Original Article

Protective Effect of Henna (Lawsonia Inermis Linn.) at Different Doses in Acetaminophen Induced Hepatotoxicity in Albino Rats

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ABSTRACT

Objective: To assess the protective effects of Henna (Lawsonia inermis Linn.) at two different doses on hepatotoxicity caused by acetaminophen in albino rats.

Study Design: Experimental study

Place and Duration of Study: This study was conducted at the Department of Anatomy, Postgraduate Medical Institute (PGMI), Lahore, Pakistan for a period of 12 days starting on June 1st, 2019.

Materials and Methods: The study comprised of 28 male adult albino rats that were divided into 4 groups A, B, C and D. Group A (control group) received 5ml/kg distilled water orally for 10 consecutive days followed by 5ml/kg normal saline intraperitoneally on day 10. Group B was given distilled water (5ml/kg) orally through gavage for 10 consecutive days followed by 750mg/kg acetaminophen dissolved in 5ml per Kg body weight normal saline intraperitoneally as single dose on day 10. Group C was given 100ml/kg Henna leaf extract dissolved in 5ml/kg distilled water given orally through gavage for 10 consecutive days followed by 750mg/kg acetaminophen dissolved in 5ml/kg normal saline intraperitoneally as single dose on day 10. Group D was given 400ml/kg Henna leaf extract dissolved in 5ml/kg distilled water orally through gavage for 10 consecutive days followed by 750ml/kg acetaminophen dissolved in 5ml/kg normal saline intraperitoneally as a single dose on day 10. All rats were sacrificed on day 12 i.e. 48 hours after administration of last dose. Livers were extracted out and sections were stained with Hematoxylin and Eosin stains. Morphological parameters such as diameter of central vein, blood vessels congestion and inflammatory cells infiltrate were studied. Biochemical parameters involved were serum ALT and AST. Results were analyzed by using SPSS version 22.0.

Results: In present study, Microscopic examination of hepatic lobules revealed abrupt increase in the diameter of central vein of Group B animals caused by acetaminophen. When this group is compared with group A (control group) while group C and D showed no significant change in diameter of central veins. Group B also showed blood vessels congestion with stagnant blood cells and disrupted endothelium causing hemorrhage within hepatic stroma. These findings were not observed in groups C & D. Signs of inflammation like infiltration of white blood cells were more appreciable in group B while this inflammation is less prominent in group C & D due to protecting effects of Henna leaf extract in these groups. Both ALT and AST were normal in control whereas raised in toxic group B. After treatment with protective agent at two different doses, ALT and AST were dropped in group C in which low dose of protective agent is used and become near to normal in group D in which high dose of protective agent was used showing hepatoprotective effects of lawsonia inermis Linn.

Conclusion: Taking in account the above mentioned observations and results, it gives a strong support that Henna Leaf (lawsonia inermis Linn.) has significant prophylactic effects on the microarchitecture of the liver that would be destroyed by the toxic effect of acetaminophen.

Key Words: Henna leaf, acetaminophen, hepatotoxicity, micrometry

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INTRODUCTION

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Acetaminophen, also called paracetamol is a commonly used analgesic and antipyretic drug.[1] It suppresses prostaglandin synthesis by acting on cycloxygenase pathway COX 2 by acting centrally.[2] It is safe when administered in therapeutic doses but overdose causes toxicity primarily in the liver. It causes mitochondrial dysfunction and centrilobular necrosis.[3] Acetaminophen produce potentially toxic effect on liver and kidneys by the formation of highly active metabolite, N-acetyl-p-benzoquinon imine (NAPQI).[4] Lawsonia inermis commonly called Henna, Mehndi is a perennial plant belongs to Family Lytheraceae. It is cultivated for

cosmetic and pharmaceutical purposes.[5] It has analgesic, antipyretic, antioxidant, antiarthretic, antiulcer, antifungal and anticancer effects.[6] It is also cited that Lawsonia inermis (Henna) has protective role in acetaminophen induced hepatotoxicity in rats [7] but its dose dependent prophylactic effects against APAP induced hepatotoxicity has not been studied yet. Keeping this in view, an experimental study was designed to observe the prophylactic effect of Lawsonia inermis leaf extract at two different doses on acetaminophen induced hepatotoxicity in adult albino rats.

MATERIALS AND METHODS

This study was conducted at the Department of Anatomy, Postgraduate Medical Institute (PGMI), Lahore, Pakistan for a period of 12 days starting on June 1st, 2019. 28 adult male albino rats that were healthy,8-10 week of age,180-220g in weight were selected. They were properly acclimatized and kept in well ventilated and temperature maintained house at 24+2ºC, humidity 55 ± 5% and dark & light cycles, each cycle of 12 hours. Rat chow and water was given to the animals-ad libitum.

Dissection and Tissue Sampling: At the end of experimental period, on 12th day, 48 hours after the administration of last dose of the agent, each rat was anaesthetized. Skin was cut by giving a midline incision. Liver was identified in the right upper abdominal region and excised carefully. The other half was fixed using 10% NBF (Neutral Buffered Formalin).

Histological Techniques

Tissue Preservation:

The liver of each animal was placed in neutral buffered formalin. Tissue was processed for up to 18 hours by using the automatic tissue processor (Histotouch III-USA). For embedding, liquid paraffin was then poured onto the tissue piece to make tissue block. By using microtome, sections of 3 µm thickness were obtained and stained with Hematoxylin and Eosin.

Parameters

1. **Biochemical parameters:**
   - **a)** serum ALT
   - **b)** serum AST
2. **Histological parameters:**
   - **a) Quantitative:**
     - i. Diameter of central vein (µm)
   - **b) Qualitative:**
     - i. Blood vessels congestion.
     - ii. Inflammatory cells infiltrates.

Histological Examination: For assessment and measurement of histological parameters, light microscope (Leica DM 1000) was used to examine the prepared tissue sections, using magnifications of 10X.

Diameter of central veins were measured with ocular micrometer. 3 hepatic sections from rat were observed. 5 hepatic lobules with central vein were identified in cross-sectional view; the cross-sectional maximum diameters of central vein were measured twice at angles perpendicular to each other i.e. horizontal & vertical diameters; the mean diameter was calculated (Fig. 1); thus 450 in total of hepatic lobules with central veins were examined and the mean diameters of central veins were noted down.

Sinusoids of each hepatic lobule was assessed for congestion and presence of inflammatory cells. 5 sinusoids in hepatic lobules in each section were examined and 3 sections from each animal were taken. Thus, the sinusoids of 450 hepatic lobules were recorded.

Statistical Analysis: All experimental data was compiled in Microsoft Word® and Excel(R) sheet. For analysis of experimental results, SPSS 22.0 (Statistical Package for Social Sciences) was used. Mean ± S.D was given for quantitative variables like diameter of central vein. Chi square was applied to observe the mean differences for qualitative variables i-e. blood vessels congestion and inflammatory cell infiltrate. For multiple comparisons, post hoc Tukey test was used. P-value of ≤0.05 was considered as statistically significant**.**

RESULTS

Diameter of Central Vein (µm): The mean diameter of central vein (µm) in all groups was determined. It was found that the diameter of central vein in all groups were significantly different (p value < 0.001) (Table 2). For multiple comparisons, post hoc Tukey test was used which indicated that diameter of central vein in group B was significantly higher when compared with group A, C and D. However, no significant difference was observed in the diameter of central vein between group A and D.

Blood Vessels Congestion: In all rats of group A, there was a normal looking endothelial lined blood vessels (central vein and sinusoids) with no congestion. In group B, blood vessels were dilated with disrupted endothelial lining and retained RBC's in lumen in all rats. In group C, blood vessel congestion was present in 5 (71.4%) rats whereas in group D, blood vessel congestion was observed in only 2 (28.6%) rats. (Table 3).

Inflammatory Cells Infiltrate: Fisher's exact test showed that there was an association between inflammatory cells infiltrate and groups. In group A, inflammatory cells infiltrate was absent in 5 (71.4%) rats while mild inflammation was observed in 2 (28.6%) rats. In group B, foci of inflammatory cells containing mainly lymphocytes were observed in all rats. In group C, mild inflammation was observed in 5 (71.4%) rats and moderate inflammation was observed in 2 (28.6%) rats whereas in group D, inflammation was absent in 5 (71.4%) rats while mild inflammation was observed in 2 (28.6%) rats. (Table 4)

Table No.1: Showing detail of animal groups and duration of therapy.

Group	Animals (N)	Intervention and Dosage	Duration of Dose	Day of Sacrifice
A	7 Control	Distilled water 5ml/kg via oral gavage	10 consecutive days	Day 12
		Normal saline 5ml/kg intraperitoneally	As a single dose on day 10	
B	7 Experimental	Distilled water 5ml/kg via oral gavage	10 consecutive days	Day 12
		Acetaminophen 750mg/kg dissolved in normal saline 5ml/kg intraperitoneally	As a single dose on day 10	
C	7 Experimental	Henna leaf extract 100mg/kg dissolved in distilled water 5ml/kg via oral gavage	10 consecutive days	Day 12
		Acetaminophen 750mg/kg dissolved in normal saline 5ml/kg intraperitoneally	As a single dose on day 10	
D	7 Experimental	Henna leaf extract 400mg/kg dissolved in distilled water 5ml/kg via oral gavage.	10 consecutive days	Day 12
		Acetaminophen 750mg/kg dissolved in normal saline 5ml/kg intraperitoneally	As a single dose on day 10	

Table No.2: Comparison of diameter of central vein (µm) among groups.

Variable	Group A Mean ± SD	Group B Mean ± SD	Group C Mean ± SD	Group D Mean ± SD	p-value
Diameter of central vein (µm) D*10	61.3 ± 8.2	150.3 ± 12.6	98.7 ± 6.8	59.6 ± 12.5	< 0.001*

One way ANOVA. *p value ≤ 0.05 is regarded as significant statistically

Table No.3: Distribution of blood vessel congestion among groups

Blood Vessel Congestion	Group A n (%)	Group B n (%)	Group C n (%)	Group D n (%)	p-value
Absent	7 (100.0%)	0 (0.0%)	2 (28.6%)	5 (71.4%)	< 0.001*
Present	0 (0.0%)	7 (100.0%)	5 (71.4%)	2 (28.6%)	

Fisher's exact test *p value ≤ 0.05 is considered statistically significant

Table No.4: Distribution of inflammatory cells infiltrate among groups.

Inflammatory Cells Infiltrate	Group A n (%)	Group B n (%)	Group C n (%)	Group D n (%)	p-value
Absent	5 (71.4%)	0 (0.0%)	0 (0.0%)	5 (71.4%)	< 0.001*
Mild	2 (28.6%)	1 (14.3%)	5 (71.4%)	2 (28.6%)	
Moderate	0 (0.0%)	3 (42.9%)	2 (28.6%)	0 (0.0%)	
Severe	0 (0.0%)	3 (42.9%)	0 (0.0%)	0 (0.0%)	

*p value ≤ 0.05 is considered statistically significant`

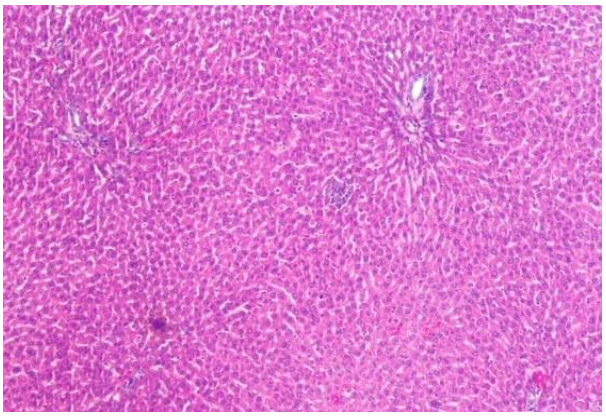

Figure No. 1: Group A showing Normal Liver Architecture

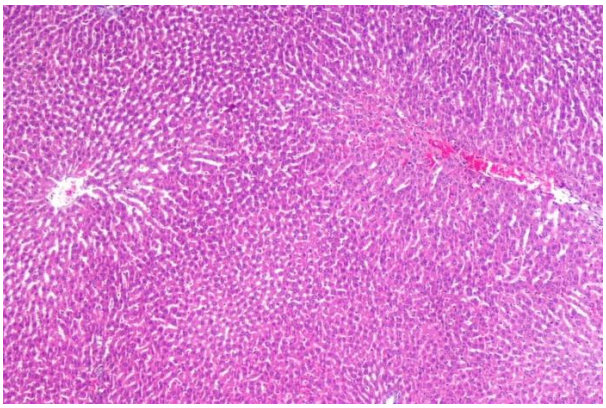

Fig No. 2: Group B showing Liver section with Congested blood vessels, wide caliber central veins full of inflammatory infiltrates

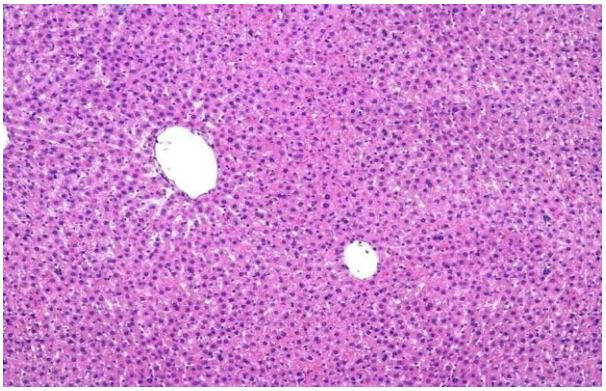

Figure No. 3: Group C showing mild congested blood vessels and moderate inflammatory cells infiltrate

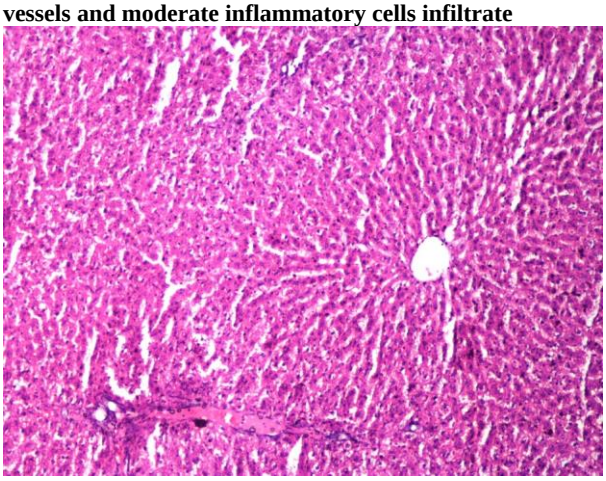

Figure No. 4: Group D showing normal caliber of central veins, normal endothelium lining of vessel and sparse presence of inflammatory cells.

Serum ALT (U/L): One way ANOVA test was used for the comparison of the serum ALT levels among groups. It was found that the mean serum ALT levels in all groups were significantly different (p value < 0.001) (Fig 5).

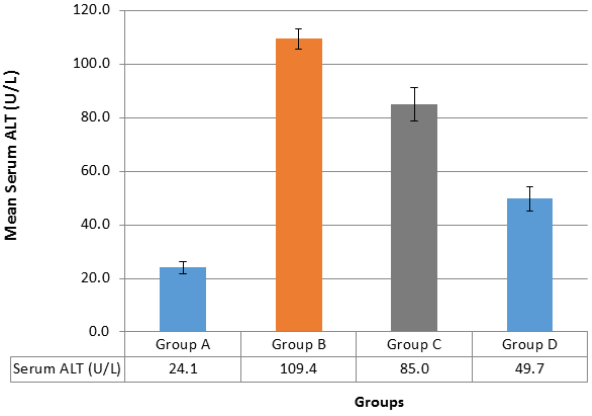

Figure No. 5: Bar chart displaying comparison of serum ALT levels among groups.

Serum AST (U/L): The mean serum AST level in all groups was determined. One way ANOVA test was used for the comparison of the serum AST levels among groups. (Fig 6).

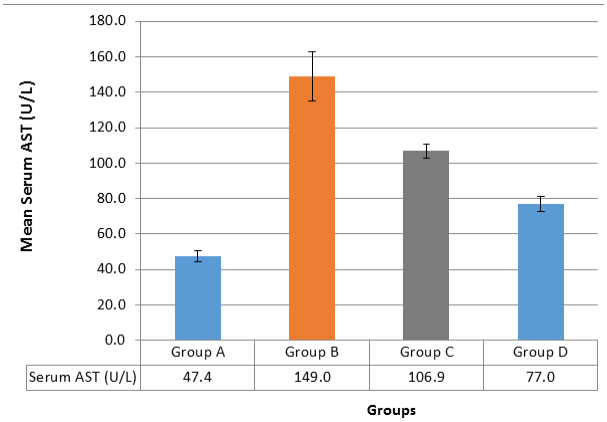

Figure No. 6: Bar chart displaying comparison of serum AST levels among groups.

DISCUSSION

Acetaminophen commonly called paracetamol is one of the most common analgesic drugs used worldwide. When dose exceeds from therapeutic level, it produces hepatotoxic effects due to excessive NAPQI (N-acetylp-benzoquinoneimine) formation.[3]

As an antioxidant, Lawsonia inermis might had reduced oxidative stress by inhibiting lipid peroxidation, quenching free radicals thus depicting protective effects on liver microvasculature.[8]

In present study, Microscopic examination of hepatic lobules revealed abrupt increase in the diameter of central vein of Group B animals caused by acetaminophen. When this group is compared with group A (control group) and group C and D (Protective groups). It shows that there is no significant increase or decrease in diameter of central veins in group A, C and D. These changes in microarchitecture correlate with previous study done on APAP or henna by Mudassir Sohail & Muzaffar, 2018.[9]

On H&E staining, signs of inflammation like infiltration of white blood cells (mainly lymphocytes) could be more appreciable in group B while this inflammation is less appreciable in group C & D due to protecting effects of Henna leaf extract in these groups. These also correlate with similar results shown in study done by Hsouna et al., 2013 in which fruit extract of lawsonia inermis was studied at dose of 250mg/kg.[10]

Blood vessels congestion with stagnant blood cells and disrupted endothelium causing hemorrhage within hepatic stroma in acetaminophen treated group B may be due to prevention of prostaglandin synthesis which could have regulated blood flow. These findings were not observed in groups C & D and correlate with studies done by Sabiba et al., 2013 who observed congestion of hepatic blood vessels following administration of acetaminphen 900mg/kg i.p.[11] Protective effects of lawsonia inermis supported by results given by Hsouna et al, 2013.[10]

Biochemical parameters used in this study were ALT and AST. Both ALT and AST are the enzymes that catalyzes the important reactions that are involved in the transfer of α-amino groups from aspartate and alanine to α-keto group of ketoglutamic acid to generate oxaloacetic and pyruvic acids respectively which are important contributers to citric acid cycle. Alanine aminotransferase is mostly present in cytoplasm of hepatocytes while AST attain both cytoplasmic and mitochondrial position. If hepatic injury occurs, it results in the release of both ALT and AST in serum causing increased level of both enzymes.[3] Both ALT and AST were normal in control whereas raised in toxic group B. After treatment with protective agent at two different doses, ALT and AST were dropped in group C in which low dose of protective agent is used and become near to normal in group D in which high dose of protective agent was used showing hepatoprotective effects of lawsonia inermis.

CONCLUSION

Taking in account the above mentioned observations and results, it gives a strong evidence that lawsonia inermis has significant prophylactic effects on the microarchitecture of the liver that would be destroyed by the toxic effect of acetaminophen. However, more long term studies can be made to further explore the genetic effects of this drug therapy on liver.

Author's Contribution:

Concept & Design of Study:	Misbah Ishtiaq
Drafting:	Abeer Anjum, Irum Naz
Data Analysis:	Aneela Ahsan, Uzma Azam
Revisiting Critically:	Misbah Ishtiaq, Abeer Anjum
Final Approval of version:	Misbah Ishtiaq

Conflict of Interest: The study has no conflict of interest to declare by any author.

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