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Research Paper

Pharmacological potential of natural flavonoid diosmin against n-nitrosodiethylamine induced hepatocellular carcinogenesis in wistar albino rats

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Abstract

The present analysis attempts to scrutinize anticancer and medicinal perspective of polyphenol diosmin against chemically induced experimental hepatocarcinogenesis. Flavonoids are one of the common components in the human diet and main resource of this polyphenol is fruits, vegetables, and seeds. Flavonoids are widely present in the genus Citrus belonging to Rutaceae family. Hepatocellular carcinoma was induced through administration of 0.01% N-Nitrosodiethylamine through drinking water for 16 weeks. Hepatocarcinogenesis shows the alterations in the body, organs weight and also levels of nucleic acids (DNA & RNA), tumour marker enzymes (AST, ALT, ALP, LDH, γ-GT and 5'NT), xenobiotic enzymes (Phase I & II) and histopathological changes of the liver and kidney. These biochemical and morphological revolutionize may be due to pathogenesis of hepatocytes induced by NDEA and it was suppressed by the management with natural bioflavonoid diosmin (200 mg/kg/b.w/p.o) for 28 days. Due to curative property of diosmin, it preserves the biochemical enzymes at customary level and convalesce the structural damage induced by the chemical carcinogen.

Keywords: Diosmin, Hepatocellular carcinoma, HCC, N-Nitrosodiethylamine, Tumour marker enzymes, Xenobiotics, Drug metabolizing enzymes.

Introduction

Hepatocellular carcinoma (HCC) arises in the hepatocytes of the liver and is the most common and lethal among other types of cancer. In this context the epidemiology of HCC is also increasing throughout the world. It is the fifth malignancy and the third most common cause of cancer related death in worldwide. Males and advanced age are associated with a higher incidence. HCC is one of the striking geographical variation in its incidence. The incidence rate of the disease is 2-3 times higher in developing countries than in developed countries. The survival of patients with HCC is influenced by many factors which include tumor size, age and sex [1].
N-nitrosodiethylamine (NDEA) is one among the N-nitroso compound and it is a genotoxic hepatocarcinogen. N-Nitrosodiethylamine is volatile, clear yellow oil that is soluble in water, alcohol,

ether, other organic solvents, and lipids. The compound is sensitive to light, especially ultraviolet light, and undergoes relatively rapid photolytic degradation. When heated to decomposition, N-Nitrosodiethylamine emits toxic fumes of nitrogen oxides [2]. Nitrate and nitrite are added to meat and fish for the purpose of preservation, as colour fixatives and as flavouring. Ingestion of nitrite and nitrate can result in the endogenous formation of nitroso compounds, particularly in the presence of nitrosatable precursors, such as primary amines, in the acidic condition of the stomach. In addition, exposure of man to preformed nitrosamines occurs due to the use of tobacco products, cosmetics, pharmaceutical products and agricultural chemicals. Average intake of nitrosamines from food by human is approximately 1 mg/day [3]. NDEA is recognized to cause perturbations in the nuclear enzymes involved in deoxyribonucleic acid repair/replication and is normally used as a carcinogen to induce liver cancer in animal models [4]. Upon metabolic activation, its active ethyl radical metabolite, and the reactive product interacts with DNA causing mutation, which would lead to carcinogenesis. Among laboratory animals, the rat has a wide variety of advantages, for example it has short life span which allows observation of NDEA transformation from its initial stage through to complete malignant cancer, and in addition, variable factors may be fixed artificially during the course of experiment. Hence it can be concluded that in addition to the ethyl adducts formation, oxidative stress resulting in the depletion of antioxidants and increase in the lipid peroxidation might be responsible for the carcinogenic effect of NDEA, and may initiate liver carcinogenesis [5].

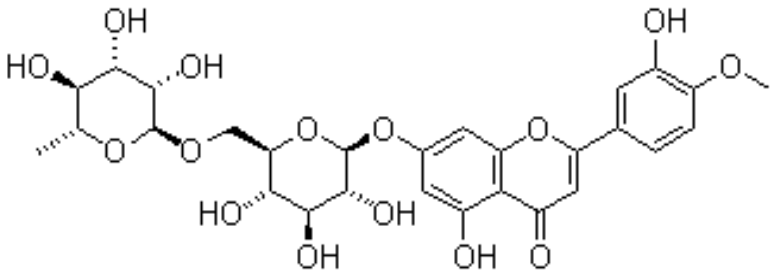
Diosmin is an important flavonoid in Citrus. It is a naturally occurring flavone glycoside i.e. a sugar molecule attached to its three ringed flavonoid structure**.** It can be isolated from various plant sources or derived from the flavonoid hesperidin. Diosmin was first isolated in 1925 from *Schrophularia nodosa*, and first introduced as a therapeutic agent in 1969. Diosmin is the major active constituent of Buchu leaf (*Barosma betulina*, *Rutaceae*) and is also found in other *Rutaceae* species. It has been used for more than 30 years as a phlebotonic and vascular-protecting agent, and has recently begun to be investigated for other therapeutic purposes including cancer, premenstrual syndrome, colitis, and diabetes [6]. It is well established that Diosmin is a free radical scavenger of reactive oxygen metabolites involved in tissue destruction occurring in inflammatory reaction [7]. The main objective of the study is to determine whether diosmin is implicated as a chemo preventive and antiprolifirative effect of diosmin in wistar rats.

Materials and Methods

Chemicals

Diosmin and N-nitrosodiethylamine were purchased from Sigma Chemical Company, St Louis, MO, U.S.A. All other chemicals including solvents used were of high purity and of analytical grade marketed by Glaxo Laboratories, Mumbai and Sisco Research Laboratories Pvt. Ltd, Mumbai, India.

Structure of Diosmin



Synonym : 3′, 5, 7-Trihydroxy-4′-methoxyflavone-7-rutinoside.

Molecular formula : $C_{28}H_{32}O_{15}$

Animals

Healthy Wistar albino male rats weighing between 180 ± 20g were obtained from the Central Animal House Facility of Dr. ALM Post Graduate Institute of Basic Medical Sciences, University of Madras, Chennai - 600 113, India. They were individually housed, maintained in clean polypropylene cages and fed with commercial rat feed and water *ad libitum*. The animal room was well ventilated and a 12 h day and light rhythm was maintained throughout the experimental period. During the course of the study, the temperature was maintained between 27°C ± 2°C. The experimental protocol was subjected to scrutiny of Institutional Animal Ethics Committee for experimental clearance (IAEC No 07/018/08.).

Diet

The animals received as balanced commercially available pelleted rat feed were provided with clean drinking water *ad libitum*. The feed contained protein (21%), lipids (5%), crude fiber (4%), ash (8%), calcium (1%), Phosphorus (0.6%), nitrogen free extract (55%) with a metabolisable energy of 3600 cal/kg.

Dose fixation

The dose of Diosmin was selected on the basis of LD_{50} was found to be 3g/kg body weight. On the basis of LD_{50}, the sub lethal dose (1/10 LD_{50}) of Diosmin 150, 200, and 250 mg/kg b/w were selected for the most of the studies. Diosmin administration of 200 mg/kg b/w per day orally for 50 days in rats did not produce any abnormalities such as a toxicity, circling, lacrimination, labowered breathing etc., in the animal throughout the experimental period[8]. Based on the reference of the study 200mg/kg b/w of diosmin was selected for the present investigation.

Experimental design

The rats were divided into four groups with six animals in each group and were given dose regimen as mention below: Group I - Normal control (vehicle treated; DMSO: 1 ml/kg b.w). Group II-Rats were induced hepatocellular carcinoma by providing 0.01% NDEA through drinking water for 16 weeks. Group III - Cancer bearing rats were treated with Diosmin (200 mg/kg/b.w/p.o) through oral gavage for 28 days. Group IV- Treated with Diosmin (200 mg/kg/b.w/p.o) alone for 28 days.

Collection of samples

At the end of the experimental period, animals were subjected to diethyl ether anaesthesia, blood was collected from retro orbital plexus and serum was separated by centrifugation. Animals were sacrificed by cervical decapitation and the tissues liver and kidney were excised, washed in ice-cold saline and blotted to dryness. A 10% homogenate of the liver and kidney tissue was prepared in 0.1 M Tris–HCl buffer (pH 7.4), centrifuged and the clear supernatant was used for further assays.

Biochemical assays

Body weight and tumor weight was estimated [9], nucleic acids: DNA and RNA were extracted, estimated by UV spectrophotometer method [10,11,12]. The marker enzymes such as Aspartate transaminase, alanine transaminase [13], alkaline phosphatase [14] as modified [15], γ-glutamyl transferase [16], lactate dehydrogenase [17], 5′-nucleotidase were also determined by using spectrophotometer method [18], The liver microsomes were separated with slight modification [19,20], the levels of Cytocyhrome P_{450} and cytochrome b_5 [21], NADPH-cytochrome C reductase [22,23], Glutathione S-transferase UDP-glucuronyl transferase [24,25] modified [26] were estimated by spectrophotometer method.

Histopathology

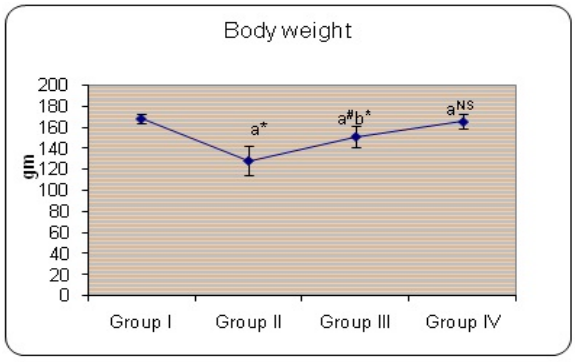
To investigate the histopathological changes in the liver and kidney tissue of various treatments, permanent mounts of the tissue samples were prepared [27]. The formalin fixed tissues were washed and subjected to dehydration in ascending grades of alcohol. The tissues were blocked in paraffin wax, sectioned into ribbon and fixed on glass slides. After staining and destaining with hematoxylin and eosin. They were permanently mounted with DPX mount for histopathological studies.

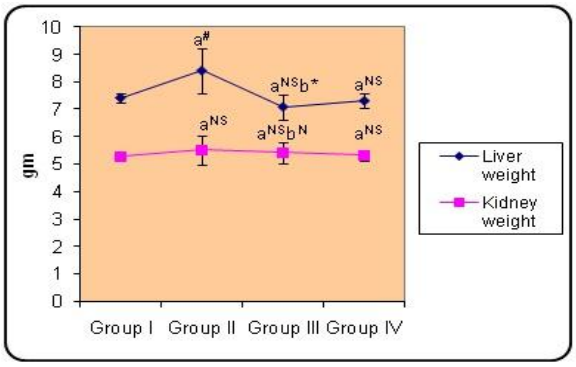
Statistical Analysis

Data are presented as the mean ± standard deviation (SD). One way analysis of variance (ANOVA) followed by Tukey's multiple comparison method was used to compare the means of different groups of by using SPSS 12.5 student's versions. Comparisons were made between group II and IV with group I and III for animal studies. P <0.05 was considerable statistically significant in all cases.

Results

The body, liver and kidney weight of the control and experimental animals are presented in figure 1. In group II NDEA induced animals, the body weight was significantly decreased (p<0.001) when compared to group I control animals. On the contrary these weight were increased significantly (p<0.001) in group III Diosmin treated animals when compared with group II cancer bearing animals. On the other hand the liver and kidney weight was found to be significantly increased (p<0.01) in group II cancer bearing animals when compared to group I control animals. Ironically the increased liver weight was significantly decreased (p<0.001) in group III diosmin treated animals when compared to group II cancer bearing animals. There is no significant change in the kidney weight in all comparison groups. Whereas there was no significant changes were observed in group IV Diosmin alone treated animals when compared to group I control animals.



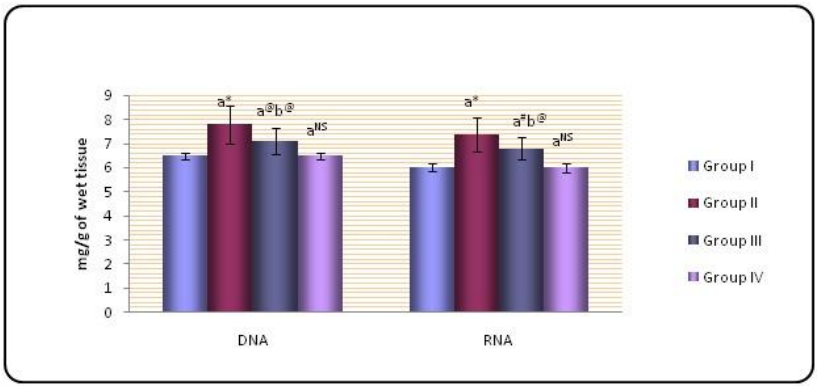


Each Value represents means ± SD of six animals
a – Group II III & IV compared with group I
b – Group III compared with Group II
*p<0.001, #p<0.01, @p<0.05, NSNot significant

Figure 1: Effect of Diosmin on body, liver and kidney weight of control and experimental animals

In the present investigation the levels of nucleic acids (DNA and RNA) in liver of control and experimental animals are presented in figure 2 .In group II animals, the level of DNA were significantly elevated (p<0.001) when compared to group I control animals. On the conversely there was a significant decrease (p<0.05) in the level of DNA was observed in group III Diosmin treated animals, when compared to group II cancer bearing animals. The level of RNA was significantly increased (p<0.001) in group II animals when compared to group I control animals. In contrast there was

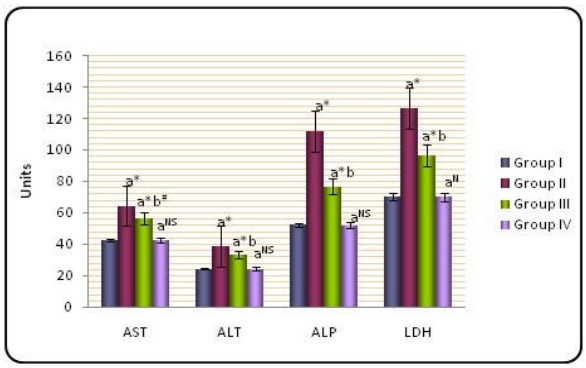
significantly decreased (p<0.05) level of RNA was observed in group III Diosmin treated animals. However, no significant changes were observed in group IV Diosmin alone treated animals when compared to the group I control animals.

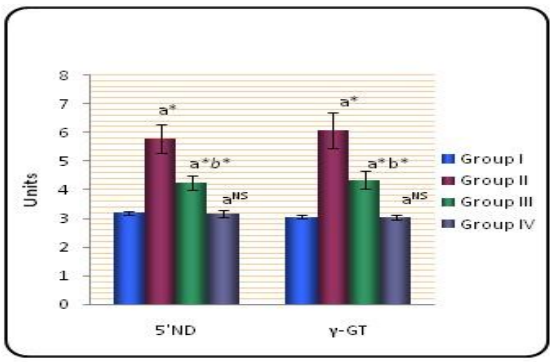


Each Value represents means ± SD of six animals
a – Group II III & IV compared with group I
b – Group III compared with Group II
*p<0.001, #p<0.01, @p<0.05, NSNot significant

Figure 2: Levels of Nucleic acids in liver of control and experimental animals

The effect of diosmin in the levels of marker enzymes in serum of experimental animals was presented in figure 3. In serum there was a significant (p<0.001) increased levels of marker enzymes in serum were observed in group II NDEA induced animals, When compared to group I control animals. Whereas diosmin treated group III animals, all the marker enzymes in serum are significantly decreased (p<0.001), and these level were reverted back to near normal, when compared to group II animals. However, there were no remarkable changes in group IV diosmin alone treated animals when compared with group I control animals.





Units: AST (µ moles of pyruvate liberated/mg protein/min); ALT (µ moles of pyruvate liberated/mg protein/min); ALP (µ moles of p-nitro phenol liberated/mg protein/min); 5'ND (µ moles of inorganic phosphate liberated/mg protein/min); γ-GT (µ moles of p-nitro aniline formed/mg protein/min); LDH (µ moles of pyruvate liberated/mg protein/min)

Each Value represents means ± SD of six animals
a – Group II III & IV compared with group I
b – Group III compared with Group II
*p<0.001, #p<0.01, @p<0.05, NSNot significant

Figure 3: Level of Tumor Marker Enzymes in serum of control and experimental animals

The level of marker enzymes in liver of control and experimental animals are presented in table 1. In liver the group II cancer bearing animals, the activities of marker enzymes were decreased significantly ($p<0.001$). On the contrary, in diosmin treated group III animals, marker enzymes level ($p<0.001$) were increased significantly ($p<0.01$). However, there was no significant change in the parameters observed in group IV drug control animals when compared with group I control animals.

Table 1: Level of tumor marker enzymes in Liver of Control and Experimental animals

Parameters	Group I (Control)	Group II (DEN)	Group III (DEN + Diosmin)	Group IV (Diosmin)
AST (µ moles of pyruvate liberated/mg protein/min)	34.00 ± 0.58	22.96 ± 2.51 ^{a*}	29.59 ± 1.91 ^{a*b*}	33.73 ± 1.23 ^{aNS}
ALT (µ moles of pyruvate liberated/mg protein/min)	11.00 ± 0.27	7.11 ± 0.84 ^{a*}	9.35 ± 0.66 ^{a*b*}	11.37 ± 0.44 ^{aNS}
ALP (µ moles of p-nitro phenol liberated/mg protein/min)	1.17 ± 0.02	1.41 ± 0.13 ^{a*}	1.27 ± 0.08 $^{a@b*}$	1.16 ± 0.04 ^{aNS}
5'ND (µ moles of inorganic phosphate liberated/mg protein/min)	3.53 ± 0.06	4.36 ± 0.44 ^{a*}	3.03 ± 0.20 $^{a\#b*}$	3.28 ± 0.13 ^{aNS}
γ-GT (µ moles of p-nitro aniline formed/mg protein/min)	3.60 ± 0.07	5.17 ± 0.52 ^{a*}	4.42 ± 0.32 $^{a*b\#}$	3.57 ± 0.14 ^{aNS}
LDH (µ moles of pyruvate liberated/mg protein/min)	4.19 ± 0.10	5.59 ± 0.65 ^{a*}	5.11 ± 0.35 $^{a\#b@}$	4.17 ± 0.18 ^{aNS}

Each value represents means ± SD of six animals
a – Group II, III & IV compared with Group I
b – Group III compared with Group II
$^{*}p<0.001$, $^{\#}p<0.01$, $^{@}p<0.05$, ^{NS}- Not Significant

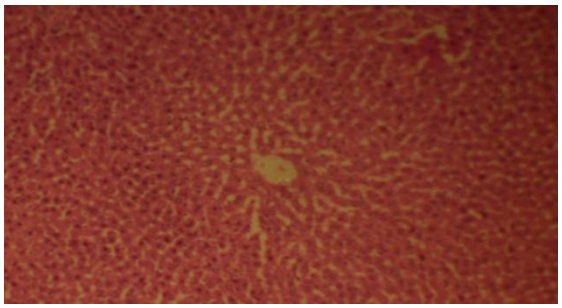
The results of the effect of diosmin on biotransfomation enzymes such as cytochrome $P_{450,}$ cytochrome b_5 , NADPH cytochrome C reductae, GST and UDPGT of liver microsomes in control and experimental animals are presented in table 2. In group II cancer bearing animals, the levels of phase I biotransformation enzymes were significantly decreased ($p<0.001$) when compared to the group I control animals. On the other hand, Phase II biotransformation enzymes were significantly increased ($p<0.001$) in group II cancer bearing animals. Ironically a significant increase ($p<0.05$) in Phase I enzymes. The phase II enzymes such as GST was concomitant decrease ($p<0.001$) and the level of UDPGT was also significantly decreased ($p<0.01$) when compared to group II animals. However, there was no significant changes in the group IV diosmin alone treated animals when compared to group I control animals.

Histopathology studies on liver control and experimental animals are presented in figure 4. Group I control animals indicated the structural unit of the liver is the normal hepatic lobule which is made up of glowing plate's cords or strands of cells forming a complex around a central vein. Group II NDEA induced hepatocellular carcinoma bearing rats display alterations in histological appearance of liver. It showed fatty degeneration with displacement of the nucleus Hydropic deterioration was also seen in harsh wounded hepatocytes. It also depict severe hepatocellular necrosis with the adjacent liver cells. Group III primary liver cancer rats treated with diosmin illustrate the damaged liver architecture was altered, necrosis healed and the cellular disintegration was found to be inferior. Group IV rats treated with diosmin alone showed the normal histological appearance of liver cells additional establish its nontoxic property.

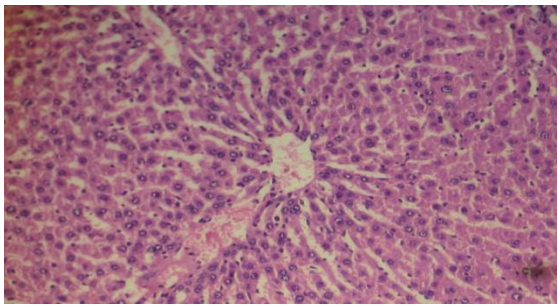
Table 2: Activities of Phase I & II biotransformation enzymes in Liver Microsomes of Control and Experimental animals

Parameters	Group I (Control)	Group II (DEN)	Group III (DEN + Diosmin)	Group IV (Diosmin)
Phase I Enzyme (Cytochrome P450 n moles/mg microsomal protein/min	0.93 ± 0.02	0.15 ± 0.05 ^{a*}	0.83 ± 0.06 $^{a\#b*}$	0.90 ± 0.03 ^{aNS}
Cytochrome b5 (n moles/mg microsomal protein/min)	0.63 ± 0.01	0.21 ± 0.02 ^{a*}	0.46 ± 0.03 ^{a*b*}	0.65 ± 0.02 ^{aNS}
NADPH Cytochrome 'C' reductase (n moles/mg microsomal protein/min)	16.33 ± 0.36	12.09 ± 1.22 ^{a*}	13.31 ± 0.90 $^{a*b@}$	17.01 ± 0.55 ^{aNS}
Phase II Enzyme Glutathione-S-transferase (n moles/mg microsomal protein/min)	1.51 ± 0.03	3.87 ± 0.41 ^{a*}	2.45 ± 0.17 ^{a*b*}	1.42 ± 0.05 ^{aNS}
UDP Glucronyl transferase (n mol/mg microsomal protein/min)	32.2 ± 0.62	47.18 ± 4.79 ^{a*}	41.71 ± 2.38 $^{a*b\#}$	33.71 ± 1.19 ^{aNS}

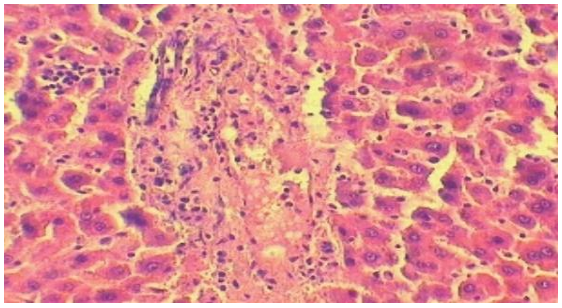
Each value represents means ± SD of six animals
a – Group II, III & IV compared with Group I
b – Group III compared with Group II
*p<0.001, $^{\#}$p<0.01, $^{@}$p<0.05, ^{NS}- Not Significant



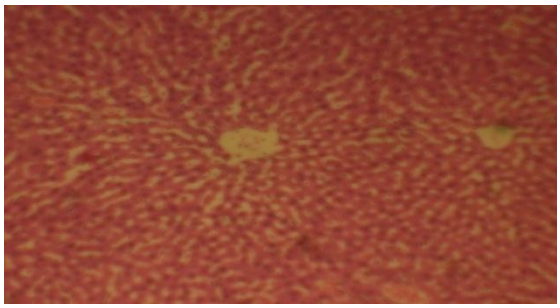
Group I - Control



Group III - NDEA + Diosmin



Group II - NDEA



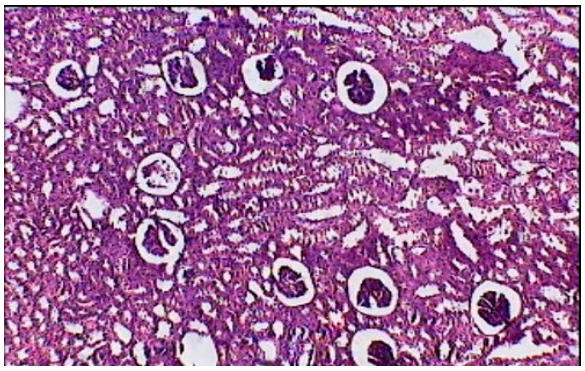
Group IV - Diosmin alone

Group I : Control animal shows normal hepatocytes and control vein.
Group II : Shows loss of architecture and focal area necrosis, with hepatocytes showing nuclear changes.

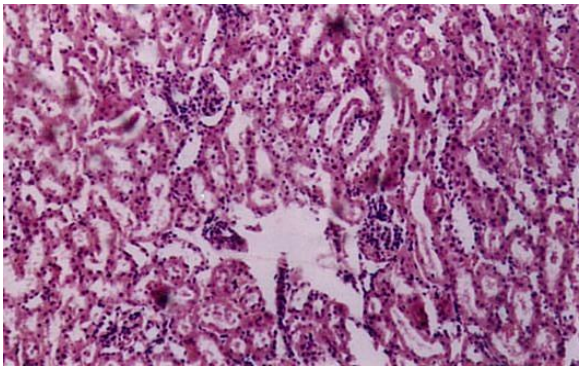
Group III : Diosmin treated shows sinusoidal dilation, lymphatic infiltration and normal hepatocytes.
Group IV : Drug control animal shows mononuclear cells and vein, hepatocytes with nuclei.

Figure 4: Histopathological sections of liver of control and experimental animals stained with hematoxylin and eosin

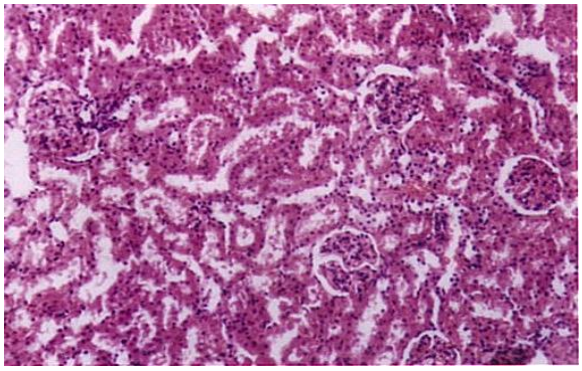
Figure 5 depicts histopathological examination of kidney of control and experimental animals. Group I Control rats showed normal architecture of kidney. Cancer bearing group II animals showed tubular epithelial damage and congested glomeruli due to NDEA administration. Diosmin treated group III animals showed recovering tubules with less congested glomeruli because of its therapeutic potential. Drug control group IV animals showed normal structural arrangements of kidney.



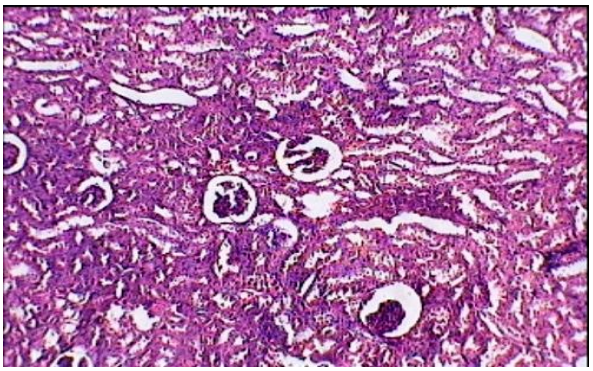
Group I – Control



Group III - NDEA + Diosmin



Group II - NDEA



Group IV - Diosmin alone

Group I : Animals showed normal architecture.
Group II : Cancer bearing animals showed tubular epithelial damage and congested glomeruli.
Group III : Diosmin treated animals shows recovering tubules with less congested glomeruli.
Group IV : Drug control animals showed almost normal architecture.

Figure 5: Histopathological sections of kidney of control and experimental animals stained with hematoxylin and eosin

Discussion

The considerable tumor progression and significant body weight loss were observed in NDEA induced rats. This may be due to decreased food intake and/or absorption efficiency of food in growing ascitic hepatoma bearing rats. In cancerous condition, the tissue level of antioxidant significantly decreased. Ultimately, it was also reported that an increased level lipidperoxidation (LPO), therefore its plays a

major role in the initiation of tumor development [28]. In addition to this, an increase in the liver, kidney weight and a regression in the body weight of tumor bearing animal show the severity of carcinogenesis in NDEA induced HCC rats [29], which may be due to the protein degradation during tumor growth. Protein metabolic perturbations in the host, although causing tissue waste may themselves favour the growth of tumor itself [30]. Antioxidant act as radical scavenger and inhibit LPO and other free radical mediated processes, thereby protecting the human body from various diseases. Administration of diosmin showed and steadily increase in the body weight of diosmin treated rats, this may be due to suppression of LPO by the way of increasing intra cellular antioxidant levels, and also up-regulation of food intake and absorption which eventually resulting in change in energy metabolism.

The diagnosis of cancer cells is based on a variety of factors related to nuclear atypia, such as increase in the nuclear to cytoplasmic ratio, variation in nuclear size, increase in the amount and irregular distribution of chromatin and increases in the amounts of nuclear deoxyribonucleic acid (DNA), ribonucleic acid (RNA) and protein resulting from the presence of the abnormal chromosomes of cancer cells [31]. Cancer arises as a result of genetic alterations leading to the activation of proto-oncogenes or inactivation of tumor suppressor genes, resulting in changes in the normal control of cell growth and differentiation. N-Nitrosodiethylamine is reported to generate reactive oxygen species that leads to an oxidative stress [32]. The interruptions in uracil nucleotide dependent biosynthesis of nucleic acid result in DNA damage. DNA content was meaningful with regard to biological and functional aspects of the tumor, as it is index of proliferative activity in tumor condition [33]. The RNA level in HCC bearing animals were also increased but not as significant as DNA In this study treatment with Diosmin probably prevented NDEA induced UTP depletion and a subsequent suppression of nucleic acids biosynthesis by restricting the formation of toxic metabolites from NDEA.

Hepatic cell participate in a variety of metabolic activities and contain a host of enzymes. In tissues, the marker enzymes are found in higher concentrations in cytoplasam and also exeunt in other organelles. Tumor marker enzymes analysis can be used as an indicator of tumor response to therapy. More sensitive and specific liver cancer marker enzymes are lactate dehydrogenase, aminotransferases, alkaline phosphatase and γ-glutamyltranspeptidase were used as indicators of liver injury [34]. In liver injury the transport function of the hepatocytes is disturbed, resulting in the leakage of plasma membrane, thereby causing an increased enzyme levels in serum.The marker enzymes such as AST, ALT, ALP, gamma GT and 5′ ND are the most sensitive markers reported in the diagnosis of hepatic damage because these are cytoplasmic in location and are released into the circulation after cellular damage [35]. The increase in serum activities of enzymes is indicative of cellular leakage and loss of functional integrity of cell membranes in liver. Previously the number of workers reported that increased serum marker enzymes well known diagnostic tool of NDEA induced HCC [36]. In the present investigation treatment with diosmin significantly reduced the activities of the above marker enzymes in group III treated rats. This indicates that Diosmin tends to prevent liver damage by maintaining the integrity of the plasma membrane, thereby suppressing the leakage of enzymes through membranes, exhibiting hepatoprotective activity. This might be the reason for the restoration in the activities of the marker enzymes during administration of Diosmin.

The body's first lines of defense against cancer are the Phase I and Phase II enzymes. These two families of enzymes are central to the body's ability to protect itself from all manner of carcinogens that routinely enter through the diet and the environment. Phase I and Phase II enzymes are capable of entering into a wide variety of chemical reactions because they encounter an enormous range of natural and man-made chemicals. Drug biotransformation is one of the most important factors that can affect the overall therapeutic and toxic profile of a drug. It can lead to detoxification and excretion of the drug, but also to bioactivation. For this reason, drug biotransformation is a pivotal factor in the early developmental stage of new drugs. Biotransformation occurs in many tissues, and the liver as the most important organ, but also the kidneys, skin, lungs, and intestine can be involved. Drug biotransformation is divided into two types of reactions, namely phase I (hydrolysis, oxidation, and reduction) and phase II reactions (conjugation) [37]. Thus the estimation of biotransformation enzymes such as Phase I and Phase II metabolizing enzymes are important in NDEA induced liver cancer. Cytochrome P_{450} plays main role in the metabolism of numerous classes of compounds, including drugs, carcinogens, and pesticides, as well as natural products. Cytochrome b_5 is involved in the Cytochrome P_{450} mediated transformation through the electron donation by NADH via Cytochrome b_5

reductase. The NADPH cytochrome reductase is involved in the metabolism of carcinogen and facilitated the microsomal peroxidation. In this study the decreased level of these enzymes was observed in cancer bearing animals. This inhibition may be due to alteration in the metabolism of NDEA. A significant increase in the action of these phase I enzymes was observed after the treatment of diosmin.

In phase II enzyme glutathione-S-transferase (GST) is a soluble protein located in cytosol and plays an important role in detoxification and excretion of xenobiotics. GST catalyzes the conjugation of the thiol functional groups of GSH to electrophilic xenobiotics and results in increasing solubility. The xenobiotic–GSH conjugate is then either eliminated or converted to mercapturic acid [38]. This enzyme catalyses the conjugation of reactive electrophilic moieties (generated by phase I enzyme systems) with the thiol group of reduced glutathione, these metabolites are thus blocked from interaction with nucleic acids and other nucleophilic sites as they are excreted as nontoxic conjugates [39]. Thus, the Diosmin increase the glutathione S-transferase activity, observed in this study and this may offer substantial protection to animals in which carcinogenicity and/or genotoxicity are mediated by electrophilic metabolities of xenobiotics. Enhancement of glutathione-S-transferase enzyme activity and a concurrent reduction in carcinogenic response has been reported by a number of workers [40]. Hepatic microsomal UDP-GT is known to be important preneoplastic and neoplastic markers to evaluate the extent of free radical damage caused by exposure to various carcinogens. It catalyse the transfer of glucuronic acid from UDP- glucuronic acid to phenol, hydroxylamines, carboxylic acid etc. These enzymes increased levels was observed in group II animals. It may be due to the synthesis of glucuronides by microsomal UDP-glucuronyl transferase is the major pathway for inactivation and excretion of both endogenous and xenobiotic organic compounds [41]. The induction of these phase 2 enzymes (UDP-GT & GST) by diosmin probably contributed to their protective effects on NDEA induced carcinogenesis by increasing the detoxifying capacity of the liver.

Conclusion

In conclusion, the present study display that diosmin have the chemotherapeutic efficacy against N-Nitrosodiethylamine induced liver carcinogenesis. Diosmin proved its therapeutic efficiency by restoring the structural modifications and biochemical enzymes damaged by NDEA.

References

1. Wang XW., Hussain SP., Huo TI., Wu CG., Forgues M., Hofseth LJ., Brechot C., Harris CC., Molecular pathogenesis of human hepatocellular carcinoma, Toxicology, 181-182, 43–7 **(2002)**

2. Bansal AK., Bansal M., Soni G., Bhatnagar D., Modulation of N-nitrosodiethylamine (NDEA) induced oxidative stress by vitamin E in rat erythrocytes, Hum Exp Toxicol, 24(6), 297-302 **(2005)**

3. Jakszyn P., Gonzalez CA., Nitrosamine and related food intake and gastric and oesophageal cancer risk: A systematic review of the epidemiological evidence, World J Gastroenterol, 12(27), 4296–303 **(2006)**

4. Bhosale P., Motiwale L., Ingle AD., Grade RV., Roa KVK., Protective effect of rhodotorula glutinis NC7M 3353 on the development of hepatic preneoplastic lesions, Current Science, 83, 303-308 **(2002)**

5. Sivalokanathan S., Ilayaraja M., Balasubramanian MP., Efficacy of Terminalia arjuna (Roxb.) on N-nitrosodiethylamine induced hepatocellular carcinoma in rats, Indian J Exp Biol, 43(3), 264-7 **(2005)**

6. Ramelet AA., Pharmacologic aspects of a phlebotropic drug in CVI-associated edema, Angiology, 51(1), 19-23 **(2000)**

7. Bergan JJ., Schmid-Schönbein GW., Takase S., Therapeutic approach to chronic venous insufficiency and its complications: place of Daflon 500 mg. Angiology, 52(Suppl 1), S43-7**(2001)**

8. Heusser J., Osswald W., Toxicological properties of diosmin and its action on the isolated venous tissue of the dog, Arch Farmacol Toxicol, 1, 33-40 **(1977)**

9. Geren RI., Greenberg NH., Mac Donald MM., Schumacher AM., Abbott BJ., Protocols for screening chemicals agents and natural products against animal tumours and other biological systems, Cancer Chemother Rep, 3, 1-103 **(1972)**

10. Schneider WC., Determination of nucleic acids in tissues by pentose analysis, In Methods in Enzymology, Colowick SP., Kaplan, NO (Eds) Academic Press New York, 3, 680-684 **(1957)**

11. Burton K., A study of the conditions and mechanism of the diphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid, Biochem J., 62, 315-323 **(1956)**

12. Rawal UM., Patel US., Rao GN., Desai RR., Clinical and biochemical studies on cateractous human lens III: Quantitative study of protein, RNA and DNA, Arogya J. Health Sci., 3, 69-72 **(1977)**

13. King J., The phosphohydrolases- acid and alkaline phosphatases, In: Practical Clinical Enzymologist, Van D. (Ed). Nostrand Company Limited London, 191-208 **(1965)**

14. Bergmeyer MU., Steroid dehydogenase, In methods of Enzymatic Analysis, Ed. HU Bergmeyer Academic Press New York, 476-477.

15. Balasubramanian MP., Dhandayuthapani S., Nellaiappan K., Ramalingam K., Comparative studies on phosphomonoesterase in helminthes, Helminthologia, 20,111-120 **(1983)**

16. Rosalki SB., Rau D., Serum gamma-glutamyl transpeptidase activity in alcoholism, Clin Chim Acta, 39(1), 41-47**(1972)**

17. King J., The transferases alanine and aspartate tranaminases, In: Practical Clinical Enzymology, Van D. (Ed.), Nostrand Company Limited London, 121-138 **(1965)**

18. Luly P., Barnabei O., Tria E. Hormonal control In vitro of plasma membrane bound (Na^+- K^+)-ATPase of rat liver, Biochim Biophys Acta, 282(1), 447-452 **(1972)**

19. Boyd MR., Burka LT., In viva studies on the relationship between target organ alkylation and the pulmonary toxicity of a chemically reactive metabolite of 4-ipomeanol, J. Pharmacol Exp. Ther, 207(3), 687-697 **(1978).**

20. Kamath SA., Narayanan KA., Iteraction of Ca^{2+} with endoplasmic reticulum of rat liver, A standardized procedure for the isolation of rat liver microsomes, Anal Biochem., 48(1), 53-61**(1972)**

21. Omura T., Sato R., The carbon monoxide binding pigment of liver microsomes, J. BiolChem., 239, 2370-2378 **(1964)**

22. Phillips AH., Langdon RG., Hepatic triphosphopyridine nucleotide-Cytochrome c Reductase: Isolation, characterization and kinetic studies, J. Biol Chem., 237, 2652-2660 **(1962)**

23. Strittmatter P., Verlick SF., A microsomal cytochrome reductase specific for diphosphopyridine nucleotide, J. Biol Chem., 221(1), 277-286 **(1956)**

24. Habig W.H., Pabst M.J., Jakoby W.B., Giutathione S-transferases, The first enzymatic step in mercapturic acid formation, J. Biol Chem., 249(22), 7130-7139 **(1974)**

25. Isselbacher K.J., Chrabas M.F., Quinn RC., The solubilization and partial purification of a glucuronyl transferase from rabbit liver microsomes, J. Biol Chem., 237, 3033-6 **(1962)**

26. Hollman S., Touster O., Alterations in tissue levels of UDP-glucose Dehydrogenase, UDP-glucurnic acid pyrophosphatase and glucuronyl transferase induced by substances influencing the production of ascorbic acid, Biochem Biophys Acta, 62, 338-352 **(1962)**

27. Bancroft J.D., Cook H.C., Manual of Histological Techniques, Churchill Livingstone Edinburgh, 19-99 **(1984)**

28. Rice-evans CA., Bourdon R., Free radical lipid interaction and their pathological consequences, Progressive Lipid Research, 12, 71-110 **(1993)**

29. Zhao J.A., Peng L., Geng C.Z., Liu Y.P., Wang X., Yang H.C., Wang S.J., Preventive Effect of Hydrazinocurcumin on Carcinogenesis of Diethylnitrosamine-induced Hepatocarcinoma in Male SD Rats, Asian Pac J Cancer Prev**,** 15(5), 2115-21 **(2014)**

30. Tessitore L., Costelli P., Bonetti G., Baccino FM., Cancer cachexia, malnutrition, and tissue protein turnover in experimental animals, Arch Biochem Biophys, 306(1), 52-8**(1993)**

31. Evans MD., Dizdaroglu M., Cooke MS., Oxidative DNA damage and disease: induction, repair and significance, Mutat Res, 567(1), 1–61**(2004)**

32. Pakkir Maideen NM., Velayutham R., Manavalan G., Role of Prosopis cineraria against N-nitrosodiethylamine-induced liver tumor in rats with reference to marker enzymes and nucleic acid contents, Bangladesh J, Pharmacol., 6, 128-132**(2011)**

33. Pinlaor S., Yongvanit P., Hiraku Y., Ma N., Semba R., Oikawa S., Murata M., Sripa B., Sithithaworn P., Kawanishi S., 8-Nitroguanine formation in the liver of hamsters infected with Opisthorchis viverrini, Biochem Biophys Res Commun., 309, 567–571**(2003)**

34. Bose CH., Mukherjee M., Enzymatic tumor markers in ovarian cancer: A multiparametric study, Cancer Lett., 77, 39-43**(1994)**

35. Sallie R., Tredger J.M., Willam R., Drugs and the liver. Biopharm Drug Dispos, 12, 251–259**(1991)**

36. Sivalokanathan S., Ilayaraja M., Balasubramanian M.P., Antioxidant activity of *Terminalia arjuna* bark extract on N-nitrosodiethylamine induced hepatocellular carcinoma in rats, Mol Cell Biochem., 281(1-2), 87-93 **(2006)**

37. Lu F.C., Biotransformation of toxicants, in: Basic Toxicology: Fundamentals, Target Organs and Risk Assessment, third Eds, Taylor and Francis Washington DC, pp. 27–39**(1996)**

38. Rao G.M., Rao C.V., Pushpangadan P., Shirwaikar A., Hepatoprotective effects of rubiadin, a major constituent of Rubia cordifolia Linn. J Ethnopharmacol, 103(3), 484-90**(2006)**

39. Bansal A.K., Bansal M., Soni G., Bhatnagar D., Protective role of vitamin E pretreatment on N-nitrosodiethylamine induced oxidative stress in rat liver, Chem Biol Interact., 156(2-3), 101 - 111**(2005)**

40. Tsuda H., Ohshima Y., Nomoto H., Fujita K., Matsuda E., Iigo M., Takasuka N., Moore MA., Cancer prevention by natural compounds, Drug Metab Pharmacokinet, 19, 245-63**(2004)**

41. Kunz H.W., Buchmann A., Schwarz M., Schmitt R., Kuhlmann W.D., Wolf C.R., Oesch F., Expression and inducibility of drug-metabolizing enzymes in preneoplastic and neoplastic lesions of rat liver during nitrosamine-induced hepatocarcinogenesis, Arch Toxicol, 60, 198-203**(1987)**