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# PARACETAMOL INDUCED HEPATOTOXICITY IN RATS: ROLE OF *AEGLE MARMELOS* (L.) CORRÊA AGAINST LIVER DAMAGE

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## ABSTRACT

The current investigation was carried out to inspect the effects of oral intake of *Aegle marmelos* (L.) Corrêa extract against liver fibrosis and cirrhosis induced by Paracetamol in male wistar rats. *Aegle marmelos* is a medicinal plant with anti-inflammatory, anti-hyperlipidemic, anti-allergic, cardioprotective and anti-carcinogenic activities. The healthy age-matched inbred strain of male wistar rats were recruited for the experiment. Three categories were formulated for the healthy, age-matched inbred strain of male wistar rats (n = 6) with first group (I) designated as control, (II) received Paracetamol 500 mg/kg body wt. daily in distilled water for 15 days, group III received Paracetamol 500 mg/kg body wt. daily in distilled water together with *Aegle marmelos* (AM) 440 mg/kg b.w. daily for 15 days. Plasma total bilirubin, ALP, AST and ALT levels were estimated and correlated with histological findings. Parcetamol induced hepatotoxicity in rats as shown by enhanced liver enzymes levels in Paracetamol group as compared with control group. *Aegle marmelos* treatment decreased hepatotoxic effects of Paracetamol by significantly reducing the elevated liver enzymes. The body weights of animal of all groups were reduced in this study. Histologic investigations revealed enlargement, paleness, portal and periportal inflammation in paracetamol treated rats whereas only enlargement and paleness were present in paracetamol + AM treated. The biochemical assays and histological results showed the moderate hepatoprotective activity of the AM and that its intervention may be beneficial in the treatment of liver pathologies.

**Key word:** *Aegle marmelos*, hepatotoxicity, Paracetamol, cirrhosis

**Abbreviation:** *Aegle marmelos*=AM*;* ALP = alkaline phosphatase; AST = aspartate aminotransferase; ALT = alanine aminotransferase; NAPQI= n-acetyl para-benzoquineimine

## INTRODUCTION

Liver metabolizes several endogenous and exogenous substances, eliminates drugs and detoxify body therefore, chances for liver toxicity are increased following malnutrition, anemia, infections, alcohol consumption, medications and xenobiotics (Mroueh *et al*., 2004).Chronic alcoholism and chronic hepatitis B and C are main causes of death in Asian parts and sub-Saharan Africa.

*Aegle marmelos* (L.) Corrêa, commonly known as the bael, is an important medicinal Asian plant. It possesses medicinal properties within every part of it and is consumed in the form of different preparations either alone or in combination with other herbs (Rishaba*et al*., 2012). The AM leaves possess anticonvulsive, antipyretic, analgesic, antimalarial, antidiuretic (Yaheya and Ismail,2018), hypothermic, anticancer (Takase *et al*., 1994), anti-inflammatory, cardiovascular protective, anti-diabetic (Maity*et al*., 2009) antihypertensive, hypolipidemic and hypoglycaemic properties (Lambole *et al* 2010). Active constituents that AM houses include alkaloids, sterols, essential oils, coumarins, tannins, glycosides, phenols and terpenoids (Venkatesan *et al*., 2009). AM leaves contain aegeline 2 which attribute anti hyperglycemic activity acids and lowering the blood glucose levels accompanied with increase in HDL-C and HDL-C/TC ratio (Narender *et al*., 2007). Kothari studied significant antidepressant and anxiolytic activities of leaf extract of *Aegle marmelos* (Kothari *et al*., 2010).

Paracetamol induces hepatotoxicity by forming toxic and highly reactive metabolite n-acetyl para-benzoquineimine (NAPQI). Herbal treatment is in limelight nowadays for traditional medicine practitioners and researchers for liver treatment. About 2000 curative plants have been isolated in herbal system with new advancements in medicine and science. This increasing awareness of natural products invites more attention than allopathic system for having lesser side effects along with multiple medicinal properties. Therefore, the present work aimed to scientifically prove the hepatoprotective nature of orally administered leaf extract of *Aegle marmelos* against paracetamol induced liver fibrosis and cirrhosis in male wistar rats.

## MATERIALS AND METHODS

**Plant Material**:

Undried *Aegle marmelos* (L.) Corrêa leaves were procured locally from Karachi, Pakistan. They were cut into small pieces and air dried, crushed in electrical grinder to turn into powder which was then stored in a covered plastic container at room temperature. The extract was made by dissolving 22.0 g percent concentration (w/v) crude homogenate in distilled water, filtered through double layer of muslin cloth and refiltered using Whatman's filter paper.

**Animals Housing:**

The International center for chemical and biological sciences, Karachi was selected for the procurement of the healthy, age matched inbred strain of male wistar rats having weight ranges between 200 ± 20g. Well ventilated animal house was used to indurate animals using macrolon cages kept at 25$^{o}$C with 14/10 h day/night cycles at the Department of Physiology, University of Karachi. They had free access to food and water.

**Ethical Guideline**

Care of all animals was made mandatory in accordance with the guidelines published by National Institute of Health named "Guide for the Care and Use of Laboratory Animals".

**STUDY DESIGN**:

Categorization was done making three groups of the animals (n = 6) treated orally for 15 days as follows:

**Group-I**: remained untreated control received orally distilled water.

**Group-II**: treated with Paracetamol at a dose of 500mg/kg body wt. in distilled water daily for 15 days.

**Group-III**: treated with Paracetamol 500mg/kg body wt. in distilled water + *A. marmelos* extract 440mg/kg b. wt. daily for 15 days.

At 16$^{th}$ day rats of all groups were sacrificed. The method of cardiac piercing was employed and heparin coated tubes were used for obtaining plasma for analysis; serum was also set apart. The liver was taken out and made free of connective tissues and blood, followed by desiccation and weighing. Finally, the tissue storage was done in freezer at -70 ºC.

**Determination of Total Plasma Bilirubin, Aspartate Aminotransferase, Alanine Aminotransferase, Alkaline Phosphate**

Plasma ALT & AST (Reitman and Frankel, 1957), ALP (Teitz *et al*., 1983) and total and direct bilirubin (Dangerfield *et al*.,1953) were estimated using Randox chemical reagent package.

**Histopathological Examination**

Fixation and embedding of hepatic tissue were made using formalin and paraffin blocks, respectively. Four µm thick sections were then made and staining with Eosine and Hematotoxylin was done for microscopic examination.

The extent of hepatocytic damage was investigated from the histological sections by means of grading and quantitative scores (French *et al*., 2000).

Score 0 = no distinguishable impairment

1 = localized hepatocyte impairment on less than 25%.

2 = localized hepatocyte impairment on 25-50%.

3 = extensive, localized hepatocyte lesion

4 = comparative hepatocyte necrosis

## RESULTS AND DISCUSSION

The reduction in body weight is seen in all three groups- Control, Paracetamol treated, AM + Paracetamol (Table 1). There is no significant change in liver weight ($P>0.05$) and relative liver ($P>0.05$) weight in Control, Paracetamol treated and AM + Paracetamol treated rats (Table 1).

D-galactosamine and Acetaminophen are among the various hepatotoxic chemicals that are known to curtail hepatic functionality with consequent piling-up of ammonia (waste material) in blood (Mao *et al.*, 2014). In this study, paracetamol successively caused hepatotoxicity in rats as endorsed by biochemical and histologic findings. A significant increase in serum enzyme AST ($p<0.05$), ALT ($p< 0.05$) and Bilirubin ($p<0.05$) was observed in Paracetamol treated rats as compared with control rats (Table 2). Rats treated with Paracetamol + *Aegle marmelos* leaf extract have been shown to decrease AST ($p >0.05$), ALP ($p<0.05$), ALT ($p>0.05$) & Bilirubin ($p<0.05$) as compared to paracetamol treated rats (Table 2).

In other studies, the hepatoprotective effect of *Aegle marmelos* in alcohol-induced liver injury was evaluated in rats using essential marker biochemical parameters which indicated that, the AM leaves have excellent hepatoprotective effects (Arul *et al.,*2005).

The histological observations in the present study are in agreement with the biochemical findings. Scoring of the morphological findings of liver are described and summarized in Control, Paracetamol treated and Paracetamol + AM treated rats (Table 2). Any altered hepatocytichistological changes was absent in liver tissues of control group (Fig.1). Hepatotoxicity induced by Paracetamol was confirmed in liver tissues which indicated

Paracetamol treated rats indicated portal and periportal inflammation with progressive fibrosis (Fig.2) that further endorse induction of hepatotoxicity in these rats. Our experimental results showed that *Aegle marmelos* treatment along with Paracetamol has partly inhibited hepatotoxic alteration and overturned the liver histological alterations induced by Paracetamol and showed only enlargement and paleness (Fig.3). Similar changes in liver tissues were noted by (Eidi *et al.,* 2012).

Table1. Comparison of Body Weight, Liver Weight and Relative Liver Weight in Control, Paracetamol treated & Paracetamol+*Aegle marmelos* treated rats.

| | **Control** | **Paracetamol treated** | **Paracetamol+AM treated** |
|---|---|---|---|
| **Initial body weight** | 181.33 ± 4.04 | 176.66 ± 10.69 | 197 ± 19.46 |
| **Final body weight** | 167 ± 1.73 | 165 ± 10.58 | 183.66 ± 17.67 |
| **Liver weight** | 3.96 ± 0.23 | 4.1 ± 0.26 | 3.71 ± 0.39 |
| **Relative liver weight** | 2.36 ± 0.16 | 2.48 ± 0.10 | 2.04 ± 0.36 |

Numerical values are presented as mean ± SD.

Table 2. Comparison of Serum marker for liver injury in Control, Paracetamol treated and Paracetamol+*Aegle marmelos* treated rats.

| **Serum markers** | **Control** | **Paracetamol treated** | **Paracetamol+ AM Treated** | **LSD 0.05** |
|---|---|---|---|---|
| AST (U/L) | 9.55 ± 1.82 c | 16.03 ± 2 a | 12.4 ± 1.04 b | 1.99 |
| ALT (U/L) | 11.42 ± 0.7967 b | 14 ± 0.46 a | 11.94 ± 0.39 b | 1.99 |
| ALP (U/L) | 95.22 ± 53.44 c | 165.6 ± 16.78 a | 105.09 ± 34.7 b | 1.99 |
| TOTAL BILIRUBIN (U/L) | 0.68 ± 0.311 b | 2.01 ± 0.26 a | 0.9 ± 0.13 ab | 1.16 |

Numerical values are presented as mean ± SD; AST = aspartate aminotransferase; ALT = alanine aminotransferase; ALP = alkaline phosphatase; Same letters are not significant in each row according to Duncan's Multiple Range Test at $P < 0.05$.

Table 3. Histopathological features in Control, Paracetamol treated and Paracetamol+*Aegle marmelos* treated rats.

| **Histopathological findings** | **Control** | **Paracetamol** | **Paracetamol + AM treated** |
|---|---|---|---|
| **Enlargement** | 1 | 1 | 1 |
| **Paleness** | 1 | 1 | 1 |
| **Fatty change** | 0 | 0 | 0 |
| **Hydropic degeneration** | 0 | 0 | 0 |
| **Periportal fibrosis** | 0 | 1 | 0 |
| **Bile duct proliferation** | 0 | 0 | 0 |
| **Dysplasia** | 0 | 0 | 0 |
| **Portal Fibrosis** | 0 | 1 | 0 |

Degree of hepatic injury is expressed as scores observed via light microscopy.
Score 0 = no distinguishable impairment.
1 = localized hepatocyte impairment on less than 25%.
2 = localized hepatocyte impairment on 25-50%.
3 = extensive, localized hepatocyte lesion; 4 = comparative hepatocyte necrosis

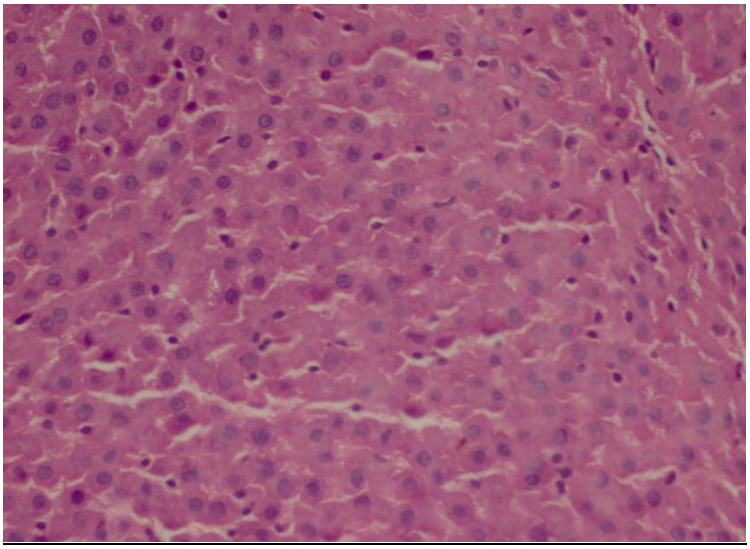

Fig.1. Histopathological features of Control rats.

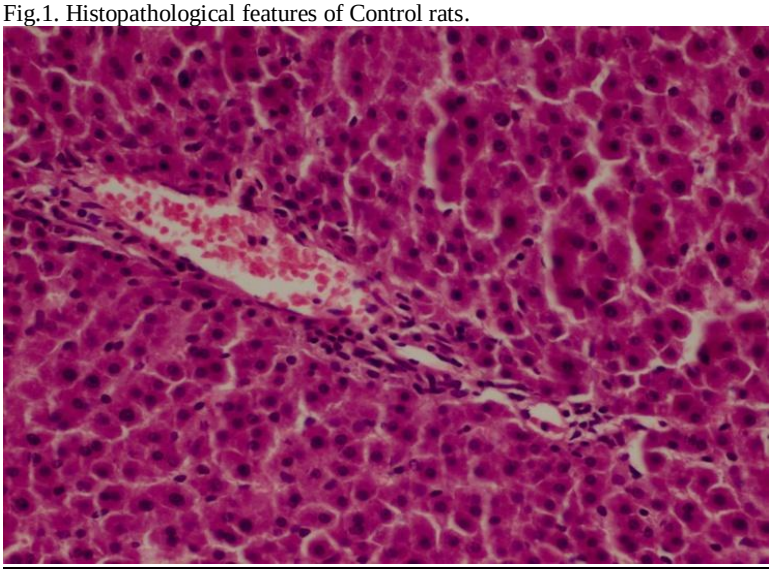

Fig.2. Histopathological features of Paracetamol treated rats.

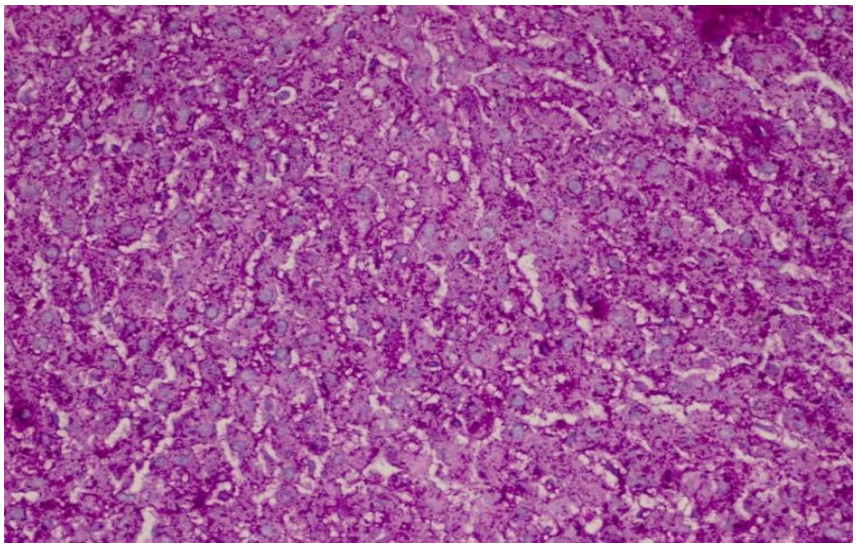

Fig.3. Histopathological features of Paracetamol +*Aegle marmelos's* treated rats.

## CONCLUSION

*Aegle marmelos* leaf extract administration for 15 days along with Paracetamol is found to moderately balance the hepatotoxicity in rats.

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