on liver function indices in normal and potassium dichromate (PDC) hepatotoxic Rats.

Groups / Parameters	Con	KCr	ZME	ZME+KCr
ALT (U/ml)	18.00±2.07	18.60±1.47 (3.33%)	19.60±0.75 (8.88%)	18.60±1.77 (3.33%)
AST (U/ml)	37.80±0.86	45.2±2.59 (19.04%)*	28.00±1.34 (-25.92%)*	26.20±0.86 (-30.68%)*[a]

ALP (U/L)	173.4±28.86	260.3±13.66 (50.11%)*	161.72±1.96 (-6.73%)	175.30±4.52 (1.09%)[a]
Bilirubin (mg/dL)	0.27±0.01	0.22±0.03 (-18.52%)*	0.27±0.01 (0.00%)	0.14±0.00 (-48.15%)[*ac]
Albumin (g/dL)	3.19±4.27	3.09±0.07 (-3.13%)	4.79±0.19 (50.15%)*	3.98±0.17 (24.76%)[*ac]
Total Protein (g/dL)	6.87±0.02	7.34±0.19 (6.84%)	7.50±0.09 (9.17%)	6.59±0.48 (-4.07%)

*: Significant change at $p < 0.05$ with respect to the control group.
a: significant change at $p< 0.05$ with respect to PDC treated rats group.
c: significant change at $p< 0.05$ with respect to ZME treated rats group
( ): per cent change with respect to control values.

Moreover, Data analysis as depicted in **Figure (1)**showed an increase of a nonsignificant change in hepatic SOD activity of KCr treated rats. Meanwhile, ZME administration alone elevated SOD activity with a significant change at ($p< 0.05$), but, inhibited its activity in ZME+KCr- intoxicated rats group as illustrated in (**Figure.1**).ZME treatment was found to enhance CAT activity markedly in the ZME+KCr- treated rats group with a significant change at $p< 0.05$ with respect to both control and ZME- treated groups.

Effect of ZME on IL-6 and TNF-α levels in livers of normal and PDC hepatotoxic Rats

As also depicted in **Figure (2)**, results of the present study shed light onof IL-6 and TNF-α levels as two pro-inflammatory markers in liver tissue homogenate to monitor cellular immune response in livers of all treated groups. Though ZME increased IL-6 level with a significant change at ($p< 0.05$), when compared to control, the ZME+PDC treated rats group revealed decreased IL-6 level with a significant change at ($p< 0.05$) when compared to of ZME treated values.

Results indicated that PDC intoxication revealed a decrease in hepatic TNF- α level with a nonsignificant change, and administrationof ZME alone for 10 days decreased TNF-α level in the hepatic tissue, with a significant change at ($p< 0.05$), compared to control values **(Figure. 2).** Meanwhile,administration of both ZME and PDC decreased hepatic TNF-α with nonsignificant change.

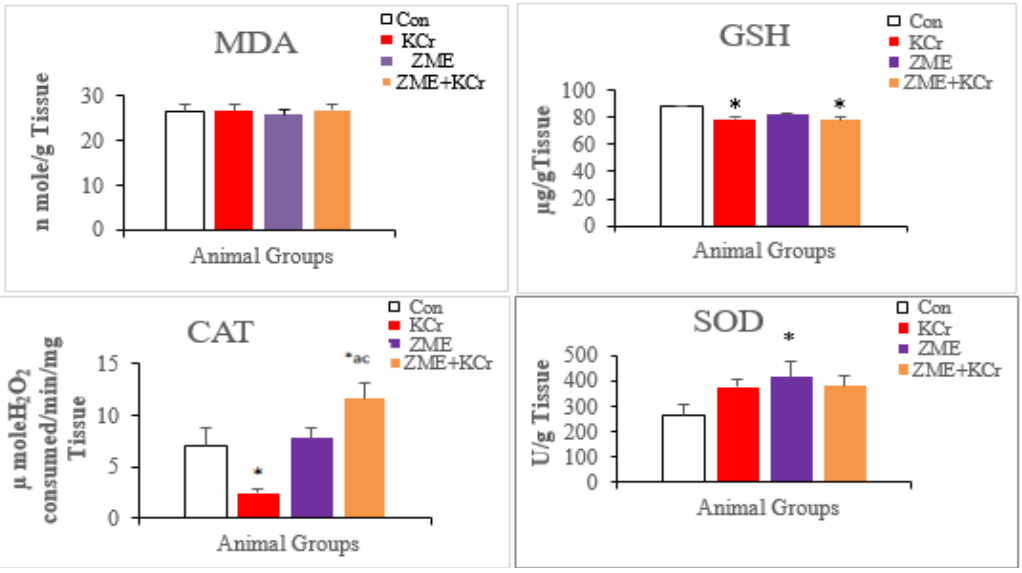

Figure (1): Effect of Methanolic Extract of *Zingiber* Dry Powder (ZME)Treatment On Malondialdehyde (MDA) and Glutathione Contents, Catalase (CAT) and Superoxide Dismutase (SOD)Activities, In Normal and Potassium Dichromate –Induced Hepato-toxic Rats. *: Significant change at $p< 0.05$ with respect to the control group. a: significant change at $p< 0.05$ with respect to KCr treated group. c: significant change at $p<0.05$ with respect to ZME treated group.

Liver histopathological examination ofZME and/or PDC Treated Rats

The microscopic examiantion of PDC treated rats liver revealed perihepatitis that was characterized by thickening of perihepatic capscule with inflammatory cells infiltration associated with vacuolization of underlying hepatocytes,in addition to activation of kupffer cells and individual hepatocellualr necrosis. Treatment of ZME only, caused portal congestion, edema and kupffer cells activation. Meanwhile, liver of ZME+KCr treated rats group showed increased severity of histopathological lesion, and perihepatitis became severed and more diffused with increased kupffer cells activation and hepatocytes necrosis.

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 $p<0.05$.

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

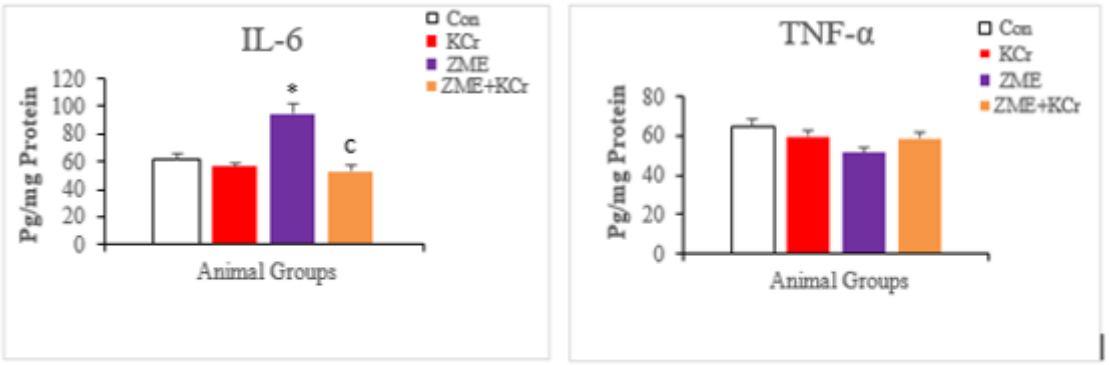

Figure (2): Effect of *Zingiber* Methanolic Extract of *Zingiber* Dry Powder (ZME) Treatment Interleukin-6 (IL-6) And Tumor Necrosis Factor-alpha (TNF-α) Levels in Livers of Normal and Potassium Dichromate-Induced Hepatotoxic Rats.*: Significant change at $p < 0.05$ with respect to the control group. a: significant change at $p< 0.05$ with respect to KCr treated group. c: significant change at $p< 0.05$ with respect to ZME treated group

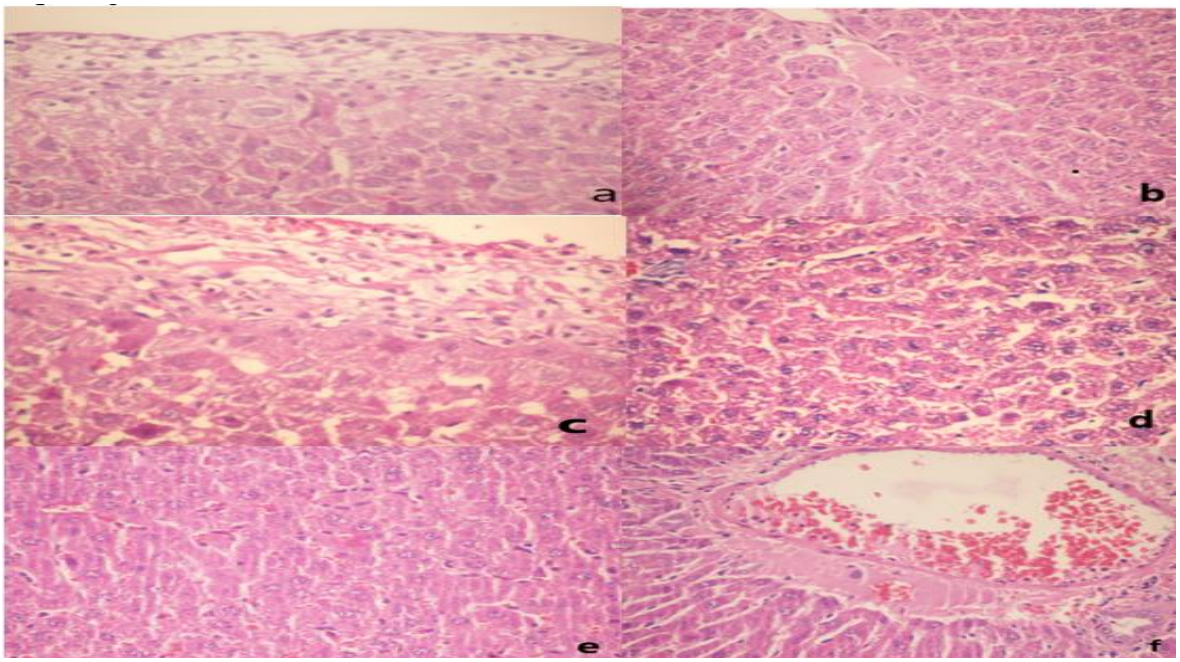

Figure (3): Micrographs of **a)** Liver of Potassium Dichromate treated rats showing perihepatitis with vacuolization (H&E, X400), **b)** Potassium Dichromate treated rats showing kupffer cell activation with individual hepatocellular necrosis (H&E,X200). **c)** Liver of *Zingiber*MethanolicExtract+PDC showing diffused thickening and inflammation of perihepatic capsule (H&E,X400), **d)** Liver of ZingiberMethanolic Extract +PDC showing vacuolization of hepatocytes with individual hepatocellular necrosis (H&E,X200). **e)** Liver of ZingiberMethanolic Extract treated rats showing portal congestion with periportaledema (H&E,X200), **f)** Liver of ZingiberMethanolic Extract showing kupffer cells activation (H&E,X200) .

IV. Discussion

Several spices have been widely used as food flavorings, and in folk medicine as well. Ginger is widely used as a common spice in foods and beverages worldwide, and in the traditional Asian medicine for digestive disorders, common cold, and rheumatism[33,34].Ginger was found to contain many bioactive ingredients giving it the antioxidant, anti-inflammatory, antifungal, anti-mycobacterial, and anti-carcinogenic properties thus having lots of beneficial health effects to treat various diseases[35, 36].

This study aimed to shed light on the effect of methanolic extract of*Zingiber* dry ginger(ZME) on normal rat liver and PDC- induced hepatotoxicity. ZME-treatment increased ALT, albumin and total protein but decreased AST and ALP in sera of ZME treated rats group. Also, decreased liver MDA content, though of non-significant change when administered alone or with PDC. Exposure to Cr (VI) was documented to be toxic, mutagenic, and carcinogenic to human and diverse animals, causing serious damage to the kidneys, liver, lungs, skin, and other vital organs[37]. The effects of Cr (VI) on the gastrointestinal system and the liver, as hepatocytes enlargement, necrosis and elevation of liver enzymes with Cr (VI) contamination was reported earlier[38]. In the present study, PDC injection induced a significant increase in liver function enzymes AST and ALP contents in sera of KCr-group. ALT, AST, and ALP are reported to be located in the cytoplasm and injuries to the liver cause the enzymes to escape into the circulatory system[39], these results were evidenced with the histopathology micrographs from the KCr treated rats group which revealedhepatic necrosis and vacoulisation.

Results of the present study indicated that PDC significantly decreased GSH content in liver tissue homogenate of KCr-group. The pathogenesis of most transition metal-induced toxicity and liver injuries is initiated by the metabolic conversion of those chemicals into reactive intermediate species (electrophilic compounds or free radicals) associated with oxidative damage which potentially alter the structure and function of cellular macromolecules[40] by acting as catalysts in the oxidative deterioration of biological molecules. The liver is being injured by PDC with a structural tissue injury, due to their diffusion across cell membranes, being easily absorbed[13]. As data showed increased MDA content of liver of KCr-treated rats, having a strong oxidative potential on tissues leading to oxidative stress and inhibition of antioxidant enzymes as previously reported [41].

ZME decreased MDA content in hepatic tissue, but showed decreased GSH content.The protection against oxidative cellular damage could be achieved through non-enzymatic GSH, which is the major soluble intracellular thiol-based antioxidant in all living aerobic cells, serving as an antioxidant by reacting directly with free radicals or by providing a substrate for the GSH-related enzyme GPx. The decreased level of non-

enzymatic antioxidant GSH could be attributed to GSH consumption through oxidative stress as it acts as an electron donor as Cr (VI) is reduced to the reactive intermediates and the inhibitory action caused by Cr VI on selenozymes[42].Cr (VI) could affect glutathione peroxidase activity differently in organs[42]. Moreover,reports indicatedinhibited thioredoxin reductase activity (critical for cell survival than glutathione) after Cr (VI) administration [42] leading to inhibition of antioxidant enzyme system, which was suggested to be due to the interaction between Cr(VI), and the selenoenzymes and glutathione peroxidase [43,42], thus enhancing the susceptibility of cells to oxidants and apoptosis and contributing to its cytotoxic effects[44].

Results of the present study revealedsharp inhibition in CAT activity due to PDC, compared to control group,which was in line with earlier reports who indicated altered gene expression, after chromate treatment, in energy metabolism, stress response, DNA repair, signaling pathways, apoptosis, and cell cycle regulation[45]. As these antioxidant enzymes (SOD and CAT) have a protective role against oxygen free radical-induced damage, their induction could be considered as an adaptive response to oxidative stress[41]reflecting the huge amounts of free radicals in liver tissue, which was accompanied by inflammation and activation of Kupffer cells, in the present study, as evidenced from the histopathological investigation of ZME treated group. The current study, also, suggests that PDC treatment led to hepatotoxicity and elevated hepatic enzymes in serum indicate the impaired liver function as documented[46]. ZME treatment reduced the levels of ALT, AST, and ALP when compared with the PDC-treated rats group values.ZME+KCr group showed elevated CAT and SOD activity showing improved antioxidant enzymatic role. The improvement in liver function in the present study could be attributed to the presence of Zingerone, Zingiberene, 6-paradol, beta-Farnesene, Farnesol, α-curcumene, β-bisabolene, cineol and Capsaicin compounds in the ZME as previously documented[4]helping ameliorating liver function indices.

Results concerning IL-6 levels in liver homogenate indicated a decrease in its level in theKCr and ZME+KCr treated groups, but showed significant increased levels in the ZME treated groups. It is documented that IL-6 controls other immune cells such as T cells, regulating hepatocytes, hematopoietic progenitor cells, the cardiovascular system, the placenta and the endocrine system[47]. Less effectiveness of ginger may be due to the synergistically high toxicity of PDC. Moreover,IL-6 showed[48] a very high expression in liver as it is an early produced pro-inflammatory cytokine, as liver kupffer cells are the main innate immune component of the liver.

The present study revealed histopathological changes in the liver tissue of PDC-treated rats group which showed focal necrosis, Kupffer cell activation, appearance of numerous vacuoles and sinusoids, and distortion of hepatic cells in the KCr treated rats group. Free radicals generated by PDC might have led to changes in the tissue architecture. These findings were in line with earlier reports, suggesting that ROS were involved in Cr(VI)-induced cell injury[41].The failure of target organelles and consequently cell death may result from insufficient protective defenses or the overwhelm of excess toxicant insult which lead to dysregulation of cell signaling pathways and dysfunction of biomolecules[41].

Fresh ginger wasdocumented to exert its anti-inflammatory properties either *via* nitric oxide (NO) suppression or attenuation of nuclear factor kappaB (NF-kB) expression-mediating inducible nitric oxide synthase (iNOS) in mouse macrophages[49]. Earlier reports suggested[50], that ginger hexane extract modulated both TNF-α and IL-1β gene expression. Moreover, The attenuation of PDC neurotoxicity using ZME was attributed to the suppression of NO level associated with elevated TNF-α and IL-6 levels[21]. Analysis of the methanolic extract of dry ginger components used in the present study, as previously documented[4] was lacking gingerols and shogaol, therefore, though ZME showed improved liver function indices it revealed no powerful protective effects on the liverin the present study, on the contrary it participated in liver damage by showing congestion, edema and activation of Kupffer cells as recorded by histopathological examination.

V. Conclusion

Dry *Zingiber*powder should be used with cautious and not in excessive amount to normal or potassium dichromate exposed individuals as it may lead to liver damaging effects which were obvious in the histopathological examination rather than biochemical analysis.

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