

Effect of *Punica Granatum* (Pomegranate) on serum ALT and AST in Carbon tetrachloride induced liver damage in Wistar Albino Rats

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Abstract

Background: Liver diseases are associated with significant morbidity and mortality. *Punica granatum* may have free radical scavenging activity and it can be used for the prevention and treatment of liver damage. **Objective:** To observe the hepatoprotective effects of *Punica granatum* on CCl₄ induced liver damage in rats. **Methods:** The experimental study was carried out in the Dept of Physiology, Dhaka Medical College, Dhaka from July 2013 to June 2014. For this purpose, 36 wistar albino rats were studied. After acclimatization for 7 days, they were divided into two groups-control and experimental group. Control group were subdivided into BC (Baseline control), CC (CCl₄ treated control) and SC (Silymarin treated control). Experimental group were subdivided into CP-APT (CCl₄ pretreated and aqueous extract of pomegranate treated), CP-EPT (CCl₄ pretreated and ethanolic extract of pomegranate treated) and APP-CT (Aqueous extract of pomegranate pretreated and CCl₄ treated). Each sub group consisted of 6 rats. All rats received basal diet for 8 days. In addition to basal diet on 8th day, BC received single dose olive oil and CC received CCl₄. Rats of SC received silymarin for 8 consecutive days. In experimental groups, CP-APT received aqueous extract of Pomegranate and CP-EPT received ethanolic extract of Pomegranate for 8 consecutive days. Moreover, APP-CT received aqueous extract of Pomegranate for 8 consecutive days and CCl₄ only on 8th day. All rats were sacrificed on 9th day and then blood samples were collected. Serum ALT and AST levels were estimated by using standard laboratory kits. Statistical analysis was done one way ANOVA and Bonferroni test. **Results:** The mean serum AST and ALT levels were significantly ($p < 0.001$) higher in CC in comparison to those of BC. Serum AST and ALT levels of all experimental groups were significantly ($P < 0.001$) lower than CC. Silymarin used as a standard reference also exhibited significant hepatoprotective activity against CCl₄ induced hepatotoxicity **Conclusion:** From the result of present study it can be concluded that, Pomegranate may have hepatoprotective effect by lowering ALT and AST levels.

Keyword: Pomegranate, CCl₄, Rats, ALT, AST

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Introduction

The liver is the largest metabolizing organ in the body which regulates homeostasis of the different body system¹. Recent

surveillance studies in the United States documented an annual incidence of newly diagnosed chronic liver disease of 72.3 per 100,000 populations. Over half (57%) of patients have hepatitis C viral infection, followed by

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alcohol induced liver disease (24%), non alcoholic fatty liver disease (9%), and hepatitis B viral infection (4%). Liver disease accounts for over 44,000 deaths per year in the United States (1.9% of all deaths), placing it as the eight leading cause of death².

The principal causative factors for the liver diseases in developing countries are environmental toxins, parasitic disease, hepatitis B and C viruses, and hepatotoxic drugs and hepatotoxic agents (carbon tetrachloride ,thiocetamide)³. Carbon tetrachloride (CCl₄) is an industrial chemical that does not occur naturally. It can cause pathological lesions in treated animals that closely resemble the symptoms of cirrhosis in human, and so it is considered an excellent model to evaluate the efficacy of hepatoprotectants. CCl₄ induced hepatotoxicity depends on dose and duration of exposure^{4,5,6}.

Pomegranate Linn. (Punicaceae), commonly known as pomegranate, recently described as nature's power fruit⁷. Pomegranate fruits are globally consumed as fresh fruit and in processed forms like juice, jam, wine, oil and also in extract supplements⁸. It is a rich source of polyphenolic compounds that include flavonoids and hydrolyzable tannins⁹. Pomegranate has been known to possess considerable pharmacological properties as pomegranate peel is a rich source of antioxidants, especially polyphenols. In some recent studies, pomegranate fruit and flower extracts exhibited free-radicals scavenging properties with simultaneous potent hepatoprotection against chemically induced liver damage in rodents^{10,11}.

Silymarin is a standardized extract of the milk thistle (*Silybum marianum*) which is used as a standard drug and exhibiting potent hepatoprotective activity at the dose range from

25-200 mg/kg in various experimental and clinical studies^{12, 13}.

Liver diseases have become a worldwide problem and are associated with significant morbidity and mortality but its medical management is currently inadequate¹⁴. Therefore, there is a growing focus to evaluate scientific basis for the traditional herbal medicines that possess hepatoprotective effects¹⁵. Very few studies has been done yet to investigate the hepatoprotective effect of pomegranate.

With the above background, the present study was carried out to observe the hepatoprotective effects of Pomegranate in experimental animals after inducing hepatotoxicity by CCl₄.

Methods

This experimental study was conducted in the Department of Physiology, Dhaka Medical College, Dhaka from July 2013 to June 2014. Total 36 apparently healthy Wister albino rats, weighing between 150 to 200 grams and age ranging from 90 to 120 days, was used for this study. The rats were purchased from the animal house of Department of Pharmacy, Jahangir Nagar University, Savar, Dhaka. The protocol of this study was approved by Ethical Review Committee of Dhaka Medical College.. The rats were kept in metallic case in the animal house of Institute of Nutrition and Food Science, University of Dhaka (DU). Before conducting the study, rats were kept in a standard laboratory condition on a 12/12 hour light/dark cycle for 7 days for acclimatization. All the rats received basal diet for 21 days. Total study period was 15 consecutive days. After selection and acclimatization for 7 days, the rats were divided into two groups, control group and experimental group. Control group were subdivided into BC (base line control group, n=6) and CC (Carbon

tetrachloride treated control group, n=6) and SC (Silymarin treated control group, n=6). Experimental groups were subdivided into CP-APT (CCl₄ pretreated and aqueous extract of pomegranate treated group, n=6), CP-EPT (CCl₄ pretreated and ethanolic extract of pomegranate treated group) and APP-CT (Aqueous extract of pomegranate pretreated and CCl₄ treated group, n=6). After grouping, initial body weight of all the rats were measured on 1st day. In addition to basal diet on 8th day, BC received single dose olive oil and CC received CCl₄. Along with basal diet, SC received silymarin for 8 consecutive days 30 minutes after administration of CCl₄. In experimental groups, in addition to basal diet, CP-APT received aqueous extract of Pomegranate for 8 consecutive days 30 minutes after administration of CCl₄ and CP-EPT received ethanolic extract of Pomegranate for 8 consecutive days 30 minutes after administration of CCl₄ along with basal diet. Moreover, APP-CT received aqueous extract of Pomegranate for 8 consecutive days and CCl₄ only on 8th day along with basal diet. 200 µl CCl₄ was diluted with 200 µl of olive oil (1:1 dilution).

Pomegranate extract preparation:

Fruits of Pomegranate were washed and the peels were manually removed, cut into small pieces and then dried at room temperature for 2 days. Dried peels of Pomegranate were grinded in an electrical grinder which was subjected to drying overnight in an air-oven electrical dryer. Then the dried sample of Pomegranate was taken in the jar. After that, ethanol and water was poured into the jar in order to prepare alcoholic and aqueous extract of Pomegranate. Then, Pomegranate extract was filtered with fine cloth. By evaporating the solvent using a rotator

evaporator under reduced pressure in water bath at a temperature of 60°C. It was stored in freeze at around 4°C and was fed to the experimental rats.

Before sacrifice, final body weights of all the rats were measured. On 9th day all the animals were anaesthetized with the help of 30% chloroform and then sacrificed. The blood samples (approximately 5ml) were collected from the heart by directly puncturing with sterile disposable syringes and taken in separate clean and dry test tubes with proper identification numbers. Then blood was centrifuged at a rate of 4000 rpm for 5 minutes. After that the supernatant serum was separated from the blood, collected in a labeled eppendroff and preserved in a refrigerator at -20°C until analytical measurement of serum for AST and ALT in Department of Pathology, DMC. Data was reported in Mean and ±SE. Statistical analyses were done by One-way ANOVA and Bonferroni test as applicable.

Results

Initial body weights of all rats were almost similar and showed no statistically significant difference of these values among the groups.

The final and % change of body weight was significantly ($p < 0.001$) lower in group CC when compared to that of group BC, SC, CP-APT and CP-EPT. (Table I).

In this study, the mean serum ALT and AST level were significantly ($P < 0.001$) higher in CC in comparison to that of BC and SC. But these levels were significantly ($p < 0.001$) lower in CP-APT, CP-EPT and APP-CT in comparison to that of CC. Again there was no significant difference in those levels between CP-APT and CP-EPT (Table II).

Table-I: Initial and final body weight and percent (%) change of body weight in different groups of rats (n=35)

Parameter	BC (n=6)	CC (n=6)	SC (n=6)	CP_APT (n=6)	CP_EPT (n=6)	APP-CT (n=5)
Initial body weight	155±2.59	168.33±4.79	160.00±5.1	170.83±5.56	158.33±4.02	186±6.85
Final body weight	210.8±2.72	195±5.16***	216.67±3.3	216.67±4.96	210±5.18	221±7.44
% change weight [F-I/I×100]	36.1±1.59	15.90±1.12***	35.85±3.06	27.06±1.77	32.72±2.12	18.9±1.17

Results are expressed as Mean±SE. One way ANOVA followed by Bonferroni test was performed to compare between groups. n = Number of rats. (***)p<0.001 CC vs BC, SC, CP-APT, CP-EPT) BC: Baseline control group CC: CCl₄ treated control group SC: Silymarin treated control group CP_APT: CCl₄ pretreated and aqueous extract of pomegranate treated group CP_APT: CCl₄ pretreated and alcoholic extract of pomegranate treated group APP-CT: Aqueous extract of pomegranate pretreated and CCl₄ treated group

Table II: Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in different groups of rats (n=35)

Parameters	BC (n=6)	CC (n=6)	SC (n=6)	CP_APT (n=6)	CP_EPT (n=6)	APP-CT (n=5)
ALT (U/L)	36.17±1.75	823.33±91.3 ^{SSS}	38.33±1.09	37.67±1.4***	34.33±1.5***	384±40.73***
AST (U/L)	34.33±1.09	663.33±70.77 ^{SSS}	34.00±1.46	35.00±1.7***	33.67±1.6***	246±12.15***

Results are expressed as MeanSE. Statistical analysis was done by One way ANOVA followed by Bonferroni test. (^{SSS}p<0.001 CC vs BC, CC vs SC) (***p<0.001 CP-APT vs CC, CP-EPT vs CC, SCvs CP-APT, CP-EPT) n = Number of rats. BC: Baseline control group CC: CCl₄ treated control SC: Silymarin treated control group, CP_APT: CCl₄ pretreated and aqueous extract of pomegranate treated group, CP_EPT: CCl₄ pretreated and alcoholic extract of pomegranate treated group APP-CT: Aqueous extract of pomegranate pretreated and CCl₄ treated group ALT: Alanine transfersae,AST: Aspartate transferase.

Discussion

In the present study, the mean % change of body weight was significantly lower in CC in comparison to that of BC. This finding is similar with those of some researcher¹⁶. Again this level was significantly higher in experimental groups in comparison to that of CC. These findings are in consistent with those of some other researchers¹⁷.

In this study, significantly higher serum AST and ALT levels in CC in comparison to that of BC was observed. This finding was in agreement with other researchers^{17,18}. Again, mean serum AST and ALT levels were significantly lower in

SC in comparison to that of CC. This finding is similar with those of some researcher¹⁹. However, no significant difference in ALT and AST was observed by some researchers⁷. This discrepancy may be due to the fact that in that study different extract of pomegranate was used. It has been suggested that most remarkable pathological characteristics of CCl₄ induced hepatotoxicity are fatty liver, cirrhosis and necrosis. These pathological lesions are due to formation of reactive intermediates such as trichloromethyl free radical (CCl₃) in the endoplasmic reticulum²⁰. In the present study, hepatic damage was observed in CCl₄ treated rats

as evidenced by an elevation of serum ALT and AST levels. Again, lower levels of serum ALT and AST observed in CP-APT, CP-EPT and APP-CT rats suggest that the possibility of Pomegranate extract to give protection against CCl₄ induced liver injury. Bodies of literature suggested that pomegranate has protective effect on liver damage due to its antioxidant properties. Antioxidant potential of pomegranate juice and peel, seeds extracts is attributed to their high polyphenolic content^{21, 22}. Polyphenolic content has strong free radicals scavenging and antioxidant properties²³. In this study effects both extracts of pomegranate are almost same. No significant difference was found between them. So both can be used as hepatoprotective. These hepatoprotective effects of pomegranate are most likely due to free radical scavenging activity.²² However, the exact mechanism involved in the hepatoprotective activity of Pomegranate extract against CCl₄ induced liver damage in rats cannot be elucidated from the present study as concentration of free radicals was not assayed.

Conclusion

From the result of the study, it may be concluded that Pomegranate may have some hepatoprotective role against CCl₄ induced liver damage.

Conflict of Interest: None

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