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ANTAGONISTIC ROLE OF MIXED HYDRO-ETHANOLIC EXTRACT OF *ALLIUM SATIVUM* ON CHROMIUM-INDUCED OXIDATIVE STRESS

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ABSTRACT

Potassium dichromate (K2Cr2O7) has been demonstrated to induce oxidative stress and carcinogenic in nature. In the present study, the protective effect of mixed hydro-ethanolic (60:40) extract of *Allium sativum* (Garlic) was studied against Cr (VI)-induced toxicity of liver and kidney of male albino rat. A group of male albino rats (80-100 g) were obtained and divided into three groups. The animals of first groups were induced by interperitonial injection with $K_2Cr_2O_7$ at a dose of 0.8 mg per 100 g body weight per day (20% LD) for a period of 28 days. The animals of second of the chromium treated groups serving as the supplemented groups were injected with mixed hydro-ethanolic (60:40) extract of *Allium sativum* at a dose of 500 mg/kg body weight daily at an interval of six hours after injection of $K_2Cr_2O_7$ for a period of 28 days. The animals of the third group received only the vehicle (0.9% physiological saline), served as control. After completion of chromium-treatment the animals were sacrifice and intact liver and kidney were dissected out for further use. Measurement of lipid peroxidation (MDA), conjugated dienes and antioxidants were used to monitor the antiperoxidative effects of mixed hydro-ethanolic (60:40) extract of *Allium sativum* in liver and kidney. The increased lipid peroxides, conjugated dienes and NO release in liver and kidney of chromium-treated rats were accompanied by a significant decrease in the levels of glutathione (GSH and GSSG) and the activities of glutathione peroxidase (GSH-Px), glutathione reductase (GR), glutathione-S-transferase (G-S-T), superoxide dismutase (SOD) and catalase (CAT). The important findings in this study corroborated the facts that mixed hydro-ethalonic (60:40) solvent extract of *Allium sativum* has greater potential benefit in maintenance of oxidative equilibrium, scavenging of ROS and augmented anti-oxidant defense against chromium-induced toxicity in liver and kidney.

KEYWORDS: Chromium, Animal, Liver, Kidney, Toxicity, Oxidative stress, *Allium sativum*.

INTRODUCTION

The occurrence of heavy metals in the environment and their enormous industrial use has led to an increase in the frequency of the human organ toxicity. Among different heavy metals chromium is one of the important heavy metal in both terrestrial and aquatic environments (Sadek, 2014). It is also a trace element which is extracts from chromate (Andleeb, 2014). Chromium presents in environment in various oxidation states. Trivalent chromium is extensively used as supplement and also a good element for glucose/insulin homeostasis (Ghalehkandi, 2018), where as hexavalent chromium is highly toxic for their easy permeation at physiological pH through the permease system (Ghalehkandi, 2018). Hepatic and renal toxicity is the most common toxicity observed in Cr (VI)-exposed workers or animals. This functional differentiation of Cr (III) and Cr (VI) is largely decided by the ionic permeability of the plasma

membrane (Wise et al, 2014). Cr (VI) compounds are the most toxic since they can be easily absorbed and transported across membranes via non-specific anion carriers (Abd-Elhakim and Mohamed, 2016). Thus, membrane damage is one of the crucial factors observed with Cr (VI) toxicity (Dey et al, 2003). Inside the cells, Cr (VI) is reduced through reactive intermediates such as Cr (V) and Cr (IV) to the more stable Cr (III) by cellular reluctant (Sun et al, 2015). This reduction process generates reactive oxygen species (ROS) and induces soft tissues' damage such as liver, pancreas, cerebellum and kidney (Dolai et al, 2016).

Allium sativum (Garlic) is a potential herb that belongs to the amaryllidaceous plant family. It is one of the most multipurpose medicinal plants used as a traditional herbal medicine to prevent and treat the variety of diseases (Prasad *et al.*, 1996). The anticarcinogenic and anti-inflammatory properties of garlic extract and its derivatives also have recently been reported by several investigators (Kalayarasan et al, 2008). *Allium sativum* compounds are having tremendous antioxidant property which exerts action by scavenging ROS, enhancing cellular antioxidant enzymes and increasing glutathione in the cells (Borek, 2001). Our previous studies showed that aqueous extract of garlic and some antioxidants like Vitamins and GSH were able to ameliorate Cr (VI)-induced membrane damage in the liver and kidneys (Dey and Dey, 2021; Dey et al, 2001; Dey et al, 2003; Dey and Roy, 2010). Therefore, the aim of this present investigation was an attempt to reduce the effects of Cr-induced tissue toxicity in liver and kidney using mixed hydro-ethalonic solvent extract of *Allium sativum* (Garlic).

MATERIALS AND METHODS

Collection of plant materials

Plant parts i.e. the fresh bulb of garlic were collected from the market. Each specimen was labelled with date of collection. Plant parts were cleaned and peeled off. Then plant parts were dried in incubator less than 40ºC.

Preparation of Hydro-ethanol extract of *Allium sativum* (Garlic)

Hydro-ethanolic (60:40) mixed solvent extract prepared from plant parts i.e. the fresh bulb of garlic by using the aqueous and ethanol.

Maintenance and treatment of animals

Male albino rats of the Wister strain (80–100 g) were fed with a lab-prepared diet, as described elsewhere (Dey et al, 2003b), with water ad *libitum*. They were maintained in accordance with the guidelines of the rule of Institutional Animal Ethics Committee. Laboratory acclimatized rats were divided into three groups of almost equal average body weight. The animals of two groups were injected intraperitoneally (*i.p.*) with Cr as $K_2Cr_2O_7$ at a dose of 0.8 mg per 100 g body weight per day (20% LD50) for 28 days, as described earlier (Dey et al, 2003b). The animals of one of the Cr-treated groups served as the supplemented group injected mixed hydro-ethanolic solvent extract of garlic (HEEG) orally at a dose of 500 mg per kg body weight daily at an interval of 6 h after injection of Cr for a period of 28 days. The animals of the remaining group received only the vehicle (0.9% NaCl), served as control.

Animals sacrifice and collection of tissues

After completion of drug treatment the animals were kept in fasted overnight prior to sacrifice. The intact liver and kidney were dissected out and adhering blood and tissue fluid were blotted dry weighted. All the samples were kept at -20^{0}C for further analysis.

Homogenization of tissues

A weighted portion of different tissues was homogenized in an ice cold 0.2 M PBS (pH 7.4) using glass homogenizer. Homogenized tissues were used for analytical assessment.

Analytical Methods

Lipid peroxidation was measured according to the method of Ohkawa et al, (1979). Malondialdehyde (MDA) was determined from the absorbance of the pink colored product (TBARS) of thiobarbituric acid-MDA reaction, at 530 mm. The reaction of MDA with TBA has been widely adopted as a sensitive method of lipid peroxidation in animal tissues. Conjugated dienes was measured according to the method of Slater (1984). NO release assays were done in liver and lungs mitochondria according to the method of Sasaki et al (1998). SOD activity was estimated by measuring the percentage inhibition of the pyrogallol auto-oxidation by SOD according to the method Marklund and Marklund (1974).

GSH (reduced glutathione) was measured according to the method of Griffith (1980). GSSG was also assayed after derivatization of GSH with 2-vinylpyridine. GSSG (oxidized glutathione) was measured by the method of Griffith (1980).

The rate of oxidation of reduced glutathione (GSH) by as catalyzed by the glutathione peroxidase (GSH-Px) is assayed for the measurement of enzyme activity. Glutathione peroxidase activity was measured according to method of Pagila and Valentine (1967). The activity of glutathione reductase was measured by the method of Miwa (1972). Glutathione S-transferase activity was also measured according to the method of Habig et al, (1974). Total protein of plasma and tissues was estimated according to the method of Lowry et al, (1951).

Statistical Analysis

The data were expressed as mean ± standard error. The significance in the differences between the means were evaluated by student's ‘t' test and probability levels of 5% or less were considered to be statistically significant.

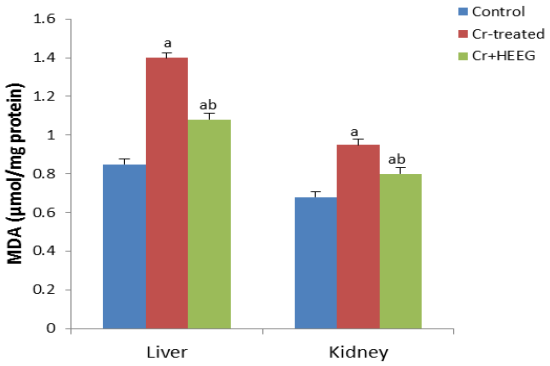
RESULTS

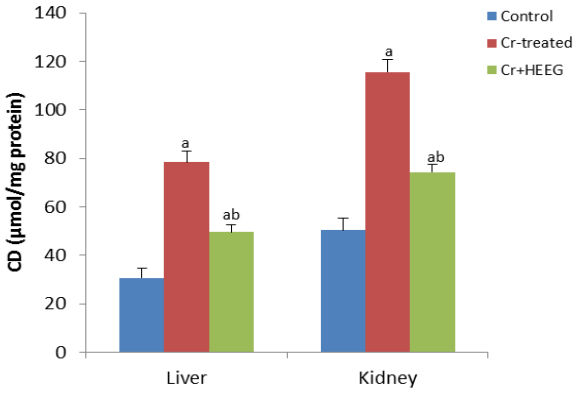
The results found that the changes of MDA, conjugated dienes and nitric oxide (NO) production were significantly increased in response to chromium but after the supplementation of mixed hydro-ethanolic extract of garlic shows the potent role to counteract the chromium-induced toxicity in liver and kidney (Figure-1, 2 & 3).

The changes of SOD and catalase activity decreased significantly in response to chromium in liver and kidney. On the other hand, mixed hydro-ethanolic extract of garlic have the potent role to counteract the chromium-induced toxicity (Figure-4 & 5).

The level of GSH and GSSG were significantly diminished in liver and kidney in response to chromium treatment (Figure-6&7) but after supplementation with mixed hydro-ethanolic extract of garlic found the potent role to counteract the chromium-induced tissue toxicity.

The activities of GPx, GR and G-S-T were significantly decreased in response to chromium when compared with control in both liver and kidney (Figure-8,9 & 10). On the other hand, it was found that the supplementation role of mixed hydro-ethanolic extract of garlic play a vital role to counteract the chromium-induced toxicity in liver and kidney.





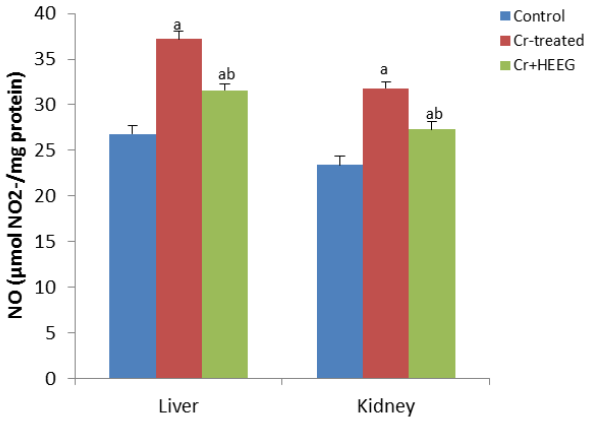
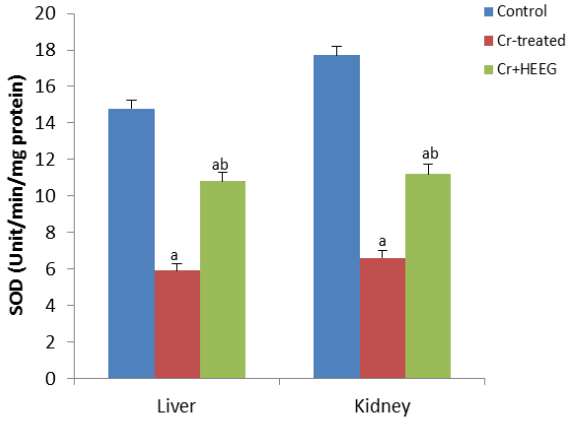


Figure- (1-3): Changes in level MDA, CD and NO concentration after co-administration of Hydro-Ethanol extract of Garlic (HEEG) in Cr-treated rats. Data represents mean ± SE, aP < 0.05 compared to control, b P < 0.05 compared to chromium.



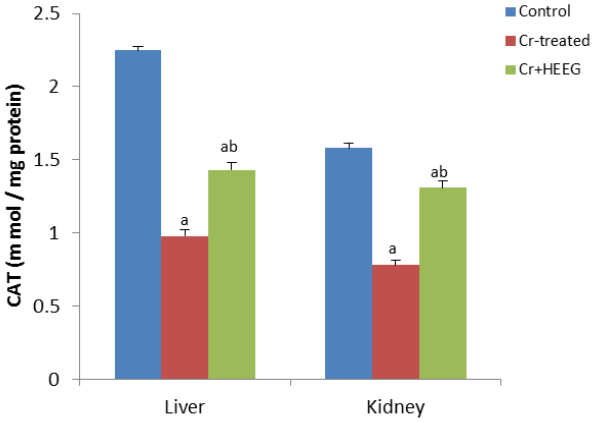
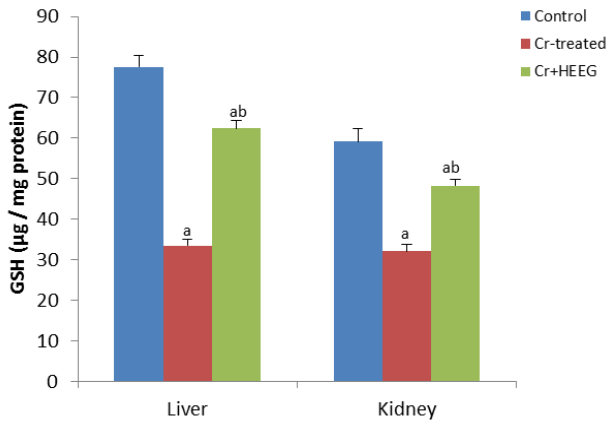
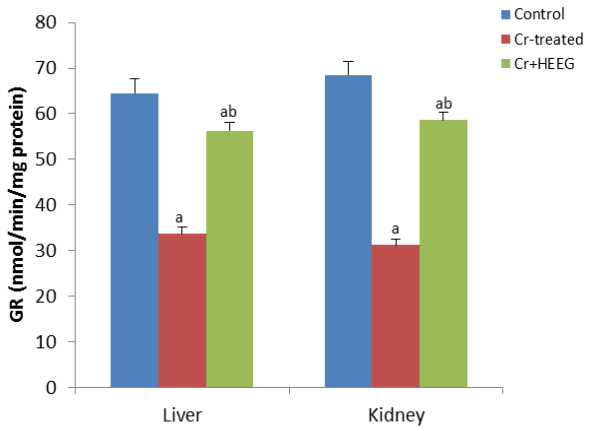
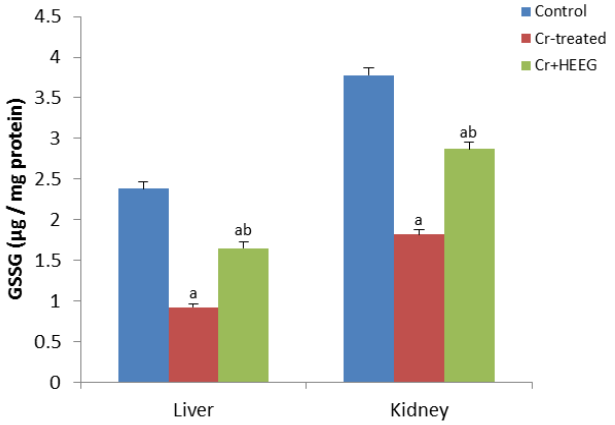


Figure- (4 & 5): Changes the activity of SOD and CAT after co-administration of Hydro-Ethanol extract of Garlic (HEEG) in Cr-treated rats. Data represents mean ± SE, aP < 0.05 compared to control, b P < 0.05 compared to chromium.







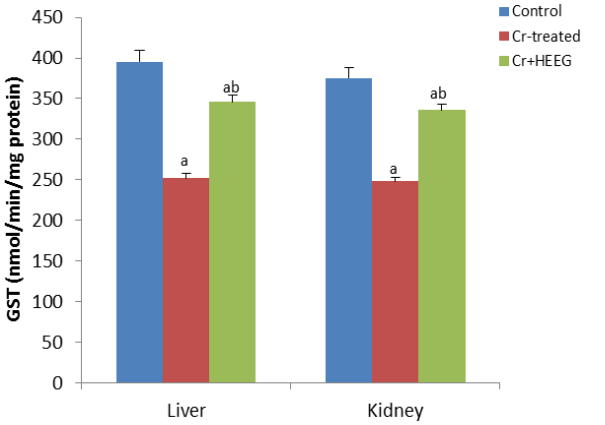
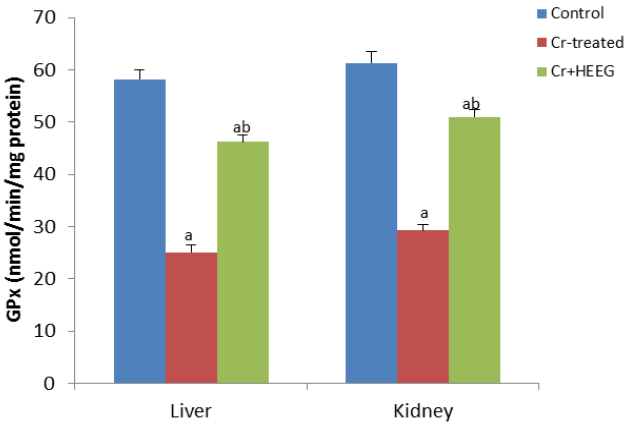


Figure- (6&7): Changes the concentration of GSH and GSSG after co-administration of Hydro- Ethanol extract of Garlic (HEEG) in Cr-treated rats. Data represents mean ± SE, aP < 0.05 compared to control, b P < 0.05 compared to chromium.

Figure- (8-10): Changes the activity of GPx, GR and GST after co-administration of Hydro-Ethanol extract of Garlic (HEEG) in Cr-treated rats. Data represents mean ± SE, aP < 0.05 compared to control, b P < 0.05 compared to chromium.



DISCUSSION

Enhanced lipid peroxidation, conjugated diene and NO production associated with antioxidant depletion in liver and kidney is a characteristic observation in response to chromium (Figure: 1-3). Chromium, a potent carcinogen, used in the present study has been reported to be oxidizing into its metabolite cotinine mainly in liver and to a significant extent in lung and plays a key role in the pathogenesis of tissues. Rana and Kumar (1984) reported enhancement of lipid peroxidation in rat liver after heavy metal poisoning with mercury, molybdenum, copper, chromium and manganese. It has been demonstrated that the chromium (V) complexes which are produced following reduction of chromium (VI) by cellular biological reductants, react with hydrogen peroxide to generate hydroxyl radicals which in turn act as the initiators of primary events in chromium (VI) cytotoxicity (Shi and Dalal, 1990a). Bagchi et al, (1995) showed that chromium (VI) induces increases in hepatic mitochondrial and microsomal lipid peroxidation. Thus, the increased level of MDA, conjugate diene and nitric oxide production in liver and kidney may be due to excessive generation of free radicals. On the other hand,

it was found that administration of mixed hydro-ethanolic extract of garlic modulated the changes induced by chromium supporting the hypothesis that plant products are effective anti-oxidative agent.

The excessive generation of free radicals can be prevented or scavenged by host antioxidant defense mechanism. In effective scavenging of free radicals due to depletion of antioxidants plays a crucial role in cell injury (Lima and Savin 2002). GSH plays a crucial role in protecting the liver and kidney from oxidative stress by detoxifying exogenous toxicants and quenching reactive oxygen species (ROS). High concentration of GSH is found in cells as the major antioxidants defense, especially in regulating the extent and duration of oxidative 'burst' (Abidi et al, 1999). GPx has a well-established role in protecting cells against oxidative injury. GPx utilizes GSH as a substrate to catalyses the reduction of organic hydroperoxides and hydrogen peroxide (Ray and Husain, 2002). On the other hand, it was found that GR activity inducing the production of GSH from GSSG. There are alternative functions for GSH in cellular metabolism independent of its antioxidant properties. GSH also participates in the detoxification of xenobiotics as a substrate for the enzyme glutathione-S-transferase. Superoxide dismutase is the antioxidant enzyme that catalyses the dismutation of the highly reactive superoxide anion to O_2 and to the less reactive species H_2O_2 (Okado-Matsumoto and Fridovich, 2001). Numerous studies have shown the importance of SOD in protecting cells against oxidative stress (Huang et al. 1997). Thus decrease in the activity of SOD (Figure-4) observed in the present study could be due to a feedback inhibition or oxidative inactivation of enzyme protein due to excess ROS generation. Catalase, which acts as preventative antioxidant plays an important role in protection against the deleterious effects of lipid peroxidation (Pigeolot et al, 1990). The inhibition of CAT activity (Figure-5) is suggestive of enhanced synthesis of superoxide anion during the ingestion of combined exposure since superoxide anion is a powerful inhibitor of catalase (Ashakumary and Vijayammal, 1996). Husain et al, (2001) have reported that chronic administration of ethanol decreased the level of GSH and activities of GPx, SOD and CAT in the liver and kidney. In the present study, it was found that the level of GSH and GSSG are decreased significantly in liver and kidney tissues (Figure: 6-7) in response to chromium in male albino rats. On the other hand, the activities of GPx, GR and GST are also significantly decreased (Figure: 8-10). This depletion of GSH, GSSG, GPx, GR, GST, SOD and CAT activities in liver and kidney in response to chromium treated rats may be due to enhanced utilization during detoxification. On the other hand, it was found that administration of mixed hydro-ethanolic solvent extract of garlic significantly enhanced the antioxidant status in liver and kidney of chromium treated rats and protect cells against the damaging effects.

CONCLUSION

Present investigation suggested that chromium induced oxidative stress could be protected or minimized through the administration with mixed hydro-ethanol solvent extracts of *Allium sativum* (Garlic). Natural antioxidants strengthen the endogenous antioxidant defenses from ROS and restored the optimal balance by neutralizing the reactive species. *Allium sativum* may play a vital role to suppress the chromium induced ROS generation and ROS mediated oxidative stress in different tissues. This finding may recommended that mixed hydro-ethanol extract of *Allium sativum* may have some important components, having the antioxidant property to diminish or prevent chromium induced toxicity.

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