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ORIGINAL ARTICLE

Hepatoprotective role of tecomella undulata bark extract in comparison with n-acetylcysteine on acetaminophen induced hepatotoxicity in albino rats.

Mehr Fatima[1], Mamoora Arslaan[2], Shabih Zehra[3], Hafiza Tuseef Sayyar[4], Muhammad Kamran[5], Ijaz Hussain Zaidi[6]

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ABSTRACT… Objective: To assess hepatoprotective effect of Tecomella Undulata bark extract on acetaminophen induced hepatotoxicity in albino rats as compared to N-Acetylcysteine. **Study Design:** Experimental Comparative Study. **Setting:** Bahria University Medical & Dental College Karachi in collaboration with Basic Medical Sciences Institute Karachi. **Period:** March 2020 till August 2020. **Material & Method:** Study included 56 Albino rats divided in four groups with 14 animals in each group. Group A was control, Group B was induced hepatotoxicity by giving Acetaminophen 500 mg as a single dose, Group C was given N–Acetylcysteine 140mg/kg intraperitoneally for 6 days and Group D was given Tecomella bark extract 200mg/kg for 15 days after acetaminophen induced liver damage. Their blood samples were collected and sent for hepatic enzyme levels. Statistical analysis was done using SPSS version 23. One way ANOVA was applied. For multiple comparison post hoc tukey's test was used. P-value <0.05 was considered significant. **Result:** The mean Serum AST, ALT and ALP levels were increased in Group-B, however, Group-D showed significant reduction in hepatic enzyme levels as compared to Group-C. Tecomella bark extract showed hepatoprotective effect by reducing hepatic enzyme levels in acetaminophen induced hepatic damage in albino rats. **Conclusion:** Thus Tecomella plant extract could act as an alternative antidote to N-acetylcysteine in acetaminophen induced hepatic damage in albino rats.

Key words: Acetaminophen, Hepatotoxicity, Hepatoprotective, N-Acetylcysteine.

INTRODUCTION

Plants were widely used to treat various ailments since pre-historic periods and it has been found that 80% of the world population uses drugs based on medicinal plants for curing illnesses.[1] Nowadays, plant based medicines are used as they are considered to be safe with minimum side effects. Tecomella Undulata, which is also known as Ammora (in English) or Rohida, desert teak, marwar teak or white cedar, is an economical and medicinally valued plant. In plant kingdom, it belongs to family Bignoniaceae. In Pakistan, it is mainly available in Sindh and Baluchistan. In traditional Indian medicine, Tecomella plant has been used for treatment of various ailments like liver disorder, abdominal diseases, infections, eye diseases. All parts of plant contain biologically important compounds like flavanoids, phytosterol, flavanols, and titerpenoids which possess antioxidant.[2]

Since liver is involved in removing harmful end products of metabolism in addition to other bodily functions, the current excessive use of drugs and alcohol is leading to increase in hepatic disease.[3] Acetaminophen causes drug induced liver injury which is dose dependant and it is mostly manifested within 1-5 days after the use of high therapeutic dosage.[4] It is one of the most commonly used drug for pain relieve. It is used almost by 60 million American at least once a week.[5] Acetaminophen is considered safe up to dosage of 4000mg/24 hours and is approved by FDA as nontoxic at these levels. However, recommended dose in case of patients with liver disease is 2000 mg or less.[6] Acetaminophen

1. MBBS, M.Phil, Assistant Professor Pharmacology, Bahria University Health Sciences, Karachi.
2. MBBS, M.Phil, Assistant Professor Pharmacology, Islamabad Medical and Dental College.
3. MBBS, FCPS, Assistant Professor Radiology, Al Tibri Medical College, Karachi.
4. M.Phil, Ph.D (Pharmacology), Pharm-D, Assistant Professor, Bahria University Health Sciences, Karachi.
5. MBBS, M.Phil, Assistant Professor Pharmacology, Bahria University Health Sciences, Karachi.
6. MBBS, M.Phil, Ph.D, Professor Pharmacology, Bahria University Health Sciences, Karachi.

Correspondence Address:
Dr. Mehr Fatima
Department of Pharmacology
Bahria University Health Sciences, Karachi.
mehr_fatima@yahoo.com

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intoxication is one of the main causes of liver failure which accounts for a high rate of emergency room visits and hospitalizations in UK.[7] Patient presents with initial symptoms of nausea, vomiting and weakness within 24 hours of ingestion. Moreover, symptoms progress to abdominal pain, jaundice, hepatomegaly, and bleeding disorders after 24 to 72 hours, with significantly raised levels of Alanine aminotransferase (ALT) and Alkaline phosphatase (ALP) within 72 to 96 hours of ingestion. This can progress to multiorgan failure, cerebral edema and renal failure.[8] However, data regarding prevalence of acetaminophen induced liver injury is lacking in Asian setup.[9]

The most commonly used antidote for acetaminophen intoxication world wide is N-Acetylcysteine (NAC).[2] However, N-Acetylcysteine is effective if it is administered early in the course of acetaminophen intoxication. It replenishes Glutathione stores thus preventing liver injury. Moreover, treatment with N-Acetylcysteine is associated with severe adverse drug reactions. In case of intravenously administered N-Acetylcysteine, severe anaphylactoid reactions occur in about a third of all cases and nausea and vomiting in about half of acetaminophen intoxicated patients.[5] N-Acetylcysteine is also found to cause variation in blood pressure and heart rate, skin rash and itching, angioedema and bronchospasm.[10]

Acetaminophen, the commonly prescribed drug usually causes liver failure which accounts for high hospitalization rate in the UK and USA. However there is a lack of data regarding the prevalence of acetaminophen induced hepatic injury in Asian setup. The most commonly used drug to treat such cases is N-Acetylcysteine which has potentially serious side-effects. On the other hand, it has been revealed that herbal plants are much safer and possess various bioactive compounds with hepatoprotective potential. Hence, there is a need to further investigate agents which can prevent liver from damage. Tecomella Undulata is an important plant whose medicinal properties are still under progress. The significance of the study is that it investigates Tecomella plant's role as an alternate hepatoprotective agent or antidote to N–Acetylcysteine in acetaminophen induced hepatotoxicity.

MATERIAL & METHODS

This was an experimental comparative study which was conducted in Bahria University Medical and Dental College (BUMDC) in collaboration with Basic Medical Sciences Institute over a 5 months duration after obtaining ethical approval from Ethical Review Committee (ERC Reference No. ERC 36/2020) of Bahria University Medical and Dental College.

A total of 56 experimental animals, which included albino rats, were randomly selected from animal house. Sample size was 56 which was calculated by G Power version 3.1.9.2 software, using formula (E=Total numbers of animals - Total numbers of groups) and (Sample size=$2SD^2$ ($Z^{a/2}+Z^{\beta})^2$ / d^2). 95% confidence interval and 5% margin of error was used to determine the required sample.[11]

The study included healthy experimental animals weighing 150- 200 gm which were not previously used for any experimentation purpose. They were kept at standard laboratory conditions. They were exposed to 12 hour light and 12 hour dark circadian cycle. They were provided free access to food and water and were acclimatized for 7 days. However, unhealthy rats which were used for any experimentation previously were excluded from the study. Experimental animals were randomly divided into four different groups with 14 animals in each group. Group A was healthy control group (n=14) which was provided normal diet and water for 15 days.

In Group B hepatotoxicity was induced by giving Acetaminophen orally 500mg/kg[12] body weight as a single dose. In Group C, animals were given Acetaminophen orally 500mg/kg[12] body weight as single dose and N- Acetylcysteine (Standard drug) 140mg/kg[13] intraperitoneally was given for 6 days. In Group D, experimental animals were given Acetaminophen orally 500mg/kg body weight as a single dose and ethanolic extract of Tecomella Undulata (Test drug) bark at the dose of 200mg/kg[14] body weight for 15 days.

They were anesthetized by putting them in a jar having cotton swab soaked with chloroform. Cardiac puncture was done by using 3 ml of disposable syringe. Blood sample of 0.3 to 0.5 ml was collected. It was kept in serum vacutainer at room temperature for 30 minutes. Centrifugation process was done at 2000 rpm for 10 minutes and serum was separated. It was stored in serum cups at -20^0 C. AST, ALT and ALP levels were analyzed using commercially available Kits.

Tecomella bark was collected from rural area of Sindh. 2000gm by weight bark of the plant was cut, air dried and ground into fine powder. The powder was then mixed with petroleum ether in order to eliminate fat. Subsequently, the same was treated with 50% ethanol by percolation, separated by filtration and extracted on rota vapour.[15] 200mg/kg of body weight Tecomella bark extract was found safe as indicated by previous studies.[14]

Statistical analysis was done using SPSS version 23. Shapiro Wilk test was done to check normality of data. Data was normally distributed. One way ANOVA was applied to observe the group mean difference in ALT, AST and ALP levels. For multiple comparison post hoc tukey's test was used. P-value <0.05 was considered significant.

RESULTS

Significant rise in serum Aspartate aminotransferase (AST) (111.96 ± 12.28)u/l was observed in Group B which was treated with toxic dose of acetaminophen, [Group C (NAC treated 31.38 ± 6.03)u/l, Group D (Tecomella treated 29.19± 5.01)]. Similarly significant rise in serum ALT levels was also observed for Group B (acetaminophen intoxicated 43.29 ± 13.08) u/l, [Group C (NAC 32.45 ± 4.72)u/l, Group D (Tecomella treated 26.08 ± 6.38)u/l. Moreover Group B also showed rise in serum ALP levels (acetaminophen intoxicated 440.96 ± 32.08)u/l, [Group C (NAC treated 245.22 ± 16.47)u/l, Group D (Tecomella plant treated 241.00 ± 17.97)u/l].

Among all groups, the lowest rise in serum AST, ALT and ALP was noted in NAC Group C and Tecomella treated Group D. Group B demonstrated the highest increase in AST, ALT and ALP levels, when compared with all other Groups, with significant difference (P- value of 0.001), while serum AST, ALT, ALP levels of control group (Group A) when compared showed significant difference with NAC (Group C), whereas, it showed non significant difference with Tecomella plant treated (Group D), with signifying the potential hepatoprotective effect of Tecomella extract as compared to NAC. As evident in Table-I.

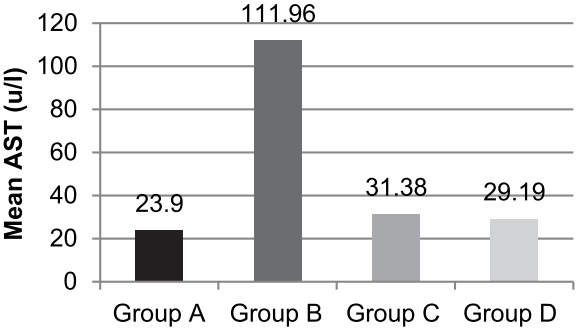

Figure-1. Mean serum AST levels

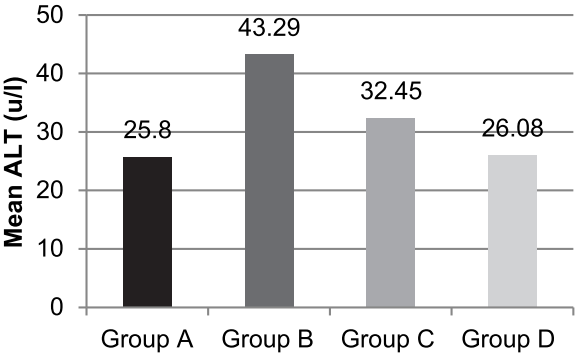

Figure-2. Mean serum ALT levels

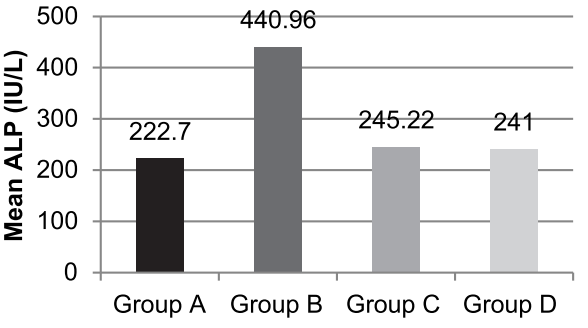

Figure-3. Mean serum ALP level

Group		AST (U/L)		ALT (U/L)		ALP (U/L)	
		Mean ± SD	P-Value	Mean ± SD	P-Value	Mean ± SD	P-Value
B	A	23.90 ±7.34	0.001*	25.80±6.99	0.001*	222.70±17.53	0.001*
	C	31.38±6.03	0.001*	32.45±4.72	0.001*	245.22±16.47	0.001*
	D	29.19±5.01	0.001*	26.08±6.38	.001*	241.00±17.97	0.001*
A	C	31.38±6.03	0.003*	32.45±4.72	0.045*	245.22±16.47	0.033*
	B	111.96±12.28	0.001*	43.29±13.08	0.001*	440.96±32.08	0.001*
	D	29.19±5.01	0.159	26.08±6.38	0.963*	241.00±17.97	0.720
D	A	23.90 ±7.34	0.159	25.80±6.99	0.963	222.70 ±17.53	0.720
	B	111.96±12.28	0.001*	43.29±13.08	0.001*	440.96±32.08	0.001*
	C	31.38±6.03	0.895	32.45±4.72	0.608	245.22±16.47	0.945
C	A	23.90±7.34	0.033*	25.80±6.99	0.045*	222.70 ±17.53	0.033*
	B	111.96±12.28	0.001*	43.29±13.08	0.001*	440.96±32.08	0.001*
	D	29.19±5.01	0.895	26.08±6.38	0.608	241.00±17.97	0.945

Table-I. Comparison among different groups
Multiple comparison test table, post hoc analysis,*P-Value : <0.05 Statistically Significant

DISCUSSION

Liver is involved in metabolic processes thereby maintaining body hemostasis. Any derangements in normal metabolic processes can provoke hepatic injury.[16] In our study, Acetaminophen was given to rat model to induce liver damage, and the hepatoprotective potential of Tecomella bark extract was compared with N-Acetylcysteine. Acetaminophen produces N-Acetyl P Benzoquinone Imine (NAPQI) when metabolized in liver. This is then changed to nonreactive compound by Glutathione. In case of excess intake of drug, its metabolism by cytochrome pathway causes formation of large amount of NAPQI. Since there is depletion of Glutathione levels in body, NAPQI cannot be changed to nonreactive compounds and causes hepatic tissue damage.[17]

Moreover, hepatic injury is always associated with hepatic enzyme release. Hepatic injury is determined by increased liver enzyme levels whereas, a subsequent reduction in enzyme levels after drug administration predicts hepatoprotective activity. In our study, Acetaminophen intoxication increased Serum AST, ALT and ALP levels on rat model and N-Acetylcysteine was used as standard drug. Findings of our study are also consistent with another study in which serum AST and ALT levels were increased in hepatotoxic rat model. Tecomella plant extract reduced all liver enzyme levels in test group. Tecomella plant extract showed hepatoprotective effect by reducing degenerative changes in test group.[18]

Since medicinal plants are associated with lesser side effects, the quest for a different plant based hepatoprotective agent is important.[19] Administration of Test drug Tecomella bark extract at dose of 200mg/kg reduced all these enzyme levels with significant P- value of 0.001 in our study.

This result is in line with another study in which Tecomella bark extract when given to experimental animals as compared to Silmyrin normalizes liver enzyme levels in rat model with drug induced hepatotoxicity.[20] Therefore, decreased level of liver enzymes indicate the membrane stabilizing role of Tecomella plant. Phytochemicals present in Tecomella plant extract play an important role in preventing hepatic tissue damage. Moreover, plant extract may produce its hepatoprotective effect by inhibiting cytochrome P-450 enzyme. Different fractions of Tecomella plant extract also showed hepatoprotective effect in Acetaminophen induced liver damage in experimental rats which can be caused by antioxidant effects of phytochemicals present in the this plant. This is demonstrated by improvement in functional capacity of hepatocytes. Glutathione is an important natural antioxidant present in the liver.[21] Moreover, N- Acetylcysteine increases glutathione level in experimental animals with

liver injury induced by acetaminophen, since, N-Acetylcysteine is the basic compound of Glutathione. Furthermore, it was found that Tecomella plant extract demonstrated its hepatoprotective effect of by rise in catalase and gluthathione levels thus maintaining cellular integrity.[2]

Our study evaluated hepatoprotective effect of Tecomella bark extract on liver enzyme levels in Acetaminophen intoxicated rats in comparison to N-Acetylcysteine. The effects of test drug on other organ functions and safety parameters were not included in this study. Moreover comparison of the test drug with other hepatoprotective agents on larger sample size can provide more evidence regarding its tecomella's role in preventing liver damage in acetaminophen induce liver damage.

CONCLUSION

The hepatoprotective effect of Tecomella bark extract has been shown by the reduction in liver enzymes Serum AST, ALT and ALP levels in acetaminophen intoxicated rats as compared to N-Acetylcysteine. Hence, Tecomella plant extract could be used as an antidote to N-acetylcysteine in acetaminophen induced hepatic damage in albino rats.

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AUTHORSHIP AND CONTRIBUTION DECLARATION

No.	Author(s) Full Name	Contribution to the paper	Author(s) Signature
1	Mehr Fatima	Literature search, Data collection, Analysis, Interpretation, drafting and final approval of article.	
2	Mamoora Arslaan	Data collection, Search of literature and revision of article.	
3	Shabih Zehra	Data analysis, Proof reading and final approval of article.	
4	Hafiza Tuseef Sayyar	Data interpretation, revision and final approval of article.	
5	Muhammad Kamran	Literature search and extraction of plant meterial.	
6	Ijaz Hussain Zaidi	Conceptulization of study design.