International Journal of Recent Trends in Science And Technology, P-ISSN 2277-2812 E-ISSN 2249-8109
Special Issue, ACAEE: 2018 pp 37-42

Original Research Article

Antioxidant potential of gallic acid against cisplatin induced oxidative damage in liver tissue

Feroz Ahmad Shergojri[1*], Bashir Ahmad Bhat[1], Madhavi Gaur[2], Qaiser Jehaan Shammi[3]

[1]Government N. M. V. College, Hoshangabad, Madhya Pradesh, INDIA.
[2]Sant Hirdaram Girls College, Sant Hirdaram Nagar, Bhopal, Madhya Pradesh, INDIA.
[3]Government Arts and Commerce P.G. College Harda, Madhya Pradesh, INDIA.
Email: ferozdass@gmail.com

Abstract

Introduction: Cisplatin is one of the most effective anticancer drugs used to treat various solid tumors. Its full usage is limited due to the side effects like hepatotoxicity and nephrotoxicity. Oxidative stress has been generally accepted to be involved in Cisplatin induced toxicity. Polyphenols have been proven very effective in oxidative stress related diseases. Gallic acid has been predicted to play an important in drug designing. We aimed to investigate whether gallic acid has the antioxidant potential against cisplatin induced oxidant damage of liver. **Methods:** 42 adult female albino mice (25-35 gm) were divided in seven groups (n = 6). Treatment groups consisted of Gallic acid (two different doses), 7,12-Dimethylbenz [a] anthracene (DMBA), Cisplatin and combination treated. Liver tissues of animals were homogenized and tested for Lipid peroxidase (LPO), Superoxide dismutase (SOD), Glutathione (GSH) and Catalase (CAT) levels. **Results:** Due to liver oxidative damage LPO levels got increased, while as SOD, GSH and CAT levels were decreased in Cisplatin treated groups. Gallic acid treatment successfully reversed the levels of all antioxidative enzymes at both doses of 200 mg/kg and 400 mg/kg b.w. **Conclusion:** The results of our study prove the antioxidant potential of gallic acid.
Key Words: Oxidative stress, Gallic acid, Cisplatin, Antioxidant potential.

***Address for Correspondence:**
Dr. Feroz Ahmad Shergojri, Government N. M. V. College, Hoshangabad, Madhya Pradesh, INDIA.
Email: ferozdass@gmail.com

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INTRODUCTION

Cisplatin is a well known chemotherapeutic drug that is widely used for the treatment of various solid tumors such as ovarian, testicular and head and neck cancers[1]. However, undesirable side effects during the cisplatin chemotherapy have been an increasing concern which limits its clinical use[2]. Among these undesirable effects, hepatotoxicity is one of the most important dose limiting side effects of cisplatin therapy[3]. Cisplatin has been elucidated to cause severe liver injury which includes dissolution of hepatic cords, focal inflammation and necrotic tissues along with periportal fibrosis, degeneration of hepatic cords and increased apoptosis[4]. Oxidative stress has been reported to be involved in liver biochemical and histological alterations[5]. Plant based foods have been found rich in polyphenols, which are the biggest group of phytochemicals[6]. Secondary metabolites of plants have shown potential benefits on human health. Since last decade, extensive research is going on plant polyphenols as antioxidants and beyond that[7]. It has been strongly supported that polyphenols play an important role in degenerative diseases, particularly cardiovascular diseases and cancers[8]. Among various polyphenols, gallic acid (3,4,5-trihydroxybenzoic acid), has emerged as a strong antioxidant agent, which is useful in diabetes, ischemic heart diseases, ulcer and other ailments[9]. Gallic acid is a naturally occurring low molecular weight triphenolic compound, present in variety of fruits and number of plants. Phytomedicines with diverse biological and pharmacological activities have large number of gallic acid derivatives present in them. Gallic acid is also used as a standard for determing phenolic content of

analytes in pharmaceutical industry[10]. Although many studies have revealed gallic acid as a strong antioxidant agent[11], however, to the best of our knowledge, the effect of gallic acid in hepatoprotection during cisplatin administration has not been reported so far. Therefore, the present study was undertaken to scrutinize the effects of gallic acid on cisplatin induced liver oxidative damage. Our study included 7, 12-dimethylbenz (a) anthracene (DMBA) induced mammary cancer and those results will be published separately.

MATERIAL AND METHODS

Experimental animals were handled according to the Institutional Animal Ethics Committee (IAEC) of PBRI, Bhopal (PBRI/IAEC/409). Female albino mice with an average weight of 20 ± 2 gm were used for the experiment. They were purchased and maintained in the animal house of Pinnacle Biomedical Research Institute (PBRI), Bhopal M.P India. Animals were maintained at ambient temperatures of 25 ± 2°C and allowed to acclimatize prior to treatment in 12/12 hours of light/dark cycle. They were fed with standard laboratory food (pelleted diet) and provided with water *ad libitum*.

Experimental Design: 42 animals were divided into seven groups of six mice each and were treated for 16 weeks according to the following procedure.

Group 1: Received olive oil throughout the experimental period and served as Normal control.

Group 2: Received DMBA (1mg DMBA in 1ml olive oil w/v) 0.2 ml/kg of body weight once a week for six weeks and served as Mammary cancer control.

Group 3: Received the daily dose of Gallic acid 200 mg/kg body weight and DMBA (1mg DMBA in 1ml olive oil w/v) 0.2 ml/kg of body weight once a week for six weeks.

Group 4: Received the daily dose of Gallic acid 400 mg/kg body weight and DMBA (1mg DMBA in 1ml olive oil w/v) 0.2 ml/kg of body weight once a week for six weeks.

Group 5: Received DMBA (1mg DMBA in 1ml olive oil w/v) 0.2 ml/kg of body weight once a week for six weeks and a single dose of Cisplatin (1mg/1ml concentration) 6 mg/kg body weight.

Group 6: Received the daily dose of Gallic acid 200 mg/kg body weight and DMBA (1mg DMBA in 1ml olive oil w/v) 0.2 ml/kg of body weight once a week for six weeks and a single dose of Cisplatin (1mg/1ml concentration) 6 mg/kg body weight.

Group 7: Received the daily dose of Gallic acid 400 mg/kg body weight and DMBA (1mg DMBA in 1ml olive oil w/v) 0.2 ml/kg of body weight once a week for six weeks and a single dose of Cisplatin (1mg/1ml concentration) 6 mg/kg body weight.

Preparation of Homogenate: Liver of the mice was dissected out, blotted off blood and rinsed in freshly prepared ice-cold saline. Liver was divided into two parts and homogenized in phosphate buffer and in tris-HCl buffer separately in Potter Elvehgen homogenizer fitted with poly Teflon plunger at high speed. Homogenate made with tris-HCl was used for the estimation of lipid peroxidation (LPO). Phosphate buffer homogenate was used for the estimation of glutathione (GSH). Homogenate made in phosphate buffer was centrifuged at 4500 rpm at 4ºC. The supernatant fraction thus obtained was used for the estimation of catalase (CAT) and superoxide dismutase (SOD) enzyme activities.

Methods For Estimation Of Antioxidant Parameters

Assay for Estimation of Lipid Peroxidation (LPO)[12]: To the 0.2 ml tissue homogenate, 0.2 ml of sodium dodecyl sulfate (8.1%), 1.5 ml of acetic acid (20%) and 1.5 ml of thiobarbituric acid (8%) was added. The volume of the mixture was made up to 4 ml by distilled water and heated on water bath at 95^{o}C for 60 minutes. After cooling 5 ml of butanol: pyridine (15: 1) was added and vortexed. The upper organic layer was separated was absorbance was read at 532 nm. The amount of MDA was calculated from MDA standard curve and expressed as nMole/gm.

Assay for Estimation of Glutathione (GSH)[13]: Tissue homogenate (0.1 ml) was added with 1.5 ml of 20% trichloro acetic acid and 1mM ethylenediamine tetra acetic acid (EDTA). The mixture was centrifuged for 10 minutes at 2000 rpm. To the 0.1ml of supernatant, 1.8 ml of Ellman's reagent [5, 50-dithio bis-2 nitrobenzoic acid (0.1mM) prepared in 0.3 M phosphate buffer] was added and mixed thoroughly. The absorbance was read at 412 nm. The amount of GSH was estimated from standard curve and expressed as nMole/gm tissue.

Assay for Estimation of Superoxide Dismutase (SOD)[14]: To the 0.1 ml supernatant, 1.2 ml of sodium pyrophosphate buffer (0.052 M), 0.1 ml phenazine methosulphate (186 µM), 0.3 ml of nitrobluetetrazolium (300 µM) and 0.2 ml of 750 µM Nicotinamide adenine dinucleotide (NADH) were added. The mixture was incubated for 90 seconds at 30^{o}C. 0.1 ml of glacial acetic acid was added and stirred with 4 ml butanol. The butanol layer was separated after centrifugation at 2000 rpm. Absorbance was taken at 560 nm and expressed as U/mg.

Assay for Estimation of Catalase (CAT)[15]: The principle of the reaction consists of the breakdown of the substrate (hydrogen peroxide) by catalase and measuring the decrease in absorbance at 240 nm. Changes in the rate of absorbance in the unit of time are an index of the activity of catalase. To the 0.1 ml supernatant, 2.9 ml of H_2O_2 (30 mM) was added in 50 nM phosphate buffer and absorbance was taken at 240 nm and expressed as U/mg.

Statistical Analysis: Data was expressed in Mean ± SD. Statistical comparison between different groups was done by using One Way ANOVA followed by Benferroni's test. P<0.05 and P<0.001 were considered as levels of significance.

RESULTS AND DISCUSSION

Antioxidant system of the body maintains a dynamic balance between the production and detoxification of free radicals under normal circumstances. However, in case of hepatic damage, oxidative stress occurs and the balance gets disturbed[16]. Lipid peroxidation occurs with the increase in free radicals, lipid peroxides break down and a great number of aldehydes are formed which includes malondialdehyde (MDA)[17]. MDA has been reviewed as a marker of Lipid peroxidation and is a highly toxic molecule. It has been mentioned as possible mutagenic and atherogenic because of its interaction with DNA and proteins[18]. As described in Table: 1 and represented in Graph: 1, Cisplatin injection highly significant (P< 0.001) increased the LPO as compared to DMBA control group. DMBA itself did not show any significant difference when compared to Normal control. Pretreatment with Gallic acid considerably (P< 0.001) reversed the effect of cisplatin at both doses. These results suggest that Gallic acid could protect against cisplatin induced liver LPO damage. Among the second line defensive mechanisms against free radical damage, Glutathione (GSH) plays an important role as non-enzymatic antioxidant system[19]. GSH is a cysteine-containing tripeptide and the central redox regulator that has a role in the detoxification of xenobiotic compounds[20]. It operates as antioxidant at both intracellular and extra cellular levels against reactive oxygen species and free radicals thus it acts as a marker of oxidative stress[21]. In this study, GSH level was estimated to assess the effect of Gallic acid against cisplatin induced liver oxidative damage. The results shown in Table: 1 and Graph: 2 indicate that cisplatin administration decreased the GSH level (P< 0.001) when compared to DMBA control group, which suggests that cisplatin may cause oxidative stress injury. Gallic acid treatment successfully ameliorated the decreased GSH level (P< 0.001) at both doses of 200 mg/kg and 400 mg/kg body weight. These results indicate that Gallic acid could encourage the production of GSH to protect against cisplatin induced liver oxidative stress damage. Antioxidants act as the primary line of defense in organisms against reactive oxygen species by catalyzing their conversion into harmless products, thus preventing them from oxidative damage. Among the oxidative enzymes, super oxide dismutase (SOD) and catalse (CAT) play the central role in detoxification of free radicals[22]. They function mutually to eliminate harmful reactive oxygen species. Superoxide produced during oxidative stress is dismutated into oxygen and hydrogen peroxide which is catalyzed by SOD[23]. Hydrogen peroxide is further detoxicated into water and oxygen by CAT without generating toxic species[24]. In our study SOD and CAT levels were decreased (P< 0.001) by the administration of cisplatin, which suggest the oxidative stress effect of cisplatin. However, their levels were ameliorated by the pretreatment with Gallic acid (200 mg/kg and 400 mg/kg body weight), which was a highly significant (P< 0.001) effect as compared to cisplatin group (Table: 1 and Graphs: 3 and 4).

Table 1: Effect of Gallic acid against Cisplatin induced oxidative stress in Liver tissue of mice

Groups	LPO (nMol/gm)	GSH (nMol/gm)	SOD (U/mg)	CAT (U/mg)
Normal Control	49.90 ± 1.424	7.03 ± 0.007	335.34 ± 7.193	8.52 ± 0.771
DMBA Control	51.66 ± 0.733	6.96 ± 0.071	321.10 ± 3.889	7.06 ± 0.403
DMBA + Gallic acid (200 mg/kg)	50.00 ± 1.245	6.97 ± 0.088	324.96 ± 1.621	7.49 ± 0.126
DMBA + Gallic acid (400 mg/kg)	48.20 ± 0.715	7.00 ± 0.025	329.60 ± 4.224	8.06 ± 0.479
DMBA + Cisplatin (6mg/kg)	236.80 ± 24.500*a	0.88 ± 0.023*a	46.47 ± 1.863*a	2.00 ± 0.045*a
DMBA + Gallic acid (200 mg/kg) + Cisplatin (6 mg/kg)	127.36 ± 6.011*b	2.09 ± 0.078*b	176.02 ± 4.322*b	5.00 ± 0.108*b
DMBA + Gallic acid (400 mg/kg) + Cisplatin (6mg/kg)	93.43 ± 2.129*b	3.85 ± 0.034*b	229.92 ± 12.768*b	6.03 ± 0.117*b

All data presented in Mean ± SD (n=6), *p < 0.001, **p < 0.05, [a] vs DMBA control, [b] vs DMBA + Cisplatin

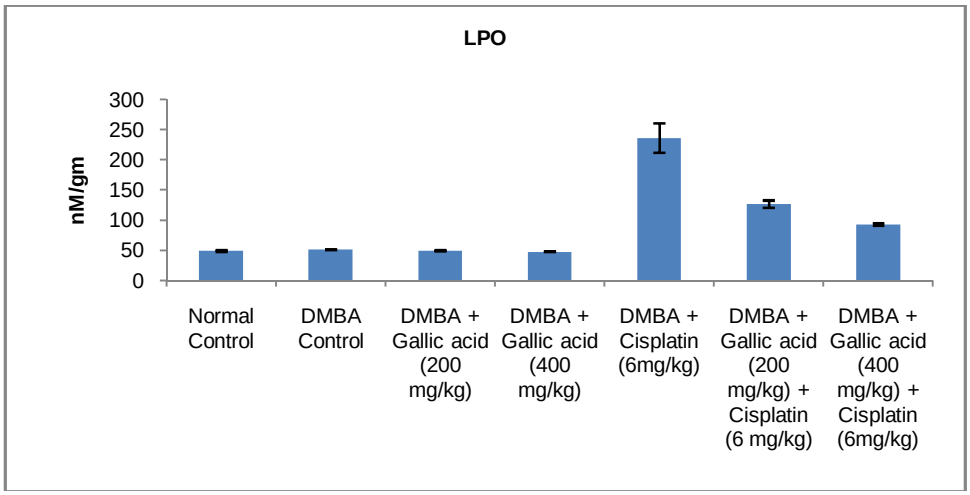

Figure 1: Cisplatin increased LPO; while Gallic acid brought it back them towards normal

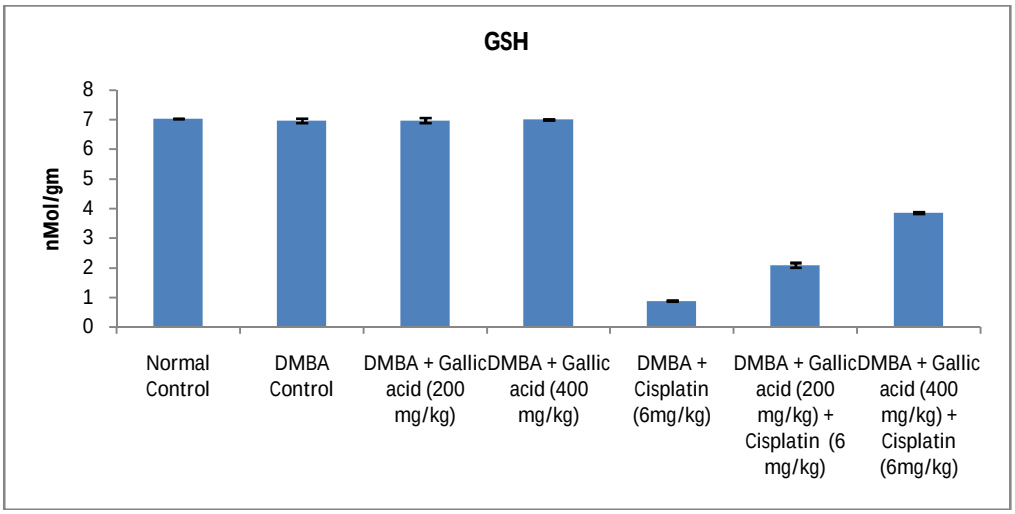

Figure 2: Cisplatin decreased GSH level; while Gallic acid significantly increased it towards normal level

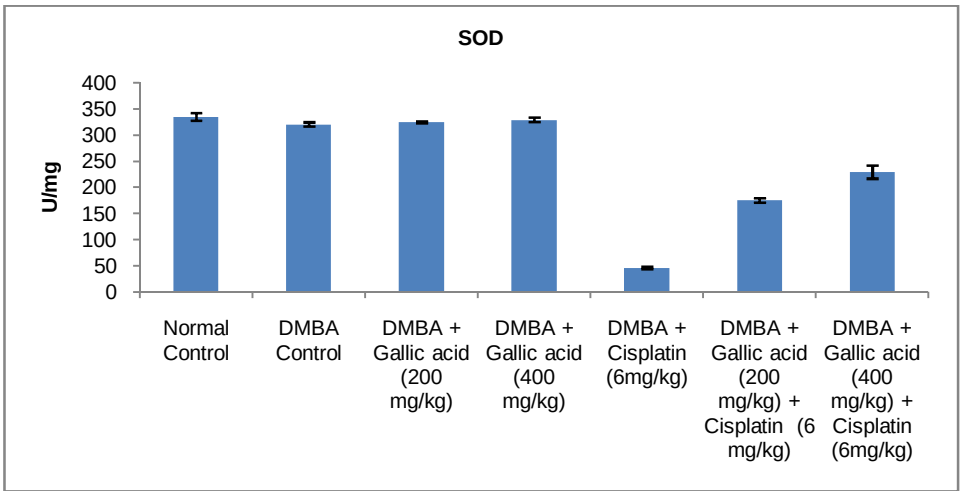

Figure 3: Cisplatin increased SOD level; while Gallic acid brought it back them towards normal

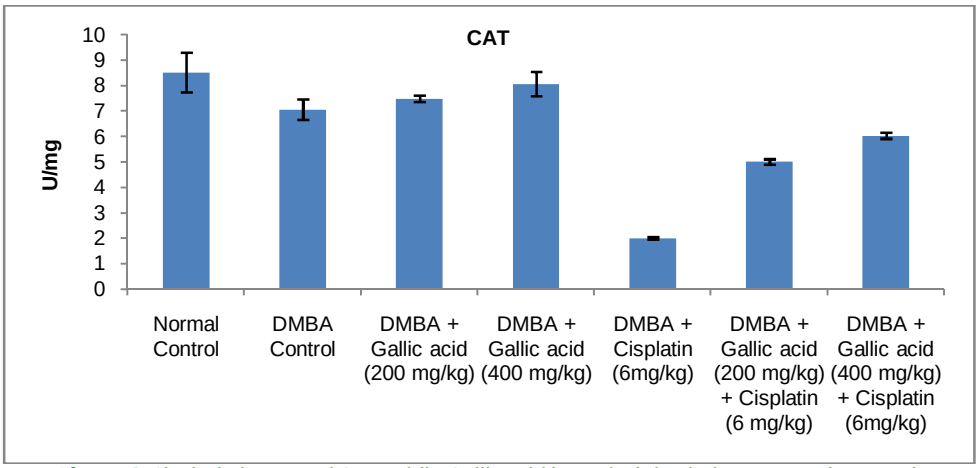

Figure 4: Cisplatin increased CAT; while Gallic acid brought it back them towards normal

CONCLUSION

The results our study revealed that Gallic acid has strong antioxidant potential and can been helpful for researches to combat the cisplatin induced liver oxidative damages.

ACKNOWLEDGEMENT

The authors are very thankful to the management of Pinnacle Biomedical Research Institute, Bhopal, M.P for providing us laboratory facilities to carry out this project.

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Source of Support: None Declared
Conflict of Interest: None Declared