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Effect of aqueous and ethanolic pomegranate peel extract on diethylnitrosamine induced changes in hematology and liver function in albino rats

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Abstract

Pomegranate (*Punica granatum* L.) an ancient fruit, widely consumed as fresh fruit and juice, has been used to investigate its anticarcinogenic effect on the biochemical enzymes, hematological parameters and histology of liver in Diethylnitrosamine (DEN) induced albino rats. Group I served as normal control. Group II and III were given low and high dose DEN for 40 days. Group IV and V were treated with low and high dose DEN + Aqueous pomegranate peel extract. Group VI and VII were treated with low and high dose DEN + Ethanalic pomegranate peel extract.Animals were sacrificed, blood collected and used for analysis of haematological parameters and biochemical enzymes. Liver tissue was taken for histopathology. On comparison with control, the level of SGOT, SGPT and ALP were significantly increased in all the treatment groups while there was no appreciable change in the level of protein. The number of leucocytes was increased in both DEN treated group while in both the supplementation groups the number of leucocytes were reduced to below control level. The number of RBCs were slightly decreased in the low dose of DEN treated and the both extract supplemented groups. The level of haemoglobin was significantly decreased in all treatment groups. The liver treated with both doses of DEN brought about dilation of the portal tract, degeneration and distruption of heptocytes as well as periventicular infiltration. Supplementation with aqueous and ethanolic pomegranate peel extract brought about occlusion of central vein, congestion of hepatocyte, exhibiting feathery, degeneration and regenerative cellular change.

Key words : Pomegranate Peel, Diethyl nitrosamine, Biochemical enzymes and Liver histology.

INTRODUCTION

Medicinal plants, herbs, spices and herbal remedies are known to Ayurveda in India since long times. The knowledge of herbal remedies which mankind has received from the past generations has to be preserved for posterity. The liver is the second largest organ in the human body after skin, and is the largest internal organ. The liver produces albumin, a protein found in blood and cholesterol, that is critical to the makeup of the outer membrane of cells. When the liver cells are damaged and cannot perform these functions, they release certain enzymes into the blood. Liver cancer is the second most common cancer in the world and most common in Asia, Africa and Southern Europe. Liver cancer or Hepatic cancer is one that originates in the liver, and are malignant tumors that grow on the surface or inside the liver. The most frequent liver cancer is hepatocellular carcinoma (HCC). Pomegranate (*Punica granatum* L.) is one of the ancient fruit that is widely consumed as fresh fruit and juice. In many traditional systems of health condition treatment, use of pomegranate for both internal and external conditions have been documented [1]. In traditional medicine, pomegranate fruits have been used to treat acidosis, dysentery, microbial infections, diarrhea, helminthiasis, haemorrhage and respiratory pathologies [2]. In recent years several pomegranate-containing products have been widely marketed for health benefits around the world, including the United [3].

MATERIALS AND METHODS

Albino rats, weighing about 130-250 grams were selected. The rats were procured and acclimatized to our laboratory conditions for two weeks. The animals were housed in a well ventilated, temperature and humidity controlled animal house, with a light schedule of fourteen hours and ten hours darkness. They were fed with standard diet and drinking water was made available *ad libitum.*

Experiments were complied with the ruling of the committee for the purpose of control and supervision of experiment on animals (CPCSEA), New Delhi, India. Registration No. 722/02a CPCSEA dt. 14.12.2006 and the study were permitted by the Institutional Ethical Committee (IEC) of the Bharathiar University.

Aqueous pomegranate peel extract(APPE):- Fresh pomegranates (*Punica granatum*) were obtained from local departmental store. The peels of the pomegranate were separated and washed with distilled water. To obtain the aqueous extract of *Punica granatum* peels, 150 gm of the air dried peels were powdered in an electric grinder to a fine powder and passed through a 24 mesh sieve and stored in plastic bags. 50 gm of powdered sample was mixed with 500 ml of distilled water for 15 minutes with continuous stirring. The resultant solution was filtered through a filter paper, and stored as a stock solution for experimentation.

Ethanolic pomegranate peel extract(EPPE):- To prepare ethanol extract of pomegranate peel, fresh fruits were peeled and washed with distilled water. 150gm of peels were weighed, powdered in an electric grinder to a fine powder and passed through a 24 mesh sieve and stored in plastic bags. 50 gm of powdered sample was taken and extract separated for 8 hours using Soxhlet's apparatus with 200 ml ethanol (99.9 %). The extract was concentrated under room temperature by evaporation and stored at 4˚c until used, designated as ethanolic extract of pomegranate peel.

Diethylnitrosoamine:- DEN was dissolved in saline and injected twice a week as intra peritoneal injection at low dose of 0.5 mg/100g BW/day and high dose of 1.0 mg/100g BW/day for 40 days to initiate hepatic carcinogenesis.

Experimental Design:- Healthy male albino rats were divided in to 7 groups of 5 animals and received the following regimen of treatments.

Group I (Control) - Animals received normal saline 1ml/100gm BW/day for 40 days and used as control.

Group II (LD) - Animals were injected DEN 0.5mg/100gm BW/day twice a week for 40 days intraperitoneally.

Group III (HD) - Animals were injected DEN 1.0mg/100gm BW/day twice a week for 40 days intraperitoneally.

Group IV (LDPA) - Animals were injected DEN 0.5mg/100g BW/day twice a week intraperitoneally and given aqueous extract of pomegranate peel orally (0.2ml/100g BW/day) daily for 40 days.

Group V (HDPA) - Animals were injected DEN 1.0mg/100g BW/day twice a week intraperitoneally and given aqueous extract of pomegranate peel orally at (0.2ml/100g BW/day) daily for 40 days.

Group VI (LDPE) - Animals were injected DEN 0.5mg/100g BW/day twice a week intraperitoneally and given ethanolic extract of pomegranate peel orally at (0.2ml/100g BW/day) daily for 40 days.

Group VII (HDPE) - Animals were injected DEN 1.0mg/100g BW/day twice a week intraperitoneally and given ethanolic extract of pomegranate peel orally at (0.2ml/100g BW/day) daily for 40 days.

All treatments were given between 9:30 to 10:00 hours in the morning. At the end of the treatment protocol, animals were anesthetized with ether and sacrificed by decapitation. Blood was collected in both EDTA coated and uncoated tubes and stored properly until analysis of hematological parameters serum enzymes. All animals were dissected and their livers were rapidly excised, washed with saline, blotted with a piece of filter paper and weighed. A bit of tissue from the region of liver was fixed in Bouin's fluid and used for histological studies.

Biochemical Analysis:- Estimation of serum glutamate oxaloacetate transaminase (SGOT) was done by Reitman and Frankel (1957) method [4].

Estimation of serum glutamate puiruvate transaminase (SGPT) was done by Reitman and Frankel (1957) method [4].

Alkaline phosphatase (ALP) is determined by colorimetric method.

The activity of protein in the blood sample was determine by (Doumas *et al.,* 1981) [5].

The Total RBC count estimated the total number of red cells in a cubic millimeter (cu.mm) of blood (Daise and Lewis 1995) [6].

The Total WBC count estimated the total number of white cells in a cubic millimeter (cu.mm) of blood (Daise and Lewis 1995) [6].

Haemoglobin estimation was done using the haemometer method (Daise & Lewis 1995) [6].

Histological studies were bone by the method of (Bancroft and Stevens, 1997) [7].

STATISTICALANALYSIS

Results obtained were tabulated. Statistical Analysis was carried out using Dunnetts "t" test. Any significant variation between the control and treated groups were recorded (Steele and Torrie, 1960) [8].

RESULTS

Effect on Body Weight (Table 1)

An increase in body weight was observed on treatment with low dose of DEN while high dose brought about a significant decrease in body weight. Supplementation with aqueous and ethanolic pomegranate peel extracts to low dose groups seems to bring about a significant increase in body weight, while the effects of the extracts on high dose groups were not significant.

Effect on Liver Weight (Table 2)

Low dose DEN treatment brought about a significant increase in liver weight, while high dose DEN caused an insignificant decrease in liver weight. The changes in liver weight brought about by both aqueous and ethanolic pomegranate peel extract supplementation were not significant, on comparison with control.

Effect on Hematological Parameters (Table 3)

WBC

The treatment with DEN both at low and high dose increased the number of leucocytes in all the treatment groups, Supplementation with aqueous and ethanolic pomegranate peel extracts to low dose DEN treated groups were observed to significantly reduce the number of leucocytes to below control

Table 1: Effect of Aqueous and Ethanolic Pomegranate peel extracts on the Body Weight of Diethylnitrosamine induced Albino rats.

BODY WEIGHT	Control	LD	HD	LDPA	HDPA	LDPE	HDPE
INITIAL	120±9.082	170±3.162	209±15.280	87±2.549	116±3.674	96±4.0	149±12.186
FINAL	138±11.683	187±3.741	178±16.324	129±1.870	127±2.0	126±2.915	142±10.319

Table 2: Effect of Aqueous and Ethanolic Pomegranate peel extracts on the Liver weight of Diethylnitrosamine induced Albino rats.

Organ Weight	Control	LD	HD	LDPA	HDPA	LDPE	HDPE
LIVER	4.17±0.338	5.88±0.216	3.65±0.133	4.93±0.234	3.76±0.213	3.85±0.216	3.50±0.147

Table 3: Effect of Aqueous and Ethanolic Pomegranate peel extracts on the RBC, WBC and HB of Diethylnitrosamine induced Albino Rats.

PARAMETERS	CONTROL	LD	HD	LDPA	HDPA	LDPE	HDPE
WBC	5.44±0.513	8.57*±0.218	9.5*±0.4	3.62*a±0.237	7.18*c±0.102	2.96*b±0.123	9.62*±0.207
RBC	9.95±0.565	8.902±1.205	10.166±0.477	7.63*a±0.196	8.506*c±0.249	7.74*b±0.305	9.004*d±0.068
HB	17.64±0.643	14.50*±7.827	15.96*±0.47	14.38*±0.622	16.3*±0.45	14.3*b±1.13	15.98*±0.867

Table 4: Effect of Aqueous and Ethanolic Pomegranate peel extracts on the Biochemical enzymes and Protein of Diethylnitrosamine induced Albino Rats.

PARMETERS	CONTROL	LD	HD	LDPA	HDPA	LDPE	HDPE
SGOT	308.9± 5.49	1025.22*± 34.46	1736.36*± 65.93	440.78*a ±10.46	712.88*c± 34.25	1443.34*b± 7.27	1007.56*d± 65.027
SGPT	210.22± 9.04	300.36*± 1.78	390.2*± 8.15	205.52a ±0.575	519.46*± 4.59	338.6*b± 9.39	490.16*d± 22.61
ALP	372.3± 11.69	234.91*± 2.60	380.5± 11.09	452.68*a ±6.87	499.86*c± 12.23	539.32*b ±9.07	476.48*d± 9.49
TOTAL PROTEIN	7.154± 0.074	6.44*± 0.268	7.08± 0.507	7.298a± 0.18	6.49*c± 0.17	6.789b± 0.13	7.27d± 0.45
ALBUMIN	4.25± 0.17	4.39± 0.18	3.14*± 0.10	3.88a± 0.36	3.62*± 0.20	4.45b± 0.24	3.896d± 0.29

Values are expressed as Mean ± S.E.M of five rats.

C Control, LD - Diethylnitrosamine (Low dose), HD - Diethylnitrosamine (High dose), LDPA - Diethylnitrosamine (Low dose) + Aqueous Pomegranate Peel Extract, HDPA- Diethylnitrosamine (High dose)+ Aqueous Pomegranate Peel extract, LDPE- Diethylnitrosamine (Low dose)+ Ethanolic Pomegranate Peel Extract, HDPE - Diethylnitrosamine (High dose) + Ethanolic Pomegranate Peel Extract.

level. But, supplementation of both extracts to high dose DEN treated groups did not bring about any significant change.

RBC

Except for a slight decrease in the low dose DEN treated, aqueous and ethanolic pomegranate peel extracts supplemented groups, no significant change was observed in the other treatment groups.

Haemoglobin

The level of haemoglobin was seen to be significantly decreased in all the treatment groups on comparison with control.

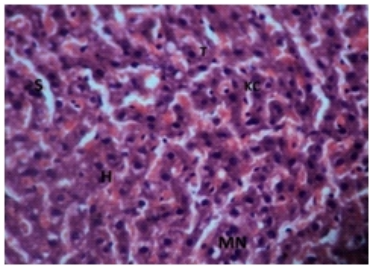
Enzyme Analysis and Total Protein (Table 4)

SGOT

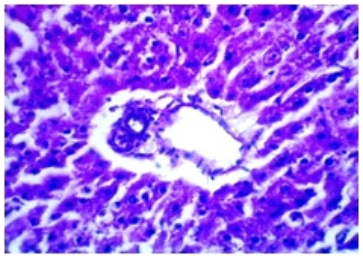
The level of SGOT enzyme was significantly increased in all

PLATE 1: TESTIS HISTOLOGY

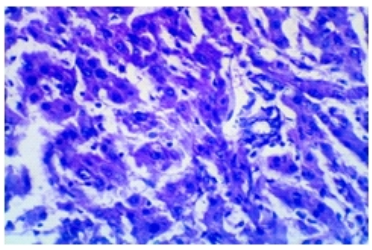
CONTROL LIVER



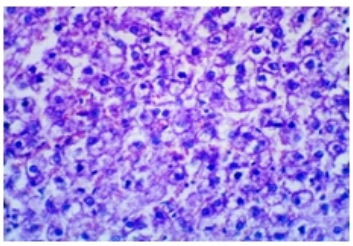
LOW DOSE DEN TREATED LIVER



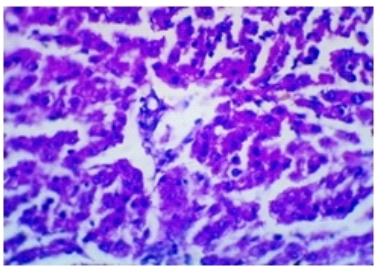
HIGH DOSE DEN TREATED LIVER



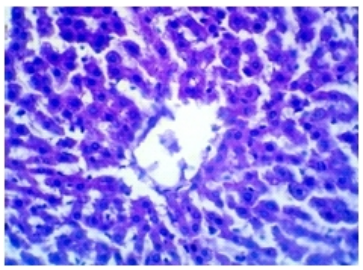
LOW DOSE DEN + APPE LIVER



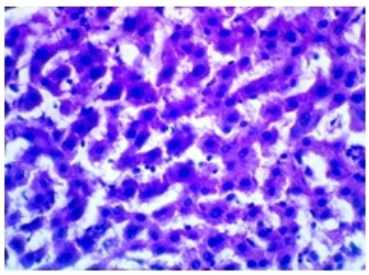
HIGH DOSE DEN + APPE LIVER



LOW DOSE DEN + EPPE LIVER



HIGH DOSE DEN + EPPE LIVER



APPE : Aqueous Pomegranate Peel Extract, EPPE : Ethanolic Pomegranate Peel Extract

the treatment groups, While this increase was lesser in the low and high dose APPE supplemented groups, both the low and high dose DEN treated as well as the low and high dose EPPE supplemented groups showed a very high increase of the enzyme level.

SGPT

The level of SGPT enzyme was also observed to be increased in almost all the treatment groups. While the low dose DEN treated and extract supplemented groups showed a lesser increase in enzyme level, the high dose of DEN treatment as well as extract supplementation are seen to bring about a higher increase of SGPT enzyme level when compared to control.

ALP

On comparison with control level, the level of ALP enzyme was observed to be significantly increased in all the pomegranate peel extract supplemented groups and low dose DEN treated groups.

Total Protein

No appreciable change in total protein level was observed in any of the treatment groups.

Effect on Liver Histology (Plate 1)

In the current study, control liver showed normal hepatocytes with by sinusoids. But DEN treatment both at low dose and high

dose brought about dilation of the portal tract, degeneration and distruption of heptocytes as well as periventicular infiltration. Supplementation with APPE-brought about occlusion of central vein, congestion of hepatocyte and sinusoidal dilation as well as swelling of hepatocytes. Supplementation with EPPE caused vascuolization and ballooning. with presence of pycnotic nuclei and intraacinar inflammatory cell infiltration, thus indicating the persistent deleterious effect of DEN on liver tissue.

DISCUSSION

Effect on Body Weight:- Orally administrated aqueous suspension of spices like *Allium cepa, Allium sativum, Capsicum annum, Carum carvi* and *Amethum graveolens* caused no significant difference in body weight and organ weight on chronic treatment [9]. But in the present study, low dose DEN treatmented as well as pomegranate extracts supplemented groups were seen to bring about an increase in body weight.

Effect on Liver Weight:- *Cinnamomum zeylanicum* treatment caused a reduction in liver weight. [10]. Cellular swelling is the first manifestation for cell injury, resulting due to shift of extracellular water into the cells. When all cells in an organ are affected, it causes some pall or increased turgor, resulting in an increase in organ weight. Fatty liver, a condition resulting from accumulation of triglycerides also results in an increase in liver size [11]. In the present investigation, the increase in liver weight on low dose DEN treatment may an initial response to cell injury and decrease in liver weight on high dose administration may be due to the degeneration of liver tissue.

Effect on Hematological Parameters:- The effect of oral administration of aqueous extract of *Occimum basilium* leaves at a dose level of 200mg/Kg. increased the amount of hemoglobin, PCV, TWBC and neutrophils levels, while lymphocytes level was decreased on administration of the extract in both male and female rats [12]. Leukocytes formed in the bone marrow enters the blood for defense mechanism. Dose-dependant increase in the level of leucocytes on *Lantana aculeatum* stem extract treatment shows the extent of shrinking cell size due to chemical intoxication[13]. According to [14] the decrease in the number of red blood corpuscles in the present study may be due to defective haemopoiesis as well as intravascular red cells damage. In the *Aloe vera* treated groups decline in RBC was lesser and an early recovery was also seen which may be due to the protection provided by the drug. These results are uphold by various reports [15] & [16].

Enzyme Analysis:- On co-exposure to sodium arsenite and ethanol the activities of ALP, ALT and AST increased in a concentration dependent manner in male Wister albino rats. Interestingly, the activities were decreased in the groups of co-administration providing a clue that the chemical interaction between sodium arsenite and ethanol is having a reversal effect. Exposure to sodium arsenite had been shown to induce ALP, AST and ALT activity , which is clearly an indication of induction of hepatotoxicity and oxidative stress in the hepatocytes [17]. As liver enzymes are usually raised in acute heptotoxity, in the present study also an increase in the activity of both transaminase and it can be observed.

Total Protein:- Evaluation of serum proteins such as albumin and globulin is a good criterion for assessing the secretory ability/functional capacity of the liver. [18]

Effect on Liver Histology:- Garlic oil (GO) administration significantly inhibited the increase of the nodule incidence and average nodule number per nodule-bearing liver induced by NDEA, improved hepatocellular architecture, and dramatically inhibited NEDA induced elevation of serum biochemical indices in a dose dependent manner[19]. On treatment with DEN liver was enlarged and hepatic architecture was completely destroyed, with appearance of minihyperplastic nodules and obvious heteromorphism. The nuclei were prominent and occupied most of the cells, with clear necrosis regents in liver visible. Appearance of necrosis,fibrosis mononuclear cell infiltration,steatosis and degeneration of hepatocytes with increase in mitotic activity in liver was evident on high dose of DEN admistration.

Supplementation with both the pomegranate extract reduced the deleterious histo pathological effects to a great extent. Initial alterations like sinusoidal capillarization and inflammatory reactions in lobules,portal and periportal areas were observed. An increase in sinusoidal endothelial cells and kupffer cells in the hepatic lobules with restoration of nuclear shape can also be observed, thus expressing the recovery from DEN induction. Similar to our current investigation, restoration of appearance of hepatocellular carcinoma induced by DEN on supplementation with blueberries have also been reported by [20].

CONCLUSION

A number of candidate drugs derived from the herbs or herbal composites formulae for chemoprevention against HCC have been experimented. There is a lack of randomized used places for controlled clinical studies regarding the use of herbal compounds .Among the herbal toxic effects hepatotoxicity in the most frequent .Therefore, in the present study pomegranate fruit was selected on the chemotherapeutic agent and aqueous and ethanolic extracts of the peel was subjected to clinical trials as supplementation to DEN induced toxic reaction on the liver tissue and haematological parameters. But amelioration of DEN induced toxicity was found to be limited . Further studies involving more intricate parameters and different dosages have to be carried out to fully advocate the use of pomegranate peel as a fullfledged chemotherapeutic agent for cancer treatment.

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