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Pharmacological Effect Of Ursolic Acid On Hepatocarcinoma Induced By N-Nitrosodiethylamine In Experimental Animals

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ABSTRACT:
Objective: *The objective of the current study was to eavlaute the protective role of ursolic acid against N-nitrosodiethylamine (DEN) induced liver cancer in Albino Wistar rats.* ***Materials and Methods:*** *When rats were given 0.01% DEN in their drinking water for 15 weeks, ursolic acid had a protective effect. Aspartate transaminase (AST), alkaline phosphatase (ALP), alanine transaminase (ALT), lactate dehydrogenase (LDH), and gamma glutamyltranspeptidase (γGT) are serum marker enzymes that are elevated in DEN. Lipid peroxide levels are high (in both serum and tissue), and the final body weight and tissue are subsequently reduced. To determine the antioxidant status, the amounts of GSH, SOD, LPO, and CAT as well as the activity of antioxidant enzymes were measured in the liver and*

hemolysate of experimental animals. By purposefully reducing these changes, ursolic acid (50 mg/kg body weight) demonstrated a potent anticancer impact in liver cancer. ***Results and discussion:*** *According to the results of the biochemical estimations, DEN was successful in inducing hepatocellular carcinoma, whereas ursolic assistance was administered to counteract the effect of DEN. By evaluating different parameters of the liver tissue homogenate, the antioxidant action of ursolic acid was demonstrated. All of these findings suggest that ursolic acid has a chemopreventive impact on hepatocellular cancer caused by DEN.* ***Conclusion:*** *The present results suggest that Ursolic acid have protective effect by modulating the antioxidant status during DEN induced hepatocarcinogenesis.*

KEYWORDS: *Ursolic acid, Antioxidant, Hepatocellular carcinoma, N-Diethylnitrosamine Antioxidants,*

INTRODUCTION

Among malignant tumours, hepatocellular carcinoma ranks sixth in frequency and is the third leading cause of cancer-related mortality.[1] It is acknowledged that men are more likely than women to develop liver tumours [2], with the average ratio of men to women being 2:1. Oestrogens were linked to a lower incidence of hepatocellular cancer among sex hormones [3, 4]. In fact, our earlier research showed that oestrogen treatment prevented the change of ERα loss linked to diethylnitrosamine (DEN)-induced hepatic tumours [5, 6]. However, long-term oestrogen use has been linked to a higher risk of liver tumours in people, and some synthetic oestrogens may promote cancer [7, 8, 9]. Additionally, a number of human investigations have found that women who use oral contraceptives are more likely to acquire both benign and malignant liver tumours [10]. As is well known, persistent alcohol use has long been linked, particularly in industrialised nations, to progressive liver disease that progresses to hepatic cirrhosis and then to HCC [11-14]. According to recent research, hepatocytes' production of reactive oxygen species (ROS) would ultimately have a harmful effect [15, 16]. Moreover, ROS-induced oxidation of target proteins or enzymes would impair regular functions and may result in hepatocarcinogenesis. Strong data also suggested that ROS might enhance hepatoma cells' capacity for invasion. Consequently, concurrent antioxidant treatment, particularly in the early phases, may represent a breakthrough in HCC therapies. [17]

N-nitroso alkyl compounds, specifically DEN, are powerful mutagens, carcinogens, and hepatotoxins [18, 19]. Tobacco products, cheddar cheese, cured and browned foods, cosmetics, agricultural toxicants, and medicinal agents all include N-nitroso compounds, which are thought to be deadly [20]. In experimental animal models, DEN has been extensively employed as a precursor to start carcinogenesis. It has been shown that DEN activation, which mostly takes place in liver microsomes, stimulates Kuepfer cells, producing large quantities of ROS that can harm liver cells and cause hepatocarcinogenesis [21]. Lipid peroxidation is a metric used to quantify ROS-induced cellular damage [22].

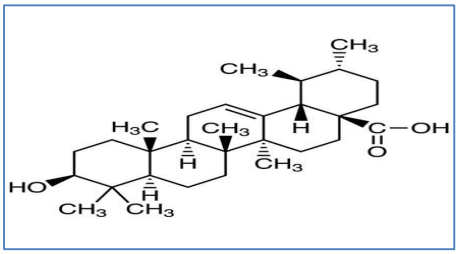


Figure 1: Structure of Ursolic acid

A beta-hydroxy group at position three replaces urs-12-en-28-oic acid to form urssolic acid, a pentacyclic triterpenoid. It serves as a geroprotector and a metabolite of plants. [23] It is a hydroxy monocarboxylic acid and a pentacyclic triterpenoid. It comes from an ursane hydride. Many plants contain ursolic acid and similar triterpene chemicals, such as oleanolic acid, betulinic acid, uvaol, or α- and β-amyrin. [24, 25] Because of the presence and activity of the enzymes that are responsible for their production, different species have different contents and compositions. One of the most promising biologically derived compounds for cancer prevention and treatment is ursolic acid. In addition to eliminating tumour cells, novel pharmaceutical approaches also alter their metabolism to stop angiogenesis and metastasis, promote cell differentiation, and shield healthy tissues from oxidative stress and inflammation that can cause neoplasms. [26] UA is a multi-purpose drug that simultaneously shields cells from carcinogens and affects multiple cell signalling enzymes. Ursolic acid has been shown in numerous studies to be crucial in preventing malignant transformation and the development of cancer, although its impact on hepatocarcinogenesis in vivo has not yet been established. [27, 28] In this study, we outlined the impact of ursolic acid on rat hepatocarcinogenesis caused by DEN.

MATERIALS AND METHODS:

Animals and Chemicals: About 155-180 g Albino Wistar rats were acquired from the Deshpande Laboratory in Bhopal, Madhya Pradesh, India. The animals were fed a commercial pelleted food and kept in cages with the right climatic conditions (M/s Hindustan Foods Ltd., Bangalore, India). Water was freely available to the animals. Every experiment was planned and carried out in compliance with the ethical standards authorised by the Institutional Animal Ethics Committee (IACE NO: 29/09/122/2024). We purchased DEN and ursolic acid from Yarrow Chem Pvt. Ltd. in Mumbai. S.D. Fine Chemicals (Mumbai, India) provided all other chemicals utilised.

Experimental Design: Four groups of six animals each were created from the experimental animals. [29, 30]

- Group 1: Normal control rats fed with standard diet and pure drinking water for 16 weeks.
- Group 2: Rats were induced with HCC by providing 0.01% DEN through drinking water for 15 weeks.
- Group 3: Rats treated with ursolic acid (50 mg/kg body weight) and administration of 0.01% DEN and continued till the end of the experiment (i.e., 16 weeks).
- Group 4: Rats were treated with ursolic acid alone by oral gavage daily at a dose of 100 mg/kg body weight for 16 weeks.

The rats underwent an overnight fast, diethyl ether anaesthesia, and cervical decapitation as a means of sacrifice following the study period. [31]

Biochemical parameters: Blood samples were allowed to clot at room temperature before being centrifuged for 10 minutes at 1500 rpm to extract the serum for biochemical assessment. The Mindray-

BS-200E Biochemical completely automated analyser was used to estimate the biochemical parameters, including AST, ALT, ALP, and ‑‑GT. 10% liver tissue homogenate (0.1 M Tris–Hcl buffer, pH 7.4) was prepared for lipid peroxidation investigations and enzymatic antioxidants. [32] The homogenate was then spun in a chilled centrifuge at 3500 r/min for 10 minutes at 4°C. The resultant supernatants were maintained in an ice bath. Superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase are antioxidant enzymes whose activity were evaluated using those methods, respectively. The methodology was followed to assess the levels of lipid peroxidation products in the serum and liver tissue homogenate. [33, 34]

Histological examination: Cervical dislocation was used to sacrifice the animals. For histological analysis, a section of the liver tissue was embedded in paraffin wax and preserved in 10% neutral buffered formalin. Haematoxylin and eosin (H&E) was applied to sections that were 5 µm thick, and they were then seen using a high power light microscope. [35, 36]

Statistical Analysis: The statistical analysis were carried out by one-way analysis of variance (ANOVA), followed by Tukey Kramer multiple comparison post-test. P values of <0.05 were considered to indicate the statistically significance. All the results were expressed as mean ± standard error (SE) for six animals in each group. [37]

RESULTS AND DISCUSSION:

Effect of ursolic acid on body weight, Liver weight and relative Liver weight: In Albino Wistar rats, the hepatoprotective effect of ursolic acid against DEN-induced HCC was clarified. The starting body weight, end body weight, liver weight, and relative liver weight of the experimental and control groups of animals are displayed in Figures 2 and 3. In comparison to group-I control animals, DEN-induced group II animals exhibit a large rise in liver weight and a considerable drop in absolute body weight. Throughout the trial, all rats shown increased tolerance to ursolic acid treatment, and the ursolic acid-treated groups III demonstrated a substantial increase in absolute body weight when compared to group-II DEN-induced animals. The relative liver weight of animals in group II is much higher than that of animals in group I, and the liver weight of animals in groups III treated with ursolic acid is significantly lower than that of animals in group II that were DEN-induced. The fact that there were no discernible differences between the control and ursolic acid-only treated groups suggests that ursolic acid is not harmful.

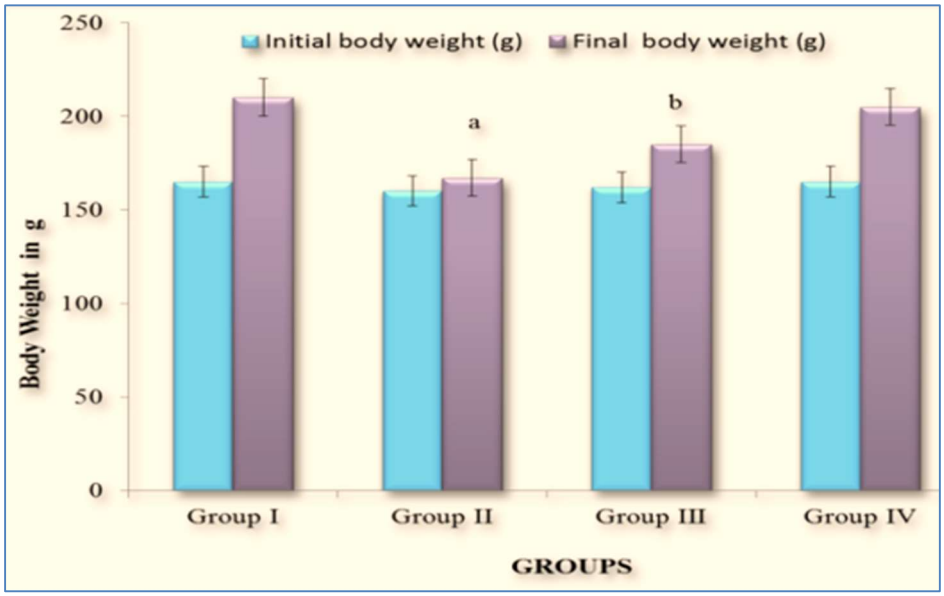


Figure 2: Body weight changes in control and experimental groups of rats.

Results are expressed as mean ± S.D for six rats in each group. Statistical significance at P>0.05 compared with [a] group 1, [b] group 2. Body weight change is expressed in grams

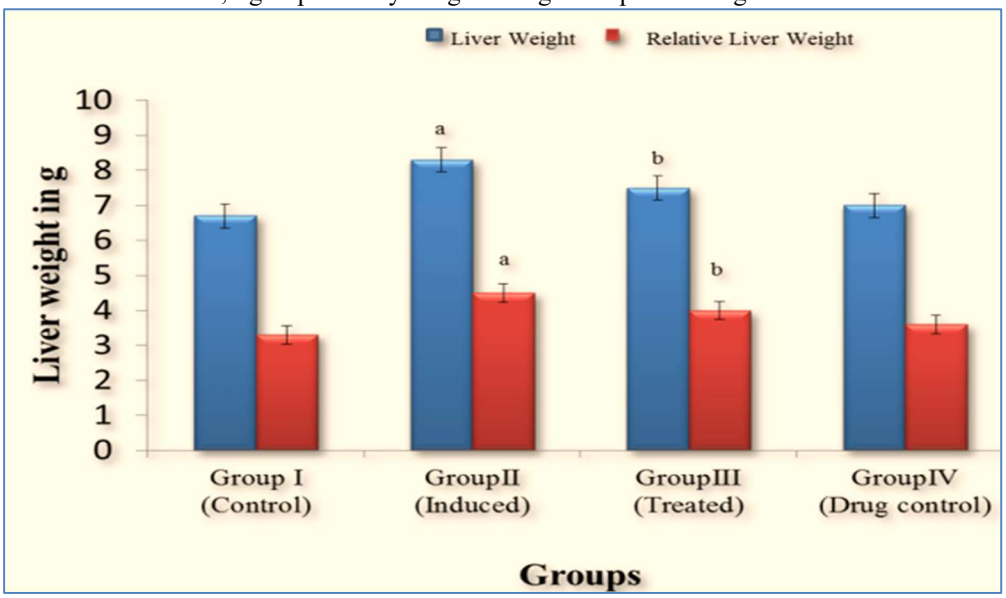


Figure 3: Liver weight and relative liver weight of control and experimental groups of rats.

Results are expressed as mean ± S.D for six rats in each group. Statistical significance at P>0.05 compared with [a] group 1, [b] group 2. Liver weight is expressed in grams. Relative liver weight is the average of liver weight at final body weight multiplied by 100.

Lipid Peroxidation: The amount of LPO in the serum and liver of the experimental and control groups of mice that underwent oxidative stress analysis is displayed in Figure 4. When compared to group I control animals, lipid peroxide levels are much higher in DEN-induced group II animals. In contrast to group II-induced animals, ursolic acid-treated group III animals exhibit a substantial reduction in lipid peroxide levels. However, when compared to group I animals, animals treated with ursolic acid

alone in group IV did not exhibit any notable changes.

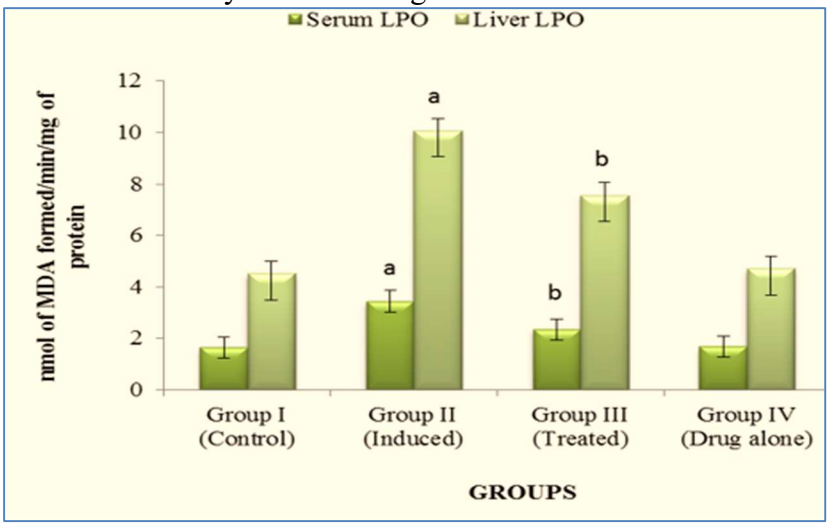
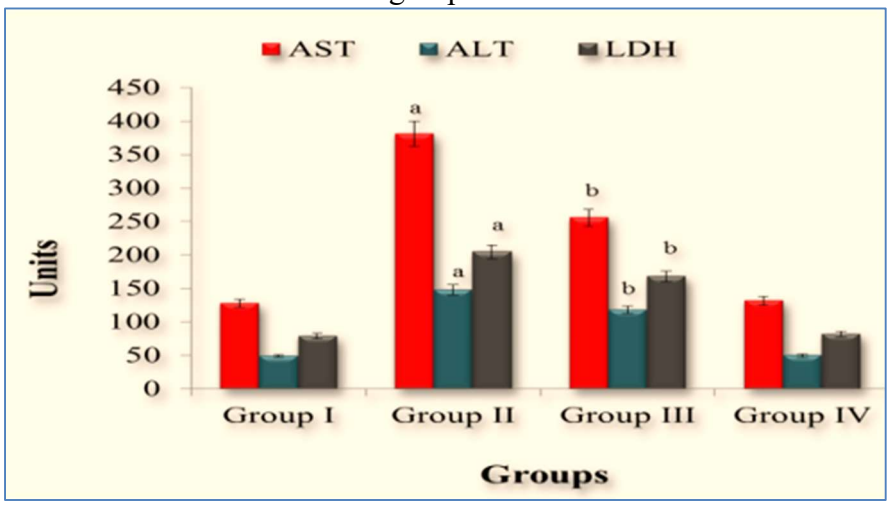


Figure 4: Effect of ursolic acid on the levels of lipid peroxides in the serum and liver of control and experimental groups of rats.

Results are expressed as mean ± S.D for six rats in each group. Statistical significance at P>0.05 compared with [a] group I, [b] group II. LPO levels are expressed as nmol of MDA formed/ min/mg protein.

Effect of ursolic acid on the activities of marker enzymes in the liver tissue of control and experimental groups of rats: The impact of ursolic acid on the levels of the marker enzymes AST, ALT, LDH, ALP, and γ-GT in the serum of the experimental and control groups of rats is depicted in Figure 5. In comparison to control mice, DEN-induced group II animals had considerably higher marker levels. In contrast to group II mice that were DEN-induced, group III treated animals showed a significant drop in marker enzyme levels. There were no discernible differences between the animals treated with ursolic acid alone and the control group.



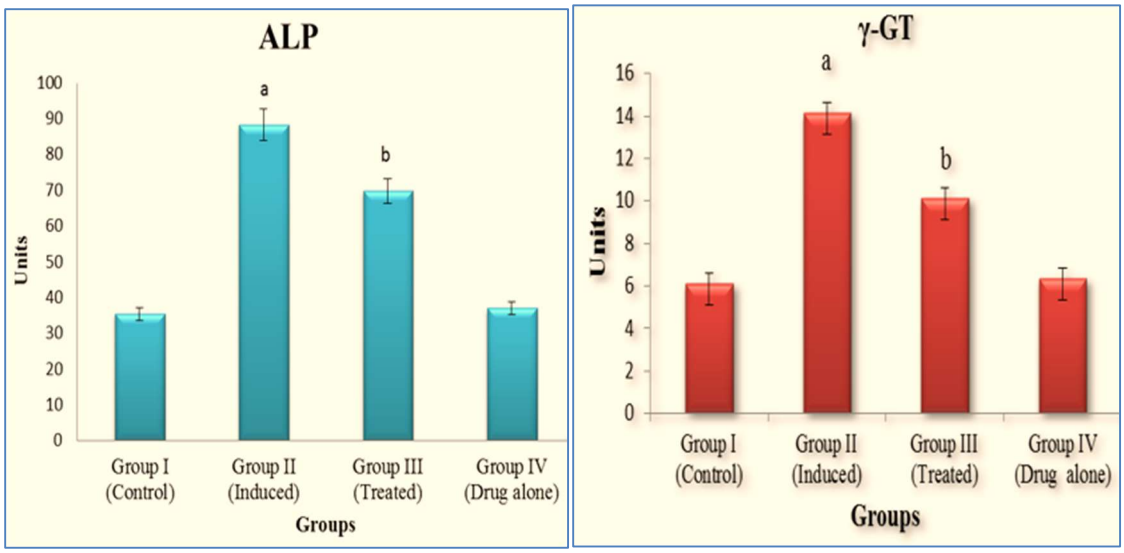


Figure 5: Effect of ursolic acid on the activities of marker enzymes in the liver tissue of control and experimental groups of rats.

Results are expressed as mean ± S.D for six rats in each group. Statistical significance at P>0.05 compared with [a] group I, [b] group II. µ moles of pyruvate liberated mg protein per min for AST, ALT and LDH; µ moles of phenol liberated mg protein per min ALP; nmoles of p-nitroaniline formed mg protein per min for GGT.

Effect of ursolic acid on the enzymic and non-enzymic antioxidant in the liver of control and experimental groups of animals: The enzymatic and non-enzymatic antioxidant activity in the livers of the experimental and control groups are shown in Table 1. When compared to control Group I mice, DEN-induced Group II animals exhibited a marked drop in the activities of enzyme antioxidants, including SOD, CAT, GPx, GR, and GST. Comparing treated group III mice to DEN-induced group II animals, however, revealed a notable rise in these enzymes. When compared to control mice, non-enzymatic antioxidants like GSH also showed noticeably lower activity during DEN-induced Group II animals. The GSH activities of treated group III animals are much higher than those of DEN-induced animals. When comparing Group IV animals treated with ursolic acid alone to the control group, no discernible changes were seen.

Table 1: Effect of ursolic acid on the enzymic and non-enzymic antioxidant in the liver of control and experimental groups of animals

Particulars	Group 1 (Control)	Group 2 (Induced)	Group 3 (Treated)	Group 4 (Drug alone)
SOD	9.11±0.65	3.45 ±0.21[a]	5.23 ±0.78[b]	8.11 ±0.21[a]
CAT	68.56 ±7.56	43.45 ±4.23 [a]	51.65 ±4.57[b]	68.64 ±7.23
GPx	98.54± 7.11	58.67 ±3.49 [a]	70.23 ±6.23[b]	96.65± 9.21
GR	158.21± 8.82	99.45 ±6.23 [a]	122.67± 6.28[b]	158.37± 6.11
GST	1.32 ±0.04	3.12± 0.03 [a]	2.54± 0.45[b]	1.55 ±0.32[a]
GSH	39.78 ±3.79	14.22 ±1.03 [a]	21.45 ±1.06 [b]	41.43 ±3.11

Results are expressed as mean ± S.D for six rats in each group. Statistical significance P>0.05 compared with [a]group 1, [b]group 2. SOD, (superoxide dismutase in units/mg protein, CAT (catalase in l mol of H_2O_2 decomposed/min/mg protein), GSH (glutathione in lg/mg protein), GPx(glutathione peroxidase in l mol of GSH utilized/min/mg protein), GR (glutathione reductase in l mol of NADPH oxidized/min/mg protein)

Histology Examination: Under a light microscope, the histopathological changes of liver tissue sections stained with haematoxylin and eosin (H&E) were evaluated. In comparison to normal control animals, animals supplied with ursolic acid alone likewise displayed a normal histological appearance and the liver's normal architecture. The DEN-induced animals displayed focal myocyte inflammation and hyalinization, but the liver treated with DEN + ursolic acid displayed nearly normal myocytes and less aberrant pathological signs. Ursolic acid's ability to protect against oxidative stress and tissue damage brought on by DEN may be the cause of these repairs.

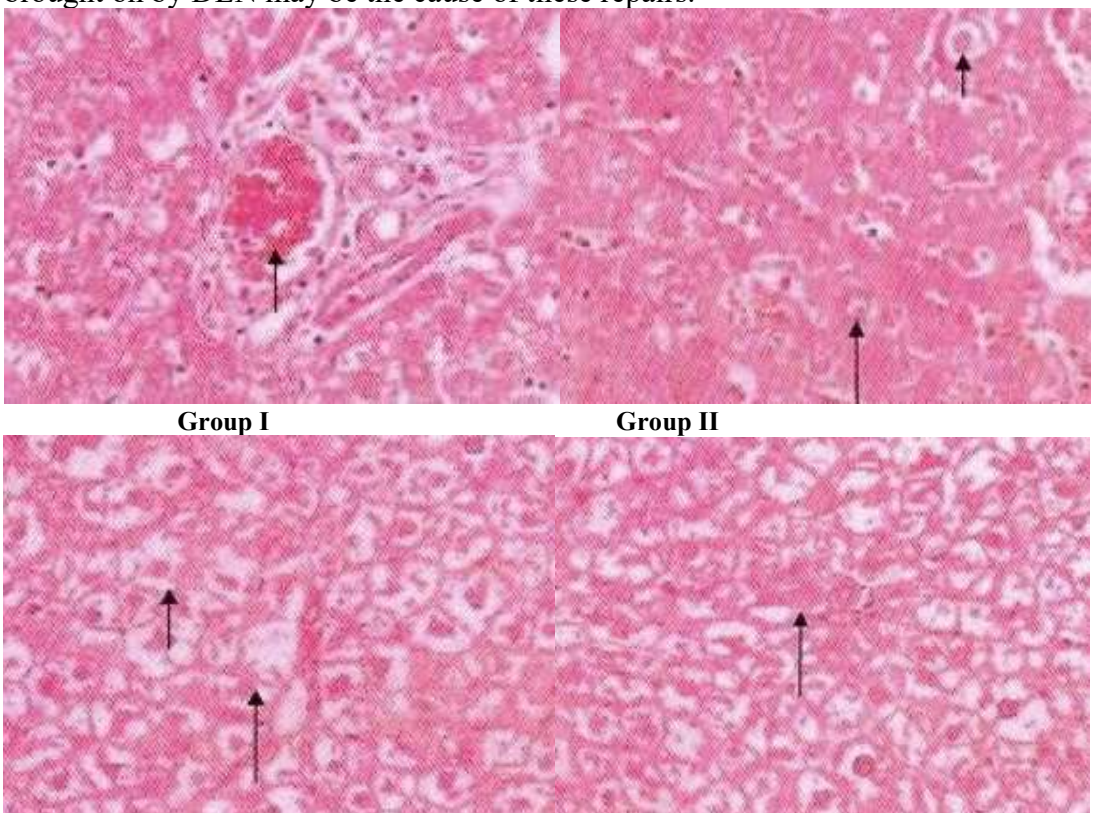


Figure 6: Histopathological examination of liver tissue in control and experimental groups of rats.

Group I-Control shows a normal architecture, Group II- DEN alone slides showing loss of architecture, a marked tendency to spread by intrahepatic veins, both hepatic and portal with significant tumor thrombi within portal vessels. Cytological tumor cells are slightly larger, have more irregular nuclei and also numerous mitotic figures. Group III-Ursolic acid treated showing few neoplastically transformed cells and hepatocytes maintaining near normal architecture. Group IV-Ursolic acid slides showing normal liver architecture. (20X, HE).

CONCLUSION:

The present investigation concludes that ursolic acid has potent antioxidant and free radical scavenging properties. According to the findings, ursolic acid effectively lowers LPO levels and dramatically boosts the body's natural antioxidant defences against DEN-induced hepatocellular carcinogenesis. Our findings further demonstrate that ursolic acid treatment prevented the significant rise in serum marker levels. Next, we propose that ursolic acid could be created as a potent chemotherapeutic substance. The molecular processes underlying ursolic acid's effectiveness as an anticancer drug are

being clarified by additional research.

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