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EVALUATION OF ANTIOXIDANT ACTIVITY OF ETHANOLIC AND AQUEOUS EXTRACT OF *OLDENLANDIA UMBELLATA AND OLDENLANDIA CORYMBOSA* ON CHROMIUM (VI) INDUCED OXIDATIVE STRESS IN ALBINO RATS

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ABSTRACT

Objective of the present study to evaluate the *in-vivo* anti-oxidant potential of ethanolic and aqueous extract of *Oldenlandia umbellata and Oldenlandia corymbosa* on chromium induced oxidative stress in albino rats. Animals were treated with plant extracts for28 days and then oxidative stress was induced with a single dose of chromium 30mg/kg. Treated with 200mg /kg (p.o) of various extracts of *Oldenlandia umbellata &Oldelandia corymbosa* and Glutathione, SOD, Catalase peroxidase and transaminase enzyme levels were determined. The present study revealed that the ethanolic and aqueous extracts of *Oldenlandia umbellata* and *Oldenlandia corymbosa* have significant *invivo* anti-oxidant activity and can be used to prevent tissues from oxidative stress .The result showed that the activities of glutathione, SOD, Catalase, peroxidase & transaminase enzymes in group treated with chromium declined significantly than that of compared with controlled group. Ethanolic and aqueous extracts of *Oldenlandia umbellata* and *Oldenlandia corymbosa* in the dose of 200mg /kg (p.o), have improved the glutathione, SOD, Catalase, peroxidase & transaminase enzyme levels significantly, which were comparable with Vit-E. Based on this study we conclude that ethanolic and aqueous extracts of O.U &O.C possesses *in vivo* antioxidant activity can be employed in protecting tissue from oxidative stress as compared with standard. However, further studies are required to establish its exact mode of action and the active principles involved in its anti-oxidant activity.

Key Words:- *Oldenlandia umbellata &Oldelandia corymbosa*, *In-vivo* Anti-oxidant Activity, Glutathione, SOD, Catalase, Peroxidase & Transaminase Enzyme.

INTRODUCTION

Reactive Oxygen Species

The formation of reactive oxygen species (ROS) / free radicals is a naturally occurring intracellular metabolic process. These harmful species are known to cause oxidative damage to a number of molecules in cells including membrane lipids, proteins and nucleic acids. The potential harmful effects of these species are controlled by the cellular antioxidant defense system (Husain *et al*., 2001).

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In recent years, the chemical importance of the herbal drugs has received considerable attention as many synthetic antioxidants have been shown to have one or the other side effects. There has been an upsurge of interest in the therapeutic potential; of the medicinal plants as antioxidants in reducing free radicals induced tissue injury. Numerous plant products have been shown to have the antioxidant activity and the antioxidant vitamins, flavonoids and polyphenolic compounds of the plant origin have been extensively reported as scavengers of free radicals and inhibitors of the lipid peroxidation (Geetha *et al*., 2003). So in the present study we have determined the antioxidant activity or potential of the plant by inducing the free radicals in the animal model with the help of chromium (VI).

Free radical induced by Chromium (VI)

Chromium ions are widely distributed in both terrestrial and aquatic environments. Chromium chloride, niacin-bound chromium or chromium polynicotinate, and chromium picolynate are used as micronutrients and dietary supplements, whereas hexavalent chromium used in diverse industries. Exist many studies in rats showing oxidative stress mediating chromium VI liver damage. Besides other methods like DNA single strand breaks (Bagchi *et al*., 2002), thiobarbituric acid based method to evaluate the lipid deterioration (Bagchi *et al*., 1997).

Chromium exists in many states of oxidation, ranging from +2 to +6, forms +3 e +6 are most stable in the environment and biologically important. Whereas trivalent chromium is an essential trace element, hexavalent chromium is non-essential, toxic, corrosive, and causes allergic reactions, lipid peroxidation, DNA damage, dermatitis, gastrointestinal tract irritation, damage to liver and kidneys, besides showing carcinogenic effects in animals.

The genotoxic, toxic and carcinogenic effect of hexavalent chromium [Cr(VI)] derived from compounds such as potassium dichromate ($K_2Cr_2O_7$) have been evaluated as many researchers (Léonard *et al*., 1994; De Flora S, 2000; Gunaratnam *et al*., 1990).

The chromate ion $[CrO_4]^{-2}$, the dominant form of Cr(VI) in neutral aqueous solutions, can readily cross cellular membranes via nonspecific anion carriers (Danielsson *et al*., 1982). Cr(V) complexes produced in the reduction of Cr(VI) by cellular reductants react with hydrogen peroxide to generate hydroxyl radicals, which may be the initiators of primary events in Cr(VI) cytotoxicity (Kawanishi *et al*., 1986; Shi X, Dalai NS, 1989). related studies suggests that hydroxyl radicals are generated from a Cr(V) intermediate that is responsible for causing DNA strand breaks (Jones *et al*., 1991). Both chromium (VI) and chromium (V) and Cr(III) are biologically active oxidation states of chromium, although Cr(VI) is more toxic and produces a greater oxidative stress. Furthermore, both oxidation states of chromium are involved in redox cycling with the production of reactive oxygen species. Further studies are required to elucidate the mechanisms involved in the regulation of tissue damaging effects by chromium complex.

$Cr(III) + O_2^{\cdot-} \longrightarrow Cr(II) + O_2$

$Cr(II) + H_2O_2 \longrightarrow Cr(III) + {}^{\cdot}OH + OH^-$

$Cr(VI) + O_2^{\cdot-} \longrightarrow Cr(V) + O_2$

$Cr(V) + H_2O_2 \longrightarrow Cr(VI) + {}^{\cdot}OH + OH^-$

MATERIALS AND METHODS

Chemicals - Thiobarbituric acid (TBA; Research-Lab fine chem. Industries Mumbai, India) nitro blue tertazolium chloride (NBT, Himedia laboratories Pvt.Ltd, Mumbai India). 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB Alfa Aesar, A Johnson Mathey company). Bovine serum albumin (Spectrochem Pvt. Ltd. Mumbai, India) carboxy methyl cellulose (Research Lab, Mumbai, India) Ethyl alcohol (absolute ethanol) all the chemical used were of analytical grade and purchased from standard manufactures (Nade *et al*., 2009).

Animals

Male albino wistar rats each weighing 180-220 were obtained from K.M. College of Pharmacy, Madurai. Rodent laboratory chow was access and water *ad libitum*, and rats were maintained on a 12 hour light/dark cycle in a temperature regulated room (20-25^{o}C) during the experimental procedures. The animals were cared for according to the guiding principles in the care & use of animals.

TREATMENT PROTOCOL

Rats were divided randomly into seven groups of six animals each and treated for four weeks i.e. 28 days (Joharapurkar *et al*., 2003) as follows:

Group 1 Served as normal control group received normal saline in a dose of 10ml/kg.

Group 2 Served as Toxic Control group and was administered chromium 30 mg/kg (30% V/V, 1-0ml/100kg) orally (Ping Yao *et al*., 2006).

Group 3 Served as a standard group and was administered Vit.E in a dose of 200mg/kg Orally (Vandana tayal *et al*., 2007; Bafna AR & Mishra SH, 2006).

Group 4 Served as the treatment control, which was treated with Aqueous Extract of *Oldenlandia umbellata* (AEOU) at 200 mg/kg body weight, in oral route.

Group 5 Served as the treatment control, which was treated with Ethanolic Extract of *Oldenlandia umbellata* (EEOU) at 200 mg/kg body weight, in oral route.
Group 6 Served as the treatment control, which was treated with Aqueous Extract of *Oldenlandia corymbosa* (AEOC) at 200 mg/kg body weight, in oral route.
Group 7 Served as the treatment control, which was treated with Ethanolic Extract of *Oldenlandia corymbosa* (EEOC) at 200 mg/kg body weight, in oral route.

Biochemical Analysis

Dissection and Homogenization

On the 29th day all animal were killed by decapitation. Blood was collected and serum was separated for estimation of alanine aminotransferase (ALT) and aspartate aminotranferase (AST) (Sundaram R, & Mitra SR., 2007). The liver was rapidly excised rinsed in ice-cold saline and a 10% W/V homogenate was prepared using (0.15MKCl) potassium chloride. Centrifuged at 800rpm for 10 min at 4^{o}C. The supernatant obtained was used for the estimation of catalase, and lipid per oxidation. Further the homogenate was centrifuged at 1000 rpm for 20 min at 4^{o}C and the supernatant was used for estimation of SOD and glutathione.

Lipid Peroxidation Assay (LPO)

Malondialdehyde (MDA), a secondary product of lipid per oxidation reacts with thiobarbituric acid at PH 3.5. The red pigment produced was extracted in n-butanol-pyridine mixture and estimated by measuring the absorbance at 532 nm (Ohkawa *et al*., 1979).

Superoxide dismutase activity (SOD)

Superoxide dismutase activity was assayed according to the method of kono (Kono Y, 1978). Where in the reduction of nitro blue tetrazolium chloride (NBT) was inhibited by superoxide dismutase and measured at 560nm spectrophotometrically. Briefly the reaction was initiated by addition of hydroxylamine hydrochloride to the reaction mixture containing NBT and post nuclear fraction of liver homogenate. The results were expressed as units per milligram of protein with one unit of enzyme defined as the amount of SOD required to inhibit the rate of reaction by 50%.

Catalase activity (CAT)

Catalase activity was assessed by the method of luck (Luck H *et al*., 1971). where the breakdown of H_2O_2 was measured at 240nm. Briefly the assay mixture consisted of 3ml of H_2O_2 phosphate buffer (0.0125M; H_2O_2) and 0.05ml of supernatant of liver homogenate and the change in the absorbance was measured at 240nm. The enzyme activity was calculated using the mill molar extension coefficient of H_2O_2 (0.07). The results were expressed as micromole of H_2O_2 decomposed per min per milligram of protein.

Estimation of Reduced Glutathione

Reduced glutathione (GSH) in the liver was assayed according to the method of Ellman (Ellman GL, 1959). Sample (0.75ml) of homogenate was precipitated with 0.75ml of 4% sulphosalicyclic acid and centrifuged at 1200g for 15 min at 4^{o}C. The assay mixture contained 0.5ml of supernatant and 4.5ml of 0.01M, DTNB. (5-5'-dithiobis (2-nitro benzoic acid)) in 0.1M, phosphate buffer (PH 8.0). The yellow colour developed was read immediately at 412nm. The results were expressed as micromole of GSH per milligram of proteins.

Determination of AST (Aspartate aminotransferase)

AST (aspartate aminotransferase) from the liver in the blood serum was assayed according to the method of Reitman'S, Frankel S.

Principle

SGOT (AST) catalyses the following reaction

α - Ketoglutarate + L-aspartate $\longrightarrow$ L-glutamate+oxaloacetate

Oxaloacetate so formed is coupled with 2, 4 Dinitrophenyl hydrazine (2, 4 DNPH) to give the corresponding hydrazine which gives brown colour in alkaline medium & this is measured colorimetrically.

Determination of ALT (Alanine aminotransferase)

ALT (alanine aminotransferase) from the liver in the blood serum was assayed according to the method of Reitman S, Frankel S.[20]

Principle

ALT catalyses the following reaction

α - ketoglutarate +L- alanine $\longrightarrow$ L-glutamate + pyruvate.

Pyruvate so formed coupled with 2, 4 Dinitrophenylhydrazine (2, 4 DNPH) to give the corresponding hydrazine which gives brown colour in alkaline medium & this can be measured colorimetrically.

Statistical Analysis

The results are expressed as mean ± SEM. Data was evaluated using one way ANOVA followed by Newman Keul's multiple range test. Probability values less than ($P<0.01$) were considered significant.

Table 1. Effect of Body Weight of Normal and Experimental Animals in Each Group

Groups	Initial body weight	Final body weight
Group-I	210.8 ±5.68	216.8 ±5.36
Group-II	206.58 ±5.26	160.20 ±3.55 a*
Group-III	216.48 ±5.22	228.35 ±4.36 b*
Group-IV	220.24 ±5.40	233.20 ±4.36 b*
Group-V	212.80± 5.20	226.4 ±2.20 b*
Group-VI	208.46± 4.38	216.2 ±4.26 b*
Group-VII	216.40± 4.68	228.8 ±4.38 b*

- Values are expressaed as mean ± SEM.
- No. of animals in each group (n) = 6
- Values were find out by using one way ANOVA followed by Newman Keul's multiple range test.
- (a*) values were significantly different from Normal control (G_1) at ($P < 0.01$).
- (b **) values were significantly different from toxic group (G_2) at ($P < 0.01$).

Table 2. Effect of AEOU, EEOU and AEOC, EEOC on Chromium Induced Free Radicals in Rats

Groups	SOD U/L	Catalase min/mg of protein	Reduced GSH mg/dl	Lipid Peroxidation nmoles/ml	AST U/L	ALT U/L
Group-I	33.92±2.43	276.4±5.45	117.30±2.45	172.25±2.76	196.42±3.26	90.96±2.45
Group-II	31.44±2.12	190.35±4.42*a	62.38±1.26*a	266.45±4.68 *a	336.85±6.52*a	220.96±5.22*a
Group-III	29.30±2.62	240.26±4.30*b	93.6±1.55*b	210.4±4.28*b	230.21±3.78*b	136.23±3.23*b
Group-IV	30.16±2.55	212.30±3.42*b	82.45±3.45*b	202.55±3.85*b	266.42±3.15*b	154.22±3.48*b
Group-V	32.48±1.40	216.32±3.28*b	83.32±3.16*b	203.42± 3.56*b	254.48±3.88*b	151.72±2.78*b
Group-VI	34.12±1.32	218.13±3.36*b	84.05±3.43*b	206.16± 3.12*b	250.24±2.70*b	149.72±2.20*b
Group-VII	31.25±1.22	220.20±3.28*b	81.15±2.56*b	202.22± 2.96*b	252.23±2.96*b	148.45±2.35*b

- Values are expressaed as mean ± SEM.
- No. of animals in each group (n) = 6
- Values were find out by using one way ANOVA followed by Newman Keul's multiple range test.
- (a*) values were significantly different from Normal control (G_1) at ($P < 0.01$).
- (b **) values were significantly different from toxic group (G_2) at ($P < 0.01$).

RESULTS

Effect of AEOU, EEOU and AEOC, EEOC on body weight, food and water consumption

The effect of AEOU, EEOU and AEOC, EEOC on body weight changes during the chromium induced oxidative stress is shown in Table No: 1. Chromium feeding resulted in significant decrease in the body weight with the duration of treatment; however, in animals fed with AEOU,EEOU and AEOC,EEOC and chromium; there was no significant change as compared to the control group. Administration of chromium did not cause any significant change in the food and water intake.

Effect of AEOU, EEOU and AEOC, EEOC on SOD and Catalase levels

Administration of chromium caused a significant increase ($p<0.01$) in the liver tissue catalase levels but did not affect SOD levels (Table No: 2).The AEOU, EEOU and AEOC, EEOC in a dose of each 200mg/kg body weight was able to restore the catalase levels to that of control values.

Effect of AEOU, EEOU and AEOC, EEOC on Reduced GSH and MDA (Lipid peroxidation)

Liver tissue GSH levels were significantly decreased following the chromium treatment, whereas significant increase in plasma MDA levels was observed (Table No: 2). Administration of AEOU, EEOU and AEOC, EEOC in 200mg/kg body weight, dose reverted the GSH and MDA levels to that of control values.

Effect of AEOU, EEOU and AEOC, EEOC on AST and ALT Levels

AST and ALT levels were increased ($p<0.01$) in all the animals treated with chromium (Table No: 2). Administration of 200mg/kg body weight dose of AEOU, EEOU and AEOC, EEOC significantly inhibited the chromium induced increase in enzyme levels and restored to that of control values.

DISCUSSION AND CONCLUSION

One of the most important early events in cell degeneration leading to necrosis, is the Lipid peroxidative damage that occurs mainly in the cell membrane. In addition, lipid peroxidation represents one of the most reaction resulting from free radicals attack on biological structures Cr(VI) and Cr(V) are both able to yield ROS.[21, 22] The majority of oxidative stress studies in rat have used TBARS as a tissue damage indicator. [23,24]

In addition there was no study relating AEOU, EEOU and AEOC, EEOC with chromium intoxication. Therefore in this study was undertaken to evaluate for the antioxidant activity against the chromium (VI) induced oxidative stress in male albino rats. The results of the present study demonstrate that the AEOU, EEOU and AEOC, EEOC at a dose of 200mg/kg body weight protected the animals significantly from the chromium induced oxidative damage.

Oral feeding of chromium resulted in a significant decrease in body weight. Chromium (IV) Compounds are well known oxidizing agents capable of directly inducing tissue damage and possess carcinogenic, mutagenic and teratogenic potency.[25] Chromium (VI) compounds are easily taken up by cells and are subsequently reduced to Cr(III) species. This reduction generates free radicals, which play major role in the adverse biological effect of these compounds[26]. Administration of chromium significantly increases the lipid peroxidation as evident by the increase in MDA levels. To cope with the oxidative stress, there was a significant decrease in reduced glutathione (GSH) and catalase level in the liver tissue. No significant change in the SOD activity was observed in the Chromium-treated animals and our results fall in confirmation with earlier studies[27].Besides activating the oxidative stress, Chromium also caused a marked increase in AST and ALT levels suggesting that the Chromium treatment also causes hepatic damage. Many workers have also demonstrated the hepato-toxic effect of Chromium (VI) [28,29], which is mainly due to the lipid peroxidation. These adverse effects of Chromium (VI) could be significantly curtailed by pre treating the animals with the AEOU, EEOU and AEOC, EEOC.

In animals fed with AEOU, EEOU and AEOC, EEOC significant protection was observed against the chromium induced oxidative stress. The AEOU, EEOU and AEOC, EEOC inhibited the chromium induced increase in MDA levels and restores the intracellular antioxidant, Like GSH and catalase levels to that control. The AEOU, EEOU and AEOC, EEOC also protected the animals significantly from the hepatotoxicity induced by chromium is revealed by the decreased AST and ALT activity compared to the chromium (VI) treated animals.

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