Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 4, 2021, Pages. 19415- 19422
Received 05 March 2021; Accepted 01 April 2021.

Potential hepatoprotective of cow ark with *Plectranthus Amboinicus* against changes in the level of CCL4-induced liver intoxication and antioxidant enzymes

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ABSTRACT

Cow ark with *Plectranthus Amboinicus* has been known to be a valuable and effective medicinal substance of biological origin, curing several diseases. CCl4 is a pharmacological agent to generate free radicals, which leads to the deleterious effects of lipid peroxidation. In the present investigation, liver homogenate was exposed to CCl4 stimulated lipid peroxidation in vivo, and determined the level of antioxidant enzymes such as Glutathione peroxidase (GPx), Catalase (CAT), SuperOxide Dismutase (SOD) and the level of lipid peroxidation was estimated. Moreover, effects of cow ark with *Plectranthus Amboinicus* on the antioxidant enzymes that led to retrieving the activities of the defense system. CCl4 attenuated the level of examined enzymes significantly, a slow and steady progress was observed in cow ark treated animals, although CCl4 injected along with cow ark was noticed. A histopathological evaluation was also attempted to affirm the findings of various examinations of cow ark efficacy. Histological observations have been confirmed and favored our obtained results.

KEY WORDS: Cow Ark, Lipid Peroxidation, Glutathione Peroxidase, CCl4.

INTRODUCTION

There has been increasing evidence that free radicals and ROS (reactive Oxygen Species) have involved varied initial regulations and progressing the liver diseases independently [1-2]. There was an evident onset of lipid peroxidation, a key physiological process of pathogenesis of liver damage, stimulated by CCl4 [3]. Recent epidemiological study revealed that alcohol-induced liver damage may be correlated with an enhanced oxidative stress and tissue damage caused by free radicals [4-6].CCl4 represented a pharmacological agent often used to attack liver cells due to the production of hydroxyl radical [7], Oxidative stress may be propagated by the auto-catalytic cycle of metabolic stress, tissue damage, cell death, resulted enhanced free radicals and exhaustion of antioxidants [8]. Lipid Peroxidation is a process, governed by free radicals such as superoxide dismutase (SOD), hydroxyl radical and H2O2, the causative agent of cellular injury [9]. Glutathione peroxidase (GPx) catalase, SOD and varied low molecular mass antioxidants offer the chief cellular defense mechanism [10]. As a major role of the metabolic process, generated free radicals are delivered ineffective by Antioxidant defense system enzymes, such as SOD, GPx, Catalase and non-enzymatic antioxidants [11].

Cow Urine Distillate is used as disinfectant and responsible for varied therapeutic purification. This is proven to be the most effective natural anti bacterial, skin diseases etc. Recent investigators reported that the rational use of cow ark products prevent non-functionality of respirational mechanism and specifically, hepatogastro intestinal system [12-13]. Cow ark has been shown to stimulate hypotensive, inflammatory, hypoglycemic, anti bacterial, antimicrobial, antibiofilm, anticancer and other effects [14-16]. Majority of the effects are associated with antioxidant and free radical scavenging abilities. In our previous studies cow ark was shown to possess remarkable potential of inhibiting serum level enzymes such as ALT and AST [17-18].

In the present investigation, the level of antioxidant enzymes such as GPx , SOD and Cat and the determination Lipid Peroxidation of CCl4 induced liver injury rat model and to

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examine whether cow ark with *Plectranthus Amboinicus* involve any protective role against CCl4 induced hepatotoxicity was attempted.

MATERIALS AND METHODS

Chemicals and reagents

All reagents and chemicals were purchased from Sigma Aldrich Chemical Company Ltd., USA.

Determination of Cow Ark dose

To assign the impact of cow urine with *Plectranthus Amboinicus* on antioxidant enzymes in the liver, the doses was calculated from the human dose as 60ml per day and multiplied with a constant factor, 0.018×5.

Experimental design

Healthy, selected male Wister rats weighed about 240-270 gm. each, were purchased from the Central Government authorized King Institute of Biological Investigation at Guindy, Chennai, Tamil Nadu, and were lodged under a 12 hour light and dark cycle with a temperature of 23^0 C with ease accessible to food and water *ad libitum*. The reared rats were grouped into 6 consisting of 5 rats in each group. Group 1 (G1) acts as a standard group, administered normal saline only (8mlKg^{-1}) for a week time, Group 2 (G2) received 3ml Kg^{-1}) 50% in vehicle, sunflower oil was used as a vehicle on the 7^{th} day of the experiment. (G3) Group 3 was administered with Sylimarin (25mg Kg^{-1}) in Ethanol of 50% concentration continuously for 7 days without any break. Group 4 (G4) animals were administered with Sylimarin for 7 days and CCl4 on the last day (7^{th} day). Group 5 (G5) animals were received with Cow Ark with *Plectranthus Amboinicus* in 50% of ethanol) orally, daily for 7 days duration whereas Group 6 was administered with Cow Ark with *Plectranthus Amboinicus* on all 7 days and CCl4 on the 7^{th} day in above mentioned concentration.

On the completion of a 7 days experiment, on the 8^{th} day after injecting CCl4 and 24 hours duration, the animals were over anesthetized and sacrificed. The liver was collected from respective groups of animals in order to determine the level of Glutathione Peroxidase, GPx, Super Oxide dismutase (SOD), Catalase (CAT) and Lipid Peroxidation. By making a wide incision, the liver was carefully separated and was processed for analytical homogenization for further analysis.

Antioxidant enzymes

The exposed liver tissues were homogenized well with 10% W/V using ice chilled Tris buffer at 7.4 pH level, followed by centrifugation at 3000 rpm for 20 minutes at 4^0C and Supernatant was carefully transformed into a separate tube for further enzymatic quantification.

Glutathione Peroxidase (GPx)

The activity of Glutathione Peroxidase was determined by following indirect coupled oxidation technique, by employing Glutathione reductase [19]. Cumene hydroperoxide (1mM) with a quantity of 0.25mM of Glutathione were served as substrate. NADPH was coupled oxidised by using Glutathione Reductase (0.25 U) in a buffer, where Tris, 50mM, with a pH of 7.6 was used and carefully observed at 340 nm.

Catalase (CAT)

The action of catalase (CAT) was measured by the method of Aebi (1983) [20]. The examined solution was mixed with 3% H_2O_2 in 0.05M phosphate buffer (pH 7.0). The reactivity was started with adding of 100 Ul of enzyme and the conformation in absorbance was noticed and read at 240 nm for 3 minutes and the level and action of catalase was expressed as in mole H_2O_2 /min/mg/protein.

Superoxide Dismutase (SOD)

SOD activity was measured by adopting the method of Marklund and Marklund, (1974) [21] using pyrogallol (2mM) autoxidation. The examined solution with pyrogallol in

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0.1M Tris buffer (pH 8.2) and the enzyme. The activity was initiated by adding substrate and the absorbance was read at 420 nm for 3 minutes with a gap of one minute each.

Lipid Peroxidation Determination

The quantification of lipid peroxidation was carried out by assessing the concentration gradient of thiobarbituric acid reactive substances (TBARS) in the exposed liver, by adopting the protocol of Ohkawa et al, (1979) [22]. The production of Malondialdehyde (MDA) acid as an indicator of Lipid peroxidation. The unit was expressed as nmol MDA mg^{-1} protein.

Histopathological Evidences

The liver of the chosen rat group was separated from the sacrificed rats, and cut into many pieces, and washed in saline water. Slices of liver were submerged in 10% formaldehyde in order to observe histopathological studies. Subsequently, pieces of liver embedded in the processed wax, by using microtome, cut into, 5Um thickness, and stained with Hematoxylin and Eosin, Observations were made based on the micro photography.

Statistical applications

Results were expressed as Mean ± SD. The significant difference between CCl4 and examined groups was calculated by applying the't' test version in software.

RESULTS AND DISCUSSIONS

The finding of the level of GPx emphasized that after receiving CCl4, there was a little bit of hike in liver GPx level as 45.68 UmL^{-1} when compared to the standard, where 37.2 Uml -1 was detected (Fig-1). The group of rats receiving silymarin, (G3), the quantity of GPx was observed as 3.68 Uml-1 were noticed as low value to the $P < 0.05$, compared with CCl4 treated groups. In G4 animals, the quantity of GPx was found to be an increasing trend when compared with the reference group. Cow Ark with plant extract treated group (G5) of rats liver, showed the level of GPx was 3.96 UmL-1 while Group 6 (G6) was found to be the closer value of Group 5**.**

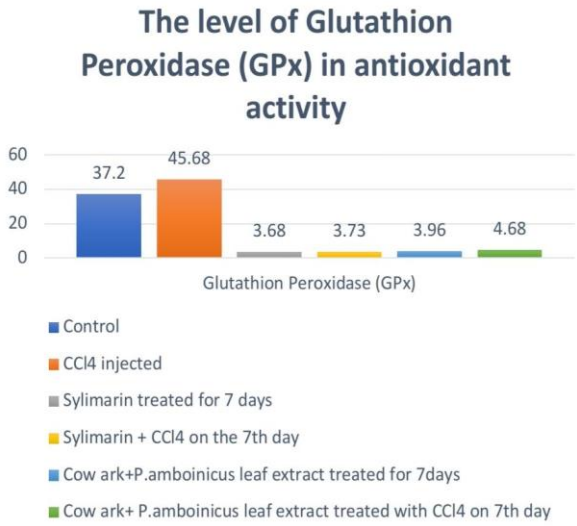


Fig -1Glutathion levels in antioxidant activity

Catalase

The quantity of Catalase level in the liver homogenate in Group 1 animals was recorded as 69.36±0.02. The level has been increased to 71.81 nmol H_2O_2 / Min/mg/protein, in Group 2 animals, due to the administration of CCl4. In group 3 rats, which received reference drugs silymarin, treated for 7 days, and the level of catalase was found to be 66.31± 0.12, which showed a slight difference in both group 1 and 2 animals. Group 4 rats, which administration CCl4 on the 7^{th} day of the experiment, showed the level of catalase was 67.63, whereas cow ark with leaf extract treated Group 5 animals exhibited a reduction in the quantity and observed as 59.86 ±0.14, which is the lowest value among all groups. Group 6

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rats were recorded as 61.33, and closer value of cow ark with plant extract treated groups (Fig-2). This showed cow ark with plant extract has exerted significant protective effects on the activity of catalase, even after CCl4 impact.

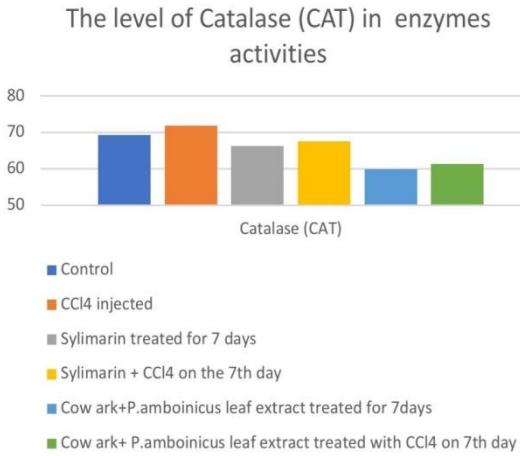


Fig -2 Catalase levels in antioxidant activity

Super Oxide Dismutase

The level of SOD in the liver homogenate, estimated as 486 ± 17 nmol L-1 in standard group, while, level of SOD showed a drastic reduction in Group 2 animals. Which received CCl4, This reduction was due to the enhancement of lipid peroxidation as found in the level of TBARS (4.88 ±0.15 nmol/MAD mg protein-1 in group 2 animals. SOD activity and the quantity was found to be 315 ± 18 in Group 3 animals, whereas cow ark treated group exhibited the value of 365 ± 42, indicating the protective role and closer value of sylimarin injected groups, where the value obtained was 329 ± 26. The activity of CCl4 on the SOD has been altered by the hepatoprotective effects of cow ark.

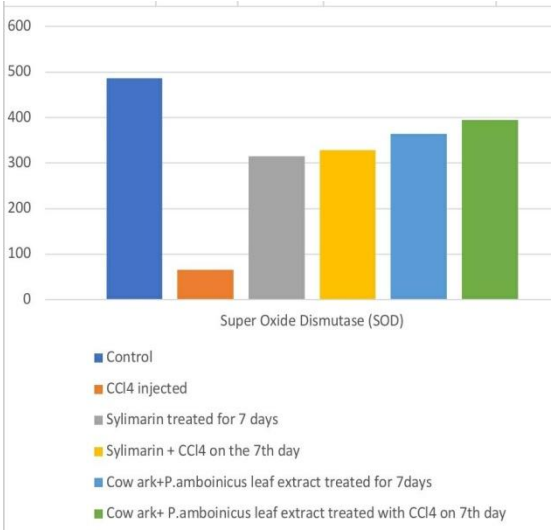


Fig -3 SOD levels in antioxidant activity

Lipid peroxidation

Consequently, the level of lipid peroxidation under CCl4 induced damage animals were observed the quantity of MDA standard Group (G1) was obtained as 3.84 ± 0.71, while, it increased to 4.88 ± 0.15, CCl4 injected groups (G2). It was obvious that cow ark with plant extract treated group (G5) has significantly reduced to 2.11 ± 0.36 due to the alteration in the lipid peroxidation functionality. Whereas G6 animals showed a value of MDA as 2.85 ± 0.21 and it was seemed to be a closer value of silymarin injected group (G3) indicating cow ark exhibiting an affordability effect against the effect of CCl4 on the last day of experiment.

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There is ample evidence for slow and steady protective effects of Cow ark with leaf extract of *P. amboinicus*, against Hepatotoxicity induced animal models.

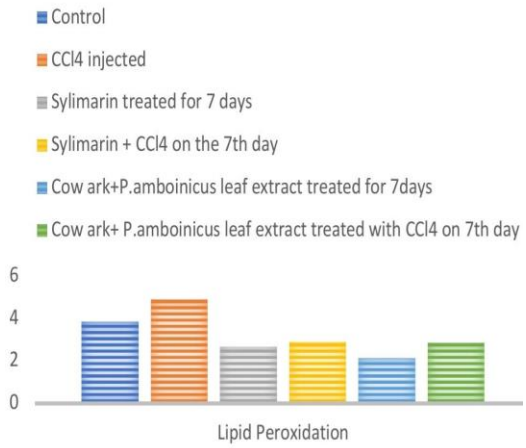


Fig -4 Lipid Peroxidation levels in antioxidant activity

The elevated level of examined enzyme level was previously detected in CCl4 treated rats, due to the hepatotoxic effects, however, the above enzyme markers were re-established towards normal, and findings are in good agreement with previous literature [23-25]. The level of MDA was significantly increased in CCl4 treatment, indicating stress compared with silymarin and cow ark with plant extract treated groups. The increased stress has been attributable to the formation of reactive metabolites and a series of reactions occurred in lipid peroxidation [26-27]. A significant decline was observed in cow ark treated group (G5) and displayed a close value of silymarin, reference value. This has been strong evidence that cow ark with herbal bioenhancer is a potential therapeutic in regulating antioxidant enzymes in liver enzymatic activities and showed a hepatoprotective role in the induced damaged liver.

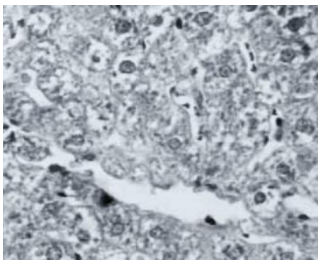
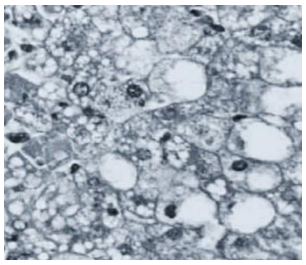
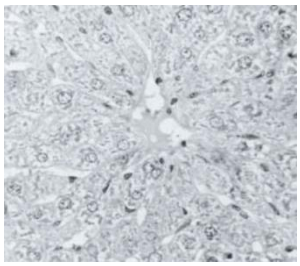
Pheromones and Pheromone compounds are present in Animal estrus urine for the potentials of many diseases. [28-30]. Many studies have been showed that free radicals implicates against diabetes, cancer, antidiarrheal, antiiflammatory, and wound healing [30-35].From the present investigation, the reactions of the antioxidant enzymes that produced *in vivo*, induced intoxication, resulted in a significant decrease in GTs, catalase, and SOD, due to the enhancement of lipid peroxidation. Glutathione level was effectively controlled in the cell by affecting pathways of its production. Decreasing the SOD level, while on the CCl4 treatment may be due to the drug enzyme interaction, leads to the deactivation of the enzyme [36]. Interestingly catalase was increased significantly, attributable to the absence of induces of SOD, such as H_2O_2 [37]. While, cow ark with *P. amboinicus* treated group showed a little bit decrease was observed than both CCl4 and reference silymarin injected animals. Surprisingly, the antioxidant enzymes level was retrieved, slow and steady to the normal conditions due to the hepatoprotective effect of cow ark with *P. amboinicus*. Observation revealed that cow ark along with *P. amboinicus* can influence lipid peroxidation activities and effectively restore the antioxidant enzymes in the liver of CCL4 induced damage.

Histopathological examination

A histological intervention was entertained on the effects of Cow ark (bio enhancer) with leaf extract of *P.amboinicus* was further supported by histological intervention (Fig-2) showed a normal appearance of liver tissue architecture. An immense alteration in lipid scattering, visualization of necrosis, a wide invasion of lymphocytes and other associated cells into the central vein were detected. Cellular edges were also interrupted in liver tissues of CCl4 administered rat group. Whereas Cow ark treated, liver tissues have shown to appear normal cell architecture, with double nucleus hepatocytes, however, a little change in the

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overall manner of hepatocytes with noticeable cytoplasm. Our histological observations have strongly affirmed the tendency of recovery in the activities of liver enzymes.

		
(i) A standard liver tissue architecture exhibiting central vein which received saline for 7day	**(ii) Observation showed that CCl4 treated liver cell**	**(iii) Bioenhancer (cow ark) treated liver tissue exhibited a recovery of original structure**

CONCLUSION

The present study finding can be treated as a reference material for further investigation in assigning cow ark with *P.amboinicus* leaf extract potential for the recovery and the fluctuations in the level of antioxidant enzyme activities.

Acknowledgements

Authors thankful to authorities of the Alagappa University, Karaikudi, Tamil Nadu, India, which was supported the research work done by the MHRD-RUSA 2.0-F.24/51/2014-U, policy (TNMulti-Gen), Dept. of Edn. Govt. of India.

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