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Amelioration of Cisplatin Induced Hepatotoxicity in Swiss Albino Mice by Gallic Acid

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ABSTRACT

Gallic acid is a natural polyphenol with strong antioxidant activity. Gallic acid protects our cells against oxidative damage by acting as an antioxidant. Cisplatin is a frequently employed broad-spectrum antineoplastic agent which remains to be a preferred treatment modality for various malignancies. However, the use of cisplatin as anticancer drug is limited due to its dose dependent toxicity. The purpose of this study was to scrutinize the potential of Gallic acid against cisplatin induced hepatotoxicity. The increased levels of aspartate aminotransferase (AST), alkaline phospatase (ALP), alanine aminotransferase (ALT), acid phosphatase (ACP), total bilirubin and direct bilirubin due to cisplatin were restored by gallic acid at both doses of 200 mg/kg and 400 mg/kg of body weight. Histopathological results of liver tissue also supported our biochemical results, thus confirming the efficacy of gallic acid to resist hepatic damage. These studies were further validated by the liver weight analysis which revealed that gallic acid successfully restored the increased liver weight due to cisplatin hepatotoxicity.

Key words: Gallic acid, Hepatotoxicity and Cisplatin.

INTRODUCTION

Liver plays an essential role in drug and xenobiotic metabolism; it occupies a pivotal position in our body and in maintaining the biological equilibrium of vertebrates [1]. The role played by this organ in removal of substances from the portal circulation makes it susceptible to first and persistent attack by offending agents like chemicals, toxins in food, peroxides, drugs, environmental pollutants etc., culminating in liver pathology.

Increasing rate of mammalian tumors and various side-effects of chemotherapeutic drugs reduce the clinical efficacy of major anticancer drugs that are currently being used. Thus the development of alternative or synergistic anticancer drugs with minimal side-effects is a constant need [2]. Cisplatin is one of the most widely used chemotherapeutic drugs for the treatment of several human cancers. However, its side effects in normal tissues and organs, notably nephrotoxicity and hepatotoxicity, limit the use of cisplatin and related platinum-based therapeutics [2], [4]. Identification of novel therapeutic natural products that reduce toxicities towards normal cells will have a significant impact upon cisplatin based therapy. Thus, combination procedure may provide a more efficient beneficial effect in various malignancies [5].

Naturally occuring polyphenols are secondary metabolites of plants which consist of a wide range of compounds divided into several classes. The most abundant antioxidants in the diet are polyphenols. These constituents are wide spread in fruits, vegetables, cereals, dry legumes, chocolate, and beverages, such as tea, coffee, or wine [6]. Our diet consists of abundant polyphenols as micronutrients, the healthy benefits depend on their bioavailability and the amount consumed. Details are emerging about the prevention of diseases such as cancer and cardiovascular diseases by polyphenols [7]. Polyphenols have shown potential health benefits in

various oxidative stress-associated diseases due to their potent antioxidant properties [8]. Gallic acid is a trihydroxybenzoic acid which is present in plants worldwide, it is also known as 3,4,5-trihydroxybenzoic acid. Various studies have shown anticancerous effects of Gallic acid in certain cancer cells. Gallic acid is also well known for its protective activity on normal cells which made gallic acid as a vital compound for cancer therapy [9].

MATERIAL AND METHODS

Experimental animals

Forty-two healthy female albino mice with an average weight of 20 ± 2 gms were purchased and maintained in the animal house of Pinnacle Biomedical Research Institute (PBRI), Bhopal M.P India. They were housed in polypropylene box type cages bedded with rice husk and were maintained at an ambient temperature of 25 ± 2°C and 12/12 hours of light–dark cycle. As per the recommended procedures, by fulfilling all the necessary ethical standards, absolute hygienic conditions were maintained in the animal house. The animals were fed with pelleted diet (purchased from Golden feeds Delhi) and water *ad libitum.* Animal experiments were performed with prior permission from Institutional Animal Ethics Committee (IAEC) of PBRI, Bhopal (PBRI/IAEC/409).

Experimental design

Animals were randomly divided into seven groups of six mice each; body weight of the animals was recorded and treated orally for the time period of 16 weeks as follows. Group 1st received only olive oil and served as Normal control. The mice in all remaining six groups received DMBA (1mg DMBA in 1ml olive oil w/v) 0.2 ml/kg of body weight once a week for six weeks. Group 2nd further received normal saline throughout the experimental period. Group 3rd and 4th mice were also daily treated with oral doses of Gallic acid 200 and 400 mg/kg of body weight respectively. Animals of group 5th received a single dose of Cisplatin (1mg/1ml concentration) 6 mg/kg of body weight. Group 6th and 7th received Gallic acid dose of 200 and 400 mg/kg of body weight respectively and single dose of Cisplatin (1mg/1ml concentration) 6 mg/kg of body weight.

Biochemical Estimations

At the end of experimental period the animals were anesthetized with light chloroform and sacrificed by cervical dislocation; blood sample and liver tissue were collected. The blood was centrifuged in remi centrifuge at 3000 rpm for 15 minutes so as to get the serum. The clear serum was collected in sterilized disposable plastic tubes and stored in a freezer set at -15^{0} for subsequent measurement of serum liver markers like Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Alkaline phosphatase (ALP) and Bilirubin (Total and Direct) with the help of commercially available kits. Liver of mice from all groups was dissected out and freed from connective tissue covering and weighed. A portion of liver was then transferred to 4% formalin solution for fixation. That portion of liver was later on processed for histopathological studies following the standard procedure [10].

Methods for estimation of Liver biomarkers

Standard methods for the determination of Aspartate aminotransferase [11], Alanine aminotransferase [11], Alkaline phosphatase [12], Total bilirubin and Direct Bilirubin [13] were followed.

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

Effect of Gallic acid on Cisplatin induced changes on liver weight

As shown in Table; 1, cisplatin (6 mg/kg) treated mice showed highly significant ($p<0.001$) increase in liver weight as compared to DMBA group. Gallic acid (200 mg/kg and 400 mg/kg) pre-treatment significantly ($p<0.05$) restored the liver weight in 6th and 7th groups, when compared to cisplatin (6 mg/kg) group. Gallic

acid (200 mg/kg and 400 mg/kg) alone did not cause any significant change in liver weight when compared to DMBA group. Also, there was no significant difference observed between normal control group and DMBA group.

Effect of Gallic on Cisplatin induced changes on liver biomarkers

As shown in Table: 2 and Table: 3, hepatotoxicity markers like ALP, ALT, AST, Total bilirubin and Direct bilirubin were highly significantly ($p<0.001$) elevated after a single dose of cisplatin (6 mg/kg) in cisplatin treated group of mice as compared to DMBA group. Pre-treatment with Gallic acid (200 mg/kg and 400 mg/kg) in groups 6^{th} and 7^{th} highly significantly ($p<0.001$) decreased the elevated levels of ALP, ALT, AST, Total bilirubin and Direct bilirubin, when compared to cisplatin (6 mg/kg) treated group. However, there was no significant difference observed between normal control and Gallic acid (200 mg/kg and 400 mg/kg) alone treated groups as compared to DMBA group. This also confirmed that Gallic acid doesn't have any side effects and is safe to use at selected doses.

Histopathological observations

Histopathological studies revealed no diformities in liver sections from normal group of animals as they had usual architecture of cells [Fig. 1]. Evaluation of cisplatin group showed swelling of hepatocytes, degenerative cells and vacoulization of cytoplasm. Additionally, there were dilations in the sinusoidal spaces. Due to increase in connective tissue, cell infiltration was also observed. Large number of kuffer cells and hepatic necrosis was also seen as a result of liver damage due to cisplatin [Fig. 5]. The combination groups showed promising protective potential of gallic acid against cisplatin induced hepatotoxicity [Fig.6], [Fig.7]. The arrangement of liver tissues and cells was usual in gallic acid and DMBA alone treated groups [Fig. 2], [Fig.3], [Fig,4]. The histophathological observations were in full agreement with those of the biochemical studies.

DISCUSSION

Gallic acid is a polyphenol which has anti-fungal and anti-viral properties. Gallic acid protects our cells against oxidative damage by acting as an antioxidant. Without harming healthy cells, gallic acid has been found to show cytotoxicity against cancer cells [14].

Cisplatin is one of the most effective and widely used chemotherapeutic drugs, which exerts mitochondrial dysfunction and cytotoxicity in cancer cells [15]. However, reversible and irreversible side effects including nephrotoxicity, bone marrow toxicity, gastrointestinal toxicity, neurotoxicity, hepatotoxicity and ototoxicity may limit its utility and therapeutic profile [16]. In this experiment, the protective role of gallic acid against Cisplatin induced hepatotoxicity was studied. Hepatotoxicity increases the level of liver markers such as alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin and direct bilirubin. These biomarkers are considered as diagnostic indicators of acute liver injury. Increased serum AST and ALT levels are indicators of cellular leakage and loss of functional capability of hepatic cell membranes in liver [17]. AST and ALT are found in higher concentrations in cytoplasm and AST in particular also exists in mitochondria of hepatic cells [18]. The transport of hepatocytes gets disturbed due to liver injury which results in the leakage of plasma membrane, thereby increasing the enzyme level in serum [19]. Soluble enzymes like AST will also be similarly released if injury involves mitochondria. Due to inflammation or liver damage, partial or full blockage of bile ducts occurs, which prevents the transport of bile from liver into gall bladder and intestine. This can cause spillage of ALP into the blood stream. Elevated levels of ALP may also be attributed to biliary cirrhosis, fatty liver and liver tumor [20]. One of the true tests of liver function is serum bilirubin, it shows the ability of liver to take up and process bilirubin into bile. Damage to the liver or blockage of excretory ducts reduces the ability of liver to excrete normal levels of bilirubin resulting in hyperbilirubinemia [20], [21]. In concurrence with the reports of other investigators [22], who investigated the effect of *Cymbopogon citrates* against cisplatin induced hepatotoxicity, data from present investigation also illustrated that cisplatin cause hepatic damage with highly significant increase in ALT, ALP, AST, Total bilrubin and Direct bilirubin levels. Treatment with gallic acid highly significantly decreased the activity of these biomarkers at both doses of 200 mg/kg and 400 mg/kg body weight. The

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studies of [23], on the protection effect of *Azadirachta indica* against cisplatin induced hepatotoxicity revealed the similar results. Our findings also collaborate the results of many other researchers [24], [25], [26] and [27].

Histopathological studies of liver in Normal group of animals revealed usual arrangement of hepatocytes with well defined nuclei, central vein, portal vein and had regular sinusoids. Our observation of the liver sections from Cisplatin treated group of animals showed drastic changes in its architecture like loss of cellular boundaries, increased diameters of sinusoids, loss of cells and fair amount of inflammation. Treatment with Gallic acid at 200 mg/kg and 400 mg/kg body weight brought significant amount of recovery in the cellular architecture of liver tissues. These observations were in accordance with many other investigators [28], [29].

Table: 1 – Combined effect of Cisplatin and Gallic acid on liver weight.

Groups	Liver Weight (gm)
Normal Control	1.17 ± 0.094
DMBA Positive Control	1.20 ± 0.017
DMBA + Gallic acid (200 mg/kg)	1.15 ± 0.027
DMBA + Gallic acid (400 mg/kg)	1.15 ± 0.028
DMBA + Cisplatin	1.32 ± 0.021*[a]
DMBA + Gallic acid (200 mg/kg) + Cisplatin (6 mg/kg)	1.22 ± 0.025**[b]
DMBA + Gallic acid (400 mg/kg) + Cisplatin (6mg/kg)	1.21 ± 0.034**[b]

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

Table: 2 – Combined effect of Cisplatin and Gallic acid on ALP, ALT and AST.

Groups	ALP (IU/L)	ALT (IU/L)	AST (IU/L)
Normal Control	58.76 ± 5.049	28.91 ± 2.102	15.32 ± 0.881
DMBA Control	76.95 ± 4.200	66.62 ± 3.044	31.82 ± 3.873
DMBA + Gallic acid (200 mg/kg)	70.98 ± 6.72	54.92 ± 3.512	25.93 ± 2.415
DMBA + Gallic acid (400 mg/kg)	65.70 ± 4.663	43.91 ± 2.777	20.62 ± 1.433
DMBA + Cisplatin (6mg/kg)	251.26 ± 17.937*[a]	1032.61 ± 37.102*[a]	202.43 ± 15.443*[a]
DMBA + Gallic acid (200 mg/kg) + Cisplatin (6 mg/kg)	153.99 ± 12.596*[b]	506.76 ± 46.559*[b]	147.62 ± 9.203*[b]
DMBA + Gallic acid (400 mg/kg) + Cisplatin (6mg/kg)	110.37 ± 9.170*[b]	316.75 ± 30.652*[b]	104.90 ± 10.349*[b]

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All data presented in Mean ± SD (n=6)

*p < 0.001

**p < 0.05

[a] vs DMBA control

[b] vs DMBA + cisplatin

Table: 3 - Combined effect of Cisplatin and Gallic acid on Total and Direct Bilirubin.

Groups	Total Bilirubin (mg/dL)	Direct Bilirubin (mg/dL)
Normal Control	0.26 ± 0.025	0.10 ± 0.007
DMBA Control	0.35 ± 0.024	0.17 ± 0.030
DMBA + Gallic acid (200 mg/kg)	0.32 ± 0.011	0.15 ± 0.013
DMBA + Gallic acid (400 mg/kg)	0.30 ± 0.021	0.12 ± 0.006
DMBA + Cisplatin (6 mg/kg)	2.81 ± 0.134*[a]	1.55 ± 0.103*[a]
DMBA + Gallic acid (200 mg/kg) + Cisplatin (6 mg/kg)	1.61 ± 0.041*[b]	1.03 ± 0.033*[b]
DMBA + Gallic acid (400 mg/kg) + Cisplatin (6mg/kg)	1.04 ± 0.028*[b]	0.55 ± 0.020*[b]

All data presented in Mean ± SD (n=6)

*p < 0.001

**p < 0.05

[a] vs DMBA control

[b] vs DMBA + cisplatin

Photomicrographs of Liver tissue stained with Haematoxylin and Eosin (40x).

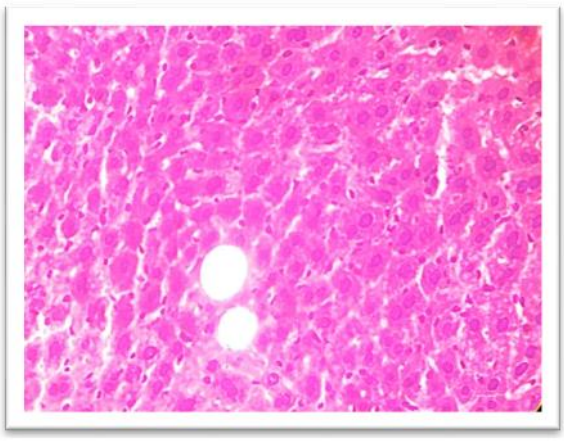

Fig. 1: Normal group

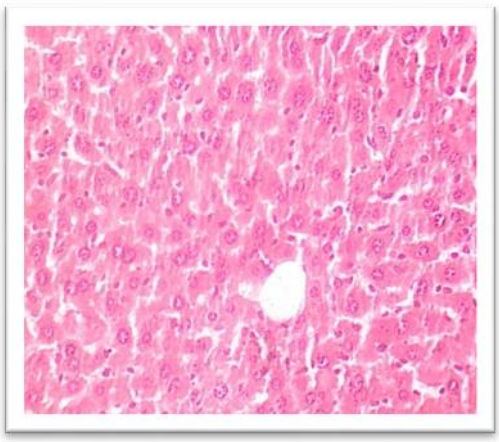

Fig. 2: DMBA group

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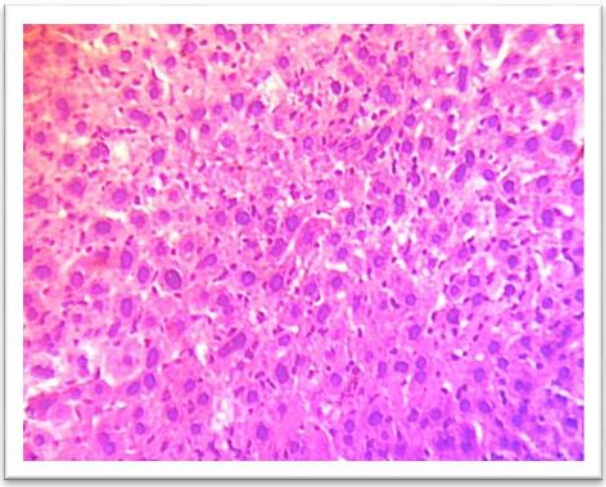

Fig.3: DMBA + Gallic acid 200 mg/kg b.w

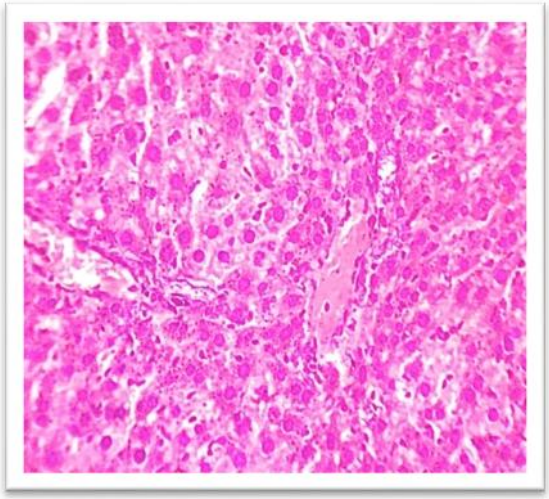

Fig.4: DMBA + Gallic acid 400 mg/kg b.w

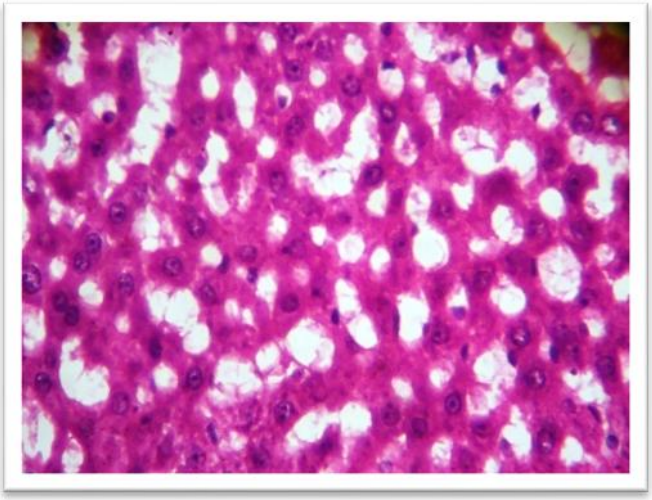

Fig. 5: DMBA + Cisplatin

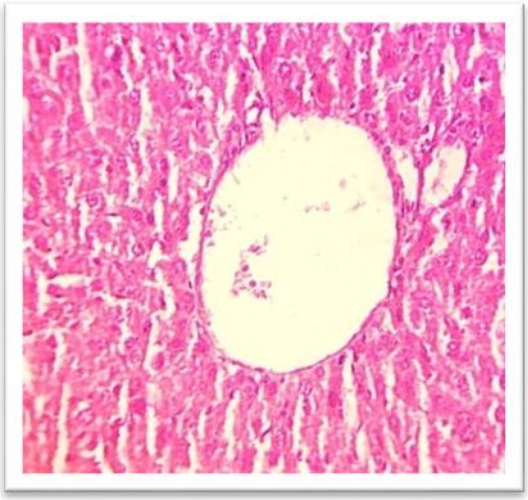

Fig. 6: DMBA + Gallic acid 200 mg/kg + cisplatin

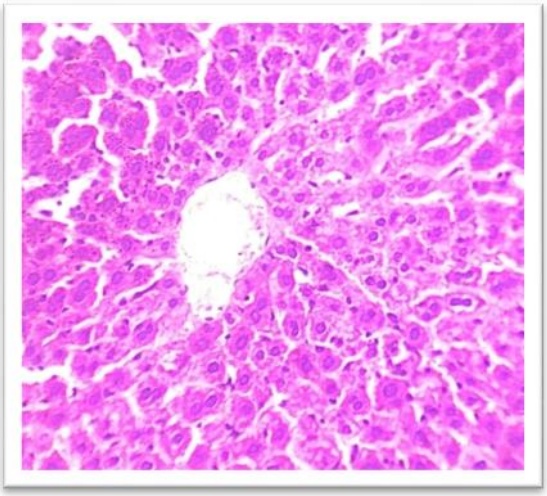

Fig. 7: DMBA + Gallic acid 400 mg/kg + Cisplatin

CONCLUSION

Finally we can conclude that our data manifestly illustrated that Gallic acid (a polyphenol) counteracts the hepatic damage caused by cisplatin in mice. The restoration of liver biomarkers may be attributed to the oxidative potential of Gallic acid.

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