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RESEARCH ARTICLE

Diosmin anti-tumour efficacious against Hepatocellular Carcinoma

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ABSTRACT:

The present study absorbed the anti-cancer activity of diosmin at 50, 100, 150, 200 µM/ml for 24h of treatment on dose dependent manner. Group II animals were administrated 0.01% NDEA to induce primary liver carcinoma. Cancer bearing experimental animals were treated orally with the drug diosmin at the dosage level of 200 mg per kg/bodyweight for 28 successive days. **Results:** Diosmin treatment on HepG2 cells constrains nearly half of the cell population and proliferation at the dose of 100 µM/ml and *in vivo* experimental studies showed remarkable repossession in biochemical and morphological characteristics. These results summarizes that both *in vivo* and *in vitro* anti-carcinogenic activity of diosmin could be an outstanding flavonoid in the treatment of HCC because of its therapeutic and pharmacological properties.

KEYWORDS: Diosmin, Flavonoids, N-nitrosodiethylamine, SEM, HCC, HepG2 cells

1. INTRODUCTION:

Liver cancer arises in the hepatocytes and is the common and fatal amongst altogether other types of cancer [1]. The incidence rate of the liver cancer is twofold higher in developing countries compared to developed countries [2]. Flavonoids are natural materials in plants that are believed to have progressive effects on human wellness. Numerous therapeutic properties have been recognized to flavonoids, particularly for their anti-carcinogenic, anti-oxidant, and anti-inflammatory properties [3,4]. In contemporary days, flavonoids as strong free radical scavengers have concerned a remarkable attention as promising therapeutics against free radical facilitated ailments [5]. Citrus flavonoid has been reported to have a wide-ranging of biological action with anti-cancer activities. In addition, it has healthiness related possessions, which including medicinal effects against many viral pathogens, favourable effects against inflammatory, effects on capillary fragility and human platelet aggregation stoppage potential [6].

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In this juncture, as a flavonoid diosmin derives to play a substantial part to extravagance numerous ailments. Diosmin is the chief constituent of *Rutaceae* species explored for additional beneficial purposes comprising anti-cancer, treating premenstrual condition, diabetes, and colitis [7,8].

2. MATERIALS AND METHODS:

2.1 *In Vitro* Investigation:

2.1.1 Cell culture:

Liver cancer cell line (HepG2) was procured from NCCS, Pune, India. Human hepatoma cells were periodically grown at 37°C as monolayer cultures in a humidified condition of 5% CO_2 in 95% O_2 in Dulbecco Modified Eagles Medium comprising 50 IU/ml of antibiotic penicillin and 50 µg/ml of streptomycin.10% (v/v) with FBS (Fetal Bovine Serum).

2.1.2 Chemicals:

Diosmin, was purchased from Sigma chemicals, DMEM medium and sodium pyruvate were bought from Biochrome, Germany. Penicillin, streptomycin and fetal bovine serum were acquired from Gibco, Germany. Trypsin-EDTA was acquired from Hi-media, India. Culture plates were purchase from TPP, Switzerland.

2.1.3 Cytotoxicity Assays:
Cell sustainability was assessed by MTT method [9]. Lactate dehydrogenase (LDH) activity was assessed [10], reduced glutathione was assayed [11].

2.2 *In vivo* Studies:
2.2.1 Chemicals:
N-nitrosodiethylamine were purchased from Sigma Chemical Company, St Louis, MO, U.S.A.

2.2.2 Animals and Dose Fixation:
The experimental protocol was approved by the Institutional Animal Ethics Committee for experimental clearance (IAEC No 07/018/08). Adult healthy male Wistar albino strain rats weighing between 160 ± 20gm were used from the Central Animal House facility of Dr. ALM PGIBMS, University of Madras, Chennai - 600 113, India. Throughout the experimentation period all the ethics guidelines were firmly followed. The dose regimen of diosmin was selected based on LD_{50} was found to be 3000mg/kg body weight [12]. Based on LD_{50}, the sub lethal dose i.e. one third of theLD_{50} (150, 200, and 250 mg/kg body weight) were designated. During the administration of diosmin at the dose of 200 mg/kg b/w did not displayed any irregularities such as toxic, rotating, lacrimation, lowered breathing etc. till end of the study. Based on the acute toxicity studies 200mg/kg b/w of diosmin was elected for the present investigation.

2.2.3 Experimental design:
The rats were divided into four separate sets with six animals in each group. Group I administrated with DMSO as a vehicle at the dose of 1 ml/kg b.w and served as control group. Group II animals were induced cancer in hepatocytes by administrating 0.01% NDEA through consumption of water for the duration of 16 weeks. Group III animals HCC rats subsequently treated with diosmin at the dose of 200 mg/kg/b.w orally for 28 successive period. Group IV animals were given diosmin alone at the dose as same as the previous group for 28 days.

2.2.4 Collection of samples:
Animals were anaesthetized at the end of the experimental period, and the blood was collected and serum was parted by centrifugation. The liver and kidney tissues were removed, washed in ice-cold saline. Using 0.1 M Tris–HCl buffer (pH 7.4), a 10% of the liver homogenate and kidney homogenate tissue were prepared for further biochemical investigation.

2.2.5 Biochemical parameters:
Estimating the superoxide dismutase activity [13], estimation of catalase activity [14], glutathione analysis was done [15], ascorbic acid estimation [16], vitamin E was assessed [17], Na^+K^+-ATPase estimation [18], Ca^{2+}-ATPase was examined [19], evaluation of Mg^{2+}-ATPase activity [20], assessment of isocitrate dehydrogenase [21] analysis of succinate dehydrogenase [22], Malate dehydrogenase assayed [23], estimation of α-ketoglutarate dehydrogenase [24], assessment of hexokinase was carried out [25], Phosphoglucoisomerase estimation [26], Aldolase was estimated [27], Glucose-6-phosphatease and Fructose-1,6-diphosphatase was assayed [28], hexose estimation [29], hexosamine activity was appraised [30], The level of hexosamine was estimated [31].

2.2.6 Scanning electron microscopic examination of liver Tissues:
Samples were fixed with modified Karnovosky's fluid 19 and 0.1 M sodium phosphate buffer at pH 7.4 was added. Fixation was performed for 10-18 h at 4°C, subsequently in fresh buffer the tissue was washed. 1% osmium tetroxide with the 0.1 M sodium phosphate buffer at 4°C the post fixation was done. After several washes, the specimens were dehydrated. Samples were additionally dried, and then the tissue samples were fixed on the aluminium stumps, and gold coating about 35 nm thicknesses of the tissue samples was attained. Finally, the samples were examined under scanning electron microscope.

3. RESULTS:
The results of the viability of control and diosmin treated (50, 100,150 and 200 μM/ml) HepG2 cells are presented in the figure 1. In this present investigation, the diosmin remarkably inhibits the hepatoma cell line (HepG2) after 24 hours of treatment. From this result, it is inferred that diosmin treatment showed marked inhibition of HepG2 cells viability in a concentration basis method. Cytosolic leakage of LDH enzymes, as a sign of cytotoxicity. The lactate dehydrogenase (LDH) levels released into the medium of control and diosmin treated (50, 100, 150 and 200 μM/ml) HepG2 cells are presented in figure 2. From this study, it was observed that LDH activities establish to be considerably elevated after 24 hours of exposure in the medium incorporated with diosmin. Glutathione is a universal molecule that plays a significant role in cellular free radical metabolism and xenobiotic detoxification mechanism. The GSH levels of normal and diosmin treated HepG2 cells were displayed in figure 3. From this present investigation, it is concluded that a substantial reduction of GSH was observed in diosmin treated HepG2 cells in a dose dependent manner.

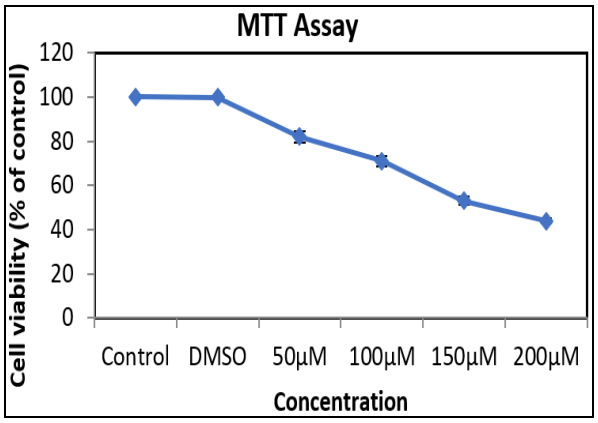

Figure 1 Effect of diosmin on HepG2 cells for 24hr –MTT Assay

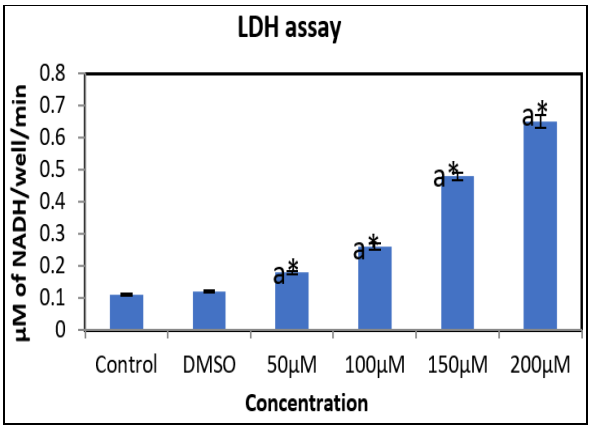

Figure 2 Level of LDH in control and Diosmin treated HepG2 cells

Each Bar represents mean ±SD of six observations. a - Control Vs DMSO, 50, 100, 150, and 200 µM 24 h, a* - p<0.05 Vs Control

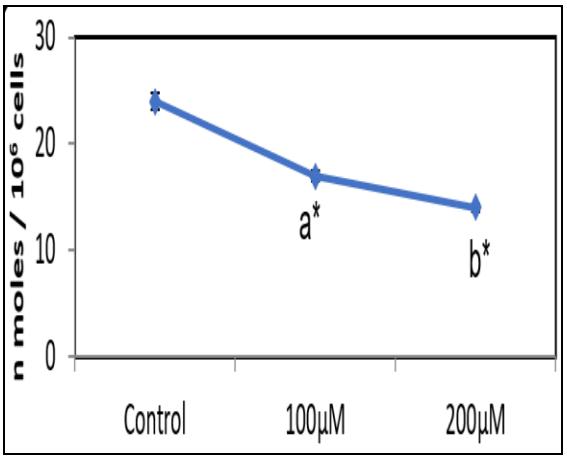

Figure 3 Level of GSH in control and Diosmin treated HepG2 cells

a – Group II compared with Group I, b – Group III compared with Group II, p<0.05

Living tissues are accomplished with antioxidant defence mechanisms encompassing enzymatic and non-enzymatic antioxidants. A reduction in the activities of these antioxidant enzymes relate to the deposit of enormously reactive free radicals and significant destructive toxic effects on cell membranes, macromolecules and their functions. In this association, the importance of diosmin on the antioxidant enzymes levels were estimated from the serum, liver and kidney were presented in Figure 4 and Tables 1 and 2 correspondingly. The NDEA induced cancer bearing animals showed a noteworthy diminution of enzymic antioxidants such as CAT, SOD, GP_X ($p<0.001$) and non-enzymic antioxidants such as Vit-C GSH and Vit-E ($p<0.001$). These antioxidants enzymes activities were considerably amplified ($p<0.01$) in drug treated rats, when compared to animals with cancer. The activities of antioxidants ($p<0.001$) in liver and kidney of cancer induced animals is noticeably decreased. These antioxidant ($p<0.001$) were significantly increased ($p<0.01$) when compared to group II animals. No such distinguished deviations were noticed in drug control group IV animals

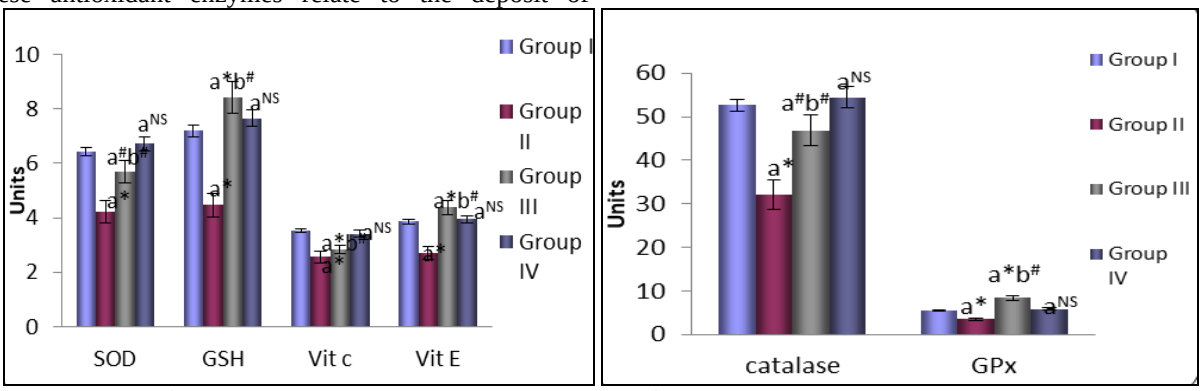

Figure 4 The Level of Enzymic and Non-enzymic Antioxidants in Serum of Control and Experimental animals

Each value represents means ± SD of six animals. Units: SOD - units/mg protein; CAT - µ moles of H_2O_2 consumed/mg protein/min; GPX - µg of GSH utilised/mg protein/min; GSH - µg of GSH/mg protein/min; Vit C – mg/dL; Vit E – mg/dL. a – Group II, III and IV compared with Group I; b – Group III compared with Group II. *p<0.001, $^{\#}$p<0.01, $^{@}$p<0.05, ^{NS}- Not Significant

Table 1 The Level of Enzymic and Non-enzymic Antioxidants in Liver of Control and Experimental animals

Parameters	Group I (Control)	Group II (DEN)	Group III (DEN + Diosmin)	Group IV (Diosmin)
SOD IU/mg protein/min	14.2 ± 0.35	7.61 ± 0.79 ^{a*}	8.92 ± 0.56$^{a@b@}$	14.28 ± 0.57^{aNS}
Catalase (µ mol of H_2O_2 consumed/mg protein/min	64.4 ± 1.87	44.91 ± 4.87 ^{a*}	51.72 ± 3.8 $^{a*b\#}$	63.97 ± 2.6^{aNS}
GPx (µg of GSH utilized/mg protein/min)	10.22 ± 0.2	7.78 ± 0.75 ^{a*}	8.95 ± 0.61$^{a\#b\#}$	10.14 ± 0.42^{aNS}
Vitamin – C (mg/dl)	3.33 ± 0.08	2 ± 0.21 ^{a*}	3.03 ± 0.22 $^{a*b\#}$	3.25 ± 0.11^{aNS}
Vitamin – E (mg/dl)	5.09 ± 0.09	3.51 ± 0.31 ^{a*}	4.1 ± 0.26 ^{a*b*}	5.22 ± 0.19^{aNS}
GSH (µg of GSH/mg protein/min)	12.55 ± 0.08	9.2 ± 0.21 ^{a*}	11.11 ± 0.22$^{a\#b*}$	12.28 ± 0.11^{aNS}

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

Table 2 The Level of Enzymic and Non-enzymic Antioxidants in Kidney of Control and Experimental animals

Parameters	Group I (Control)	Group II (DEN)	Group III (DEN + Diosmin)	Group IV (Diosmin)
SOD (IU/mg protein/min)	5.34 ± 0.14	4.41 ± 0.48 ^{a*}	4.92 ± 0.37$^{a@b@}$	5.38 ± 0.27^{aNS}
CAT (µ mol of H_2O_2 consumed/mg protein/min)	46.14 ± 1.80	30.24 ± 3.34 ^{a*}	39.61 ± 2.85 a*b*	46.01 ± 2.37 ^{aNS}
GPx (µg of GSH utilized/mg protein/min)	3.23 ± 0.06	2.69 ± 0.26 ^{a*}	2.94 ± 0.22$^{a@b@}$	3.27 ± 0.14^{aNS}
Vitamin – C (mg/dL)	2.41 ± 0.08	1.52 ± 0.15 ^{a*}	2.18 ± 0.16$^{a@b*}$	2.45 ± 0.11^{aNS}
Vitamin – E (mg/dL)	3.37 ± 0.08	2.21 ± 0.18 ^{a*}	2.62 ± 0.16 ^{a*b*}	3.40 ± 0.11^{aNS}
GSH (µg of GSH/mg protein/min)	2.72 ± 0.08	1.56 ± 0.14 ^{a*}	2.24 ± 0.15 ^{a*b*}	2.81 ± 0.12^{aNS}

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

ATPase's have been referred to as conspicuous energy linked enzymes perceived in all living organisms which supplies metabolic energy. It is also susceptible to oxyradical-linked impairment and lipid peroxidation. The membrane bound ATPase (Ca^{2+}, Na^{+}/K^{+}, and Mg^{2+}) in erythrocyte membrane and in liver tissues were presented in figure 5 and Table 3. A statistically substantial decrease (p<0.001) of Na^{+}/K^{+} and Mg^{2+} ATPase levels were perceived in group II cancer bearing animals, when compared to group I rats. The level of serum and liver Ca^{2+} ATPase was significantly augmented (p<0.001) in group II cancer bearing rats. In contrast the level of Ca^{2+} ATPase was considerably decreased (p<0.001) in diosmin treated group rats. Interestingly, these abnormal levels were brought back to near normal (p<0.001) in diosmin treated animals. Nevertheless, there was no substantial modification were observed in group IV diosmin alone treated rats when equated to the group I control.

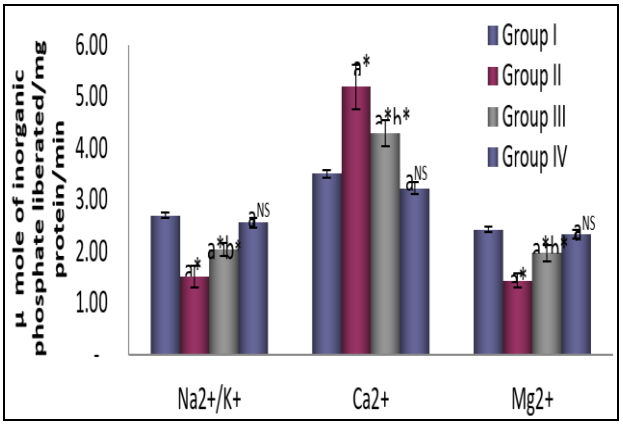

Figure 5 Activities of ATPases in Erythrocyte membrane of Control and Experimental animals

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

Table 3 The level of ATPase in the Liver of Control and Experimental animals

Parameters (µ mol of inorganic phosphate liberated/mg protein/min)	**Group I (Control)**	**Group II (DEN)**	**Group III (DEN + Diosmin)**	**Group IV (Diosmin)**
$Na^{2+/}K^{+}$ ATPase	0.32 ± 0.007	0.19 ± 0.01 ^{a*}	0.25 ± 0.01 ^{a*b*}	0.30 ± 0.01 ^{aNS}
Ca^{2+} ATPase	0.29 ± 0.007	0.54 ± 0.05 ^{a*}	0.39 ± 0.03 ^{a*b*}	0.31 ± 0.01 ^{aNS}
Mg^{2+} ATPase	0.23 ± 0.05	0.12 ± 0.01 ^{a*}	0.17 ± 0.01 ^{a*b*}	0.25 ± 0.01 ^{aNS}

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

Reduction in the activities of TCA cycle enzymes might be owing to the modification in cell structure, morphology and role of mitochondria to endure metabolic alterations and mitochondrial quantity is considerably abridged in malignancy cells. The levels of TCA cycle enzymes in the liver of experimental and control animals was illustrated in figure 6. Substantial decline (p<0.001) in the activities of SDH, ICDH, α-KGDH and MDH enzymes were detected in group II cancer animals, when compared to control group I. On the contrary, the levels of SDH, ICDH, α-KGDH and MDH were expressively increased (p<0.001) in diosmin treated animals. No evident alteration was detected in diosmin alone treated animals.

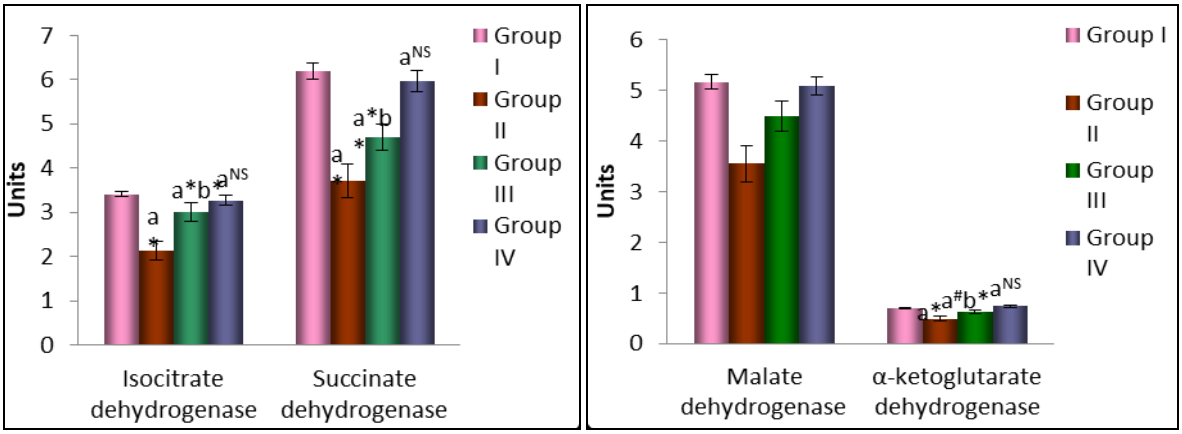

Figure 6 The Level of Mitochondrial TCA Cycle Enzymes in the Liver of Control and Experimental Animals

Each value represents means ± SD of six animals. Units: ICDH – n moles of α-ketoglutarate formed/mg protein/min; SDH – µ moles of succinate oxidised/mg protein/min); MDH – n moles of NADH oxidised/mg protein/min; α-KGDH – µ moles of potassium ferrocyanide liberated/mg protein/min

a – Group II, III and IV compared with Group I; b – Group III compared with Group II *p<0.001, $^{\#}$p<0.01, $^{@}$p<0.05, ^{NS}- Not Significant

In Table 4 the activities of diosmin on carbohydrate metabolizing enzymes in liver of control and experimental animals were presented. The activities of these enzymes such as aldolase hexokinase and phosphoglucoisomerase were significantly (p<0.001) increased. On the other hand, enzymes like glucose -6-phosphatase and fructose-1-6-diphosphatase were reduced (p<0.001) in malignance bearing rats when compared with normal group I rats. Instead, all the above-mentioned enzymes were considerably (p<0.001) reverted to near normal in diosmin treated rats. No remarkable variations were spotted in diosmin treated group IV rats.

Table 4 The level of Carbohydrate metabolizing enzyme in Liver of Control and Experimental animals

Parameters	Group I (Control)	Group II (DEN)	Group III (DEN + Diosmin)	Group IV (Diosmin)
Hexokinase (n mol of glucose-6-phosphate liberated/mg protein/min)	13.79 ± 0.30	27.19 ± 2.96 ^{a*}	19.2 ± 1.46 ^{a*b*}	13.39 ± 0.35^{aNS}
Phospho-glucoisomerase (n mol of fructose liberated/mg protein/min)	26.40 ± 0.72	42.4 ± 3.56 ^{a*}	32.06 ± 2.39 ^{a*b*}	27.38 ± 1.03^{aNS}
Aldolase (n mol of glyceraldehyde liberated/mg protein/min)	21.5 ± 0.42	36.44 ± 3.51 ^{a*}	27.62 ± 1.73 ^{a*b*}	22.8 ± 0.84^{aNS}
Glucose-6-phosphatase (n mol of inorganic phosphate liberated/mg protein/min)	22.37 ± 0.61	14.63 ± 1.57 ^{a*}	18.69 ± 1.28 ^{a*b*}	21.17 ± 0.84^{aNS}
Fructose-1,6-diphosphatase (n mol of fructose diphosphate liberated/mg protein/min)	31.35 ± 0.74	24.36 ± 2.23 ^{a*}	28.3 ± 2.05$^{a\#b*}$	31.84 ± 0.98^{aNS}

Each value represents means ± SD of six animals

a – Group II, III and IV compared with Group I; b – Group III compared with Group II. *p<0.001, $^{\#}$p<0.01, $^{@}$p<0.05, ^{NS}- Not Significant

Elevation of glycoprotein substances are significant indicator of cancerous conditions and these variations modify the rigidity of cell membranes. Tumour cell plasma membrane alteration and their outflow may be the reason for the detected raises of these glycoproteins during uncontrolled cell proliferation condition. The level of the sialic acid hexose and hexosamine in liver of experimental and control groups were depicted in figure 7. The level of sialic acid hexose and hexosamine were found to be augmented considerably in cancer bearing animals (p<0.001). In contrast, the level of glycoproteins was reverted to normal level in diosmin treated animals (p<0.05). There was no noteworthy variation in diosmin alone treated animals.

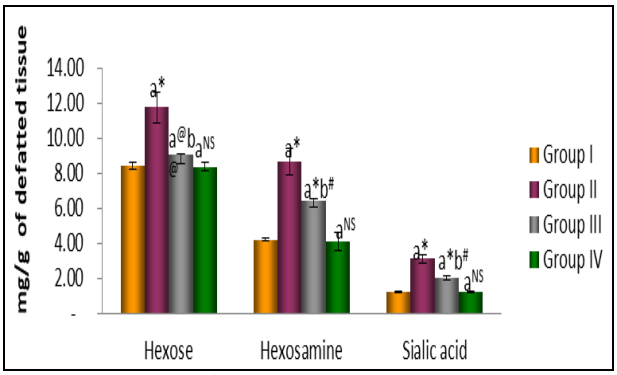

Figure 7 The Levels of Glycoproteins in the Liver of Control and Experimental animals

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

Scanning electron microscopic results were depicted in figure 8. Under microscopic observation, group I control and drug control group IV liver tissues presented normal architecture of hepatocytes with regular nuclei whereas in cancer induced group III exhibited disturbed and abnormal hepatocytes with binucleated and enlargement in the size of the cells. Carcinogen treated liver tissue section presented asymmetrical sinusoids, nuclei were injured cracked hepatocytes and tumour vacuoles. These structural abnormalities were recovered in drug treated liver sections under microscopic examination. SEM analysis displayed morphological restoration of liver tissues in drug treated animals.

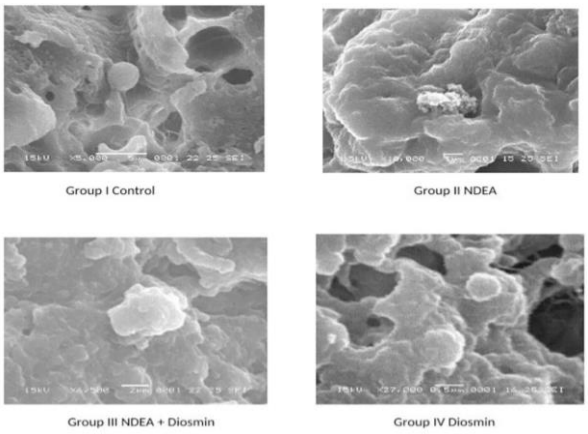

Figure 8: Scanning electron microscopic investigation of in the Liver of Control and Experimental Animals

Group I control and iv Drug control display normal morphological feature of liver cells

Group II exhibit cellular rearrangements and morphology of the hepatic cells due to carcinogen pathological effect

Group III demonstrate the anti-cancer activity of diosmin through recovering the degree of differentiation, structural aberrations and reconstruction of sinusoid

4. DISCUSSION:

In this present investigation, cytotoxic assays like MTT, LDH leakage and GSH was performed to screen diosmin anti-tumour activity on HepG2 cell line. The results of these cytotoxic assays such as MTT, LDH and GSH diosmin showed a remarkable reduction in the cell viability and levels of marker enzymes in dose and time dependent manner [32]. The reduced cell viability and proliferation might be due to anti-cancer and anti-oxidant potential of diosmin. Since it has been reported that pharmacological potential of medicinal plants and their bioactive principles have cytotoxic effect on various cancer cells [33]. Estimation of lactate dehydrogenase (LDH) leakage, an example of intracellular enzyme is a noteworthy parameter in assessing the cytotoxic nature of the drug. The HepG2 cells were treated with diosmin for 24h of time, which display enhanced LDH levels in the medium might be due to medicinal characteristics of the drug. Since LDH is recognised as a notable marker for diseased condition. Reduced glutathione is considered as a non-enzymatic antioxidant involving in plummeting various toxic metabolic products such as peroxides and radicals. The abridged intracellular GSH quantities leads to the development of ROS in cells treated with anti-tumour drugs which in turn leads to the damage of DNA and macromolecules [34]. In this analysis, GSH levels were expressively diminished in diosmin treated human hepatoma cells at the dosage of 100 and 200 μM/ml. Therefore, it is conceivable HepG2 cells with diosmin treatment decline the GSH level and promotes oxidation induction which regulate through programmed cell death or apoptosis.

It has been reported that the levels of SOD were significantly lower in malignant cells and excess formation of radicals [35]. Oxidative stress in cells can be estimated through the levels of these antioxidant enzymes which safeguard the cells and tissues from reactive oxygen species induced cellular damage [36]. In the present investigation, NDEA administration decreased the antioxidant enzymes levels could be credited to the extreme application of these antioxidants in inactivating the free radicals generated during the breakdown of NDEA. Activities of the enzymic antioxidants are reverted to regular status in diosmin treated animals which clearly designates the antioxidant potency of the drug. The non-enzymic scavenger's namely glutathione, ascorbic acid, and α-tocopherol, which scavenge residual free radicals escaping from decomposition [37]. The dropped glutathione in NDEA induced rats signifies the increased consumption of glutathione because of oxidative stress. It is reported that vitamins like E, C has several biological activities such as immunomodulation, and modification of metabolic stimulation of carcinogens [38]. In this study, Vitamins C and E were found to be suggestively declined in malignance condition when compared to control. In contrast, upon treatment with diosmin the levels tend to become usual. From the results of our investigation, diosmin have remarkable characteristic of anti-radical and antioxidant activities.

$Ca2^{+}$-ATPases Mg^{2+}-ATPase and Na^{+}/K^{+}-ATPase responsible for the maintenance of cell structure during diseased condition particularly cancerous stage cell membrane has been disturbed due to irregularity in the levels of membrane bound ATPase. During diethyl nitrosamine administration these enzymes showed decreased activities due to the sensitivity to hydroperoxides and superoxide radicals [39]. In group II animals, because of membrane damage caused by NDEA there was an abnormality in the levels of ATPase. The amount of membrane bound ATPase was found to be pointedly normalised in erythrocyte membrane and hepatocytes of diosmin treated animals could be due to

the membrane stabilizing activity of diosmin by preventing peroxidation of membrane lipids.

The mitochondrial enzymes were disturbed by ROS which ends in uncharacteristic mitochondrial substrate oxidation and cellular energy depletion. There is a massive morphological and functional difference between normal cell and tumour cell mitochondria [40]. In this present study, it was noted that the condensed actions of TCA cycle key enzymes in the liver of tumour bearing animals due to modifications in malignancy cell morphology and mitochondria undergo metabolic variations and the quantity of mitochondria tremendously condensed in cancer cells. Upon treatment with diosmin the mitochondrial abnormalities were reverted near normal due to anti-tumour property of the bioactive compound diosmin. During hepatic cancerous condition, it has been observed that glucose metabolizing enzymes levels were nonstandard in the transformation of normal liver to high glucose utilization. The activities of glycolytic and gluconeogenic enzymes and tumour progression have strong association and this glucose metabolizing enzymes levels can be used as effective pointers of diagnosis and prognosis [41]. In the present investigation, the cancer animals illustrated uncharacteristic level of carbohydrate metabolising enzymes in liver may be due to the higher rate of glycolysis in the liver and subsequent leakage of this enzyme into the blood. These metabolising enzymes were brought back moreover to normal level because of medicinal potency of diosmin.

In tumour tissues, it was found that, the augmented lipid peroxidation consequent in dropped antioxidant activity, unusual glycosylation and augmented lysosomal hydrolases as well as proteases are the major route cause for higher glycoprotein levels such as sialic acid, hexoses and hexosamine [42]. In the present investigation, the increased levels of glycoproteins in liver tissues of cancerous animals were detected. Upon treated with diosmin these glycoproteins levels were reverted to near normal. Since diosmin has previously been confirmed to inhibit tumour growth, the current indication additionally supports the anti-cancer belongings of diosmin. Scanning Electron microscopic analysis in NDEA treated animals displayed amorphous nuclei and unbalanced cytoplasmic content due to carcinogenic property of NDEA [43]. The hepatocytes were totally damaged in the carcinogen treated group II animals. The architecture of liver was damaged in HCC bearing animals due to the neoplastic conditions which was recovered after drug treatment. Due to ameliorative potential of diosmin the damaged cells were recovered in the means of cell shape, nuclei shaped and regular cytoplasm. This proves the anti-cancer properties of the diosmin.

5. CONCLUSION:

Our findings strongly reveal the anti-carcinogenic potential of diosmin which may be related directly or indirectly to its antioxidant properties. This novel approach will throw more light on the cognitive mechanism of diosmin against chemically induced hepatoma. To establish a broader implication, studies are needed in other cancer models. We expect that diosmin may be established as a hopeful anticancer agent in clinical practice in future.

6. CONFLICT OF INTEREST:

The authors have declared that there is no conflict of interest.

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