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ORIGINAL ARTICLE

Histopathological effects associated with marwar teak (tecomella undulata) bark extract and N-acetylcysteine on acetaminophen induced hepatotoxicity in albino rats.

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ABSTRACT… Objective: To determine histopathological effects associated with Marwar Teak Tecomella Undulata bark extract and N-Acetylcysteine on Acetaminophen induced liver damage in rats. **Study Design:** Experimental Comparative Study. **Setting:** Bahria University Medical and Dental College Karachi in Collaboration with Basic Medical Sciences Institute (BMSI) Karachi. **Period:** March 2020 till August 2020. **Material & Methods:** Study included 56 healthy albino rats which were randomly divided into four groups. Each group comprised of 14 experimental animals which included (Group-1) Control Group, (Group-2) hepatotoxicity induced rats, which were given Acetaminophen 500 mg as a single dose orally, (Group 3) which was induced hepatotoxicity by Acetaminophen 500mg orally as a single dose, then they were given N-Acetylcysteine standard drug at dosage of 140mg/ kg intraperitonealy for 6 days and (Group 4), which was induced hepatotoxicity by Acetaminophen 500 mg orally as a single dose, then they were given Tecomella bark extract at the dose of 200 mg/kg orally for 15 days. Experimental animals were dissected. Liver samples were sectioned (3-5μ) thickness and were stained with H&E. Histopathological findings were noted. SPSS version 23 was used for statistical analysis. P-value <0.05 was considered significant. **Result:** Deranged liver architecture with presence of moderate inflammatory cells were observed in Acetaminophen induced hepatotoxic rats (Group-2). However, there was presence of moderate inflammatory cells with normal liver architecture in rats treated with standard drug (Group–3). There was improvement in liver histology in Tecomella bark extract treated group (p-<0.05) Group-4. **Conclusion:** The Tecomella Bark extract improved histoarchitecture on acetaminophen induced hepatotoxicity in albino rats in comparison with N-Acetylcysteine.

Key words: Acetaminophen, Hepatoprotective, Hepatotoxic.

INTRODUCTION

Hepatic disease accounts for 2 million deaths annually worldwide with East Asia and South Asia placed 13th and 11th with increasing number of total deaths.[1] Since all bodily chemical processes take place in liver, it is readily affected by immoderate use of medicines and alcohol. Various inflammatory and non-inflammatory factors are responsible for hepatic diseases. Hence, hepatic diseases remain an important health related matter.[2] Hepatic injury caused by drugs are reported in both Asian and Western parts of the world. Since various drugs, plant supplements and chemicals are detoxified in liver and can cause liver damage.[3] Drug induced liver injury is one of the main reason for withdrawal of 18% of drugs from market. Moreover incidence of drug induced liver injury has risen by 6 to 8 times than the previous values and is currently at 13.9 to 19 cases per 100,000.[1]

Hepatic injury caused by Acetaminophen is manifested after giving dosages at high therapeutic levels. It causes intrinsic type of drug induced liver injury which usually is seen within 1-5 days of drug ingestion.[4] Acetaminophen is highly recommended analgesic which is mostly used by Americans almost every week.[5] In UK, Acetaminophen intoxication is the main cause of liver injury with emergency hospitalizations

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which account for 1000 hospitalizations and 5000 hospital room visits.[6] There is an overall lack of data on liver injury caused by acetaminophen intoxication in Asian countries.[7]

The most effective and safe antidote / treatment in case of acetaminophen overdose is N-Acetylcysteine since 1979.[8] Even though N-Acetylcysteine is one of the most commonly used antidote, but it still tends to produce common side effects. Administration of N-Acetylcysteine causes hypotension, nausea, vomiting, urticaria, flushing, bronchoconstriction and anaphylactoid reactions.[9]

Plant based medicines have been widely used since ancient times and also are used by large number of people even today. It is estimated that almost 7000 different plants are used for medicinal uses in India. Different types of treatments based on various plants for curing illness are extensively used by Ayuverdic and Unnani medicines. It has been found that, in USA, almost 21% people affected by liver disorder use plant based medicines, since liver is the prime organ involved in detoxification of drugs. It has been estimated that around 80% of individuals worldwide use plant based medicines generally.[10] A medicinally important plant found in Pakistan is Tecomella Undulata. It is commonly known as Desert Teak or Marwar Teak. It is part of the plant family Bignoniaceae. It is mainly available in parts of Sindh and Baluchistan. Apart from Pakistan, this medicinally and economically important tree, is also found in India and Saudi Arabia and is used for various medicinal uses.[11] All parts of the Tecomella Undulata plant possess various medicinally important compounds such as flavanoids, phytosterols, titerpenoids, fatty acids and glucosides among others. An important antioxidant present in the bark extract of this plant is Betullinic acid. It has been found that the plant exhibits multiple biological activities such as anti-oxidative, anti-inflammatory, anti-bacterial, anti-HIV and hepatoprotective effects. Furthermore, this plant has been traditionally used for the treatment of liver and spleen disorders, diseases of the abdomen, for various healing purposes and eye diseases.[12]

Acetaminophen is one of the leading cause of hepatic injury. Moreover, its main antidote N-acetylcysteine is associated with side effects and plants based medicines are safe and associated with less side effects. So, there is a need to search for an alternate hepatoprotective agent for acetaminophen induced hepatotoxicity. Hence, the study was conducted to determine the histopathological effects of Marwar Teak (Tecomella Undulata) bark extract and N-Acetylcysteine on Acetaminophen induced hepatotoxicity in albino rats.

MATERIAL & METHODS

This experimental comparative study was conducted at Bahria University Medical and Dental College (BUMDC) in collaboration with Basic Medical Sciences Institute over a 5 month duration, from March 2020 till August 2020, after approval by Ethical Review Committee (ERC Reference No. ERC 36/2020) of Bahria University Medical and Dental College. The study animals were randomly selected from the animal house. The sample size was 56 albino rats. Sample size was calculated by G Power version 3.1.9.2 software. The sample size was calculated using 95% confidence interval and 5% margin of error.[13] This study included healthy albino rats having a weight of 150-200gm, which were previously not used for any other study. All experimental animals were provided with the standard laboratory conditions and were given free access to food and water. However, rats which were diseased and were previously used for any experimentation were excluded from study.

Tecomella bark extract weighing 2000 gm was obtained from Sindh. Subsequently, it was dried and crushed to form a fine powder. After mixing it with petroleum ether, it was also mixed with 50% ethanol by the process of percolation. Then it was filtrated and extracted on rota vapour.[14]
Acute toxicity test of other studies found 200 mg/Kg of Tecomalla bark extract to be the safe dose.[15]

All experimental animals were divided into four groups. Each group comprised of 14 animals. Group-1 was the control group with healthy animals. Group-2 was given acetaminophen

orally and hepatotoxicity was produced at the single dose of 500mg/Kg[16] body weight. In Group-3, hepatotoxicity was produced by giving acetaminophen orally as a single dose 500mg/Kg body weight and then the experimental animals were given N-Acetylcysteine intraperitoneally 140mg/ Kg[17] for 6 days. In Group 4, experimental animals were given acetaminophen 500mg/kg orally as a single dose to induce hepatotoxicity. Later on they were administered Tecomella ethanolic bark extract at the dose of 200mg/Kg[15] body weight for 15 days orally. Experimental animals were sacrificed and their liver sections were sent for histopathological analysis.

Routine Tissue Processing

Fixation of liver Tissues were done using 10% formalin. They were kept in 10% formalin for 24 to 48 hours in order to avoid tissue degeneration. They were then processed through different dilutions of ethyl alcohol. Tissue was sectioned of approximately 3-5μ by microtome. They were stained with Hematoxylin and Eosin and microscopic slides were prepared and mounted.

Histopathological findings were graded as normal=0, mild tissue injury=1, moderate tissue injury=2, severe tissue injury=3^{+}. Mild, moderate and severe tissue injury was defined as inflammation, inflammatory cells, congestion, hemorrhage and presence of necrotic tissue. Statistical analysis was done by SPSS version 23, using one way Annova. P-value <0.05 was considered significant.

RESULTS

Histopathological Findings

Group-1 (Control Group)

Liver tissue showed normal internal architecture. Normal arrangement of hepatic lobule was observed. They were polygonal on cross section. Lobules were separated by sinusoids. No congestion around Portal tract, central vein, bile duct and hepatic artery was observed. They all were normal and intact. No inflammatory cells were seen. Necrosis was not observed.

Group-2 (Acetaminophen Induced Toxicity Group)

Liver architecture was deranged with infiltration of inflammatory cells predominantly lymphocytes. Congestion around the portal tract was observed. Congestion of blood vessels, bridging necrosis and hemorrhage was also found.

Group-3 (N-Acetylcysteine Treated Group)

Liver tissue showed liver architecture with mild to moderate infiltration of inflammatory cells of liver tissue. However, mild sinusoidal congestion was also noted.

Group- 4 (Tecomella Extract Treated Group)

Liver tissue showed liver architecture with normal looking hepatic cells. There was mild infiltration of inflammatory cells predominantly lymphocytes. Necrotic tissue was not seen.

Parameter	Groups				P-Value
	1	2	3	4	
Inflammation	0	3^{+}	2	1	0.006
Congestion	0	3^{+}	2	1	0.006
Hemorrhage	0	3	1	1	0.003
Necrosis	0	3	1	0	0.001

Table-I. Histological findings in all groups.
0=normal, 1=mild injury, 2= moderate injury, 3+= severe injury

Following histopathological findings were observed in hepatic tissues.

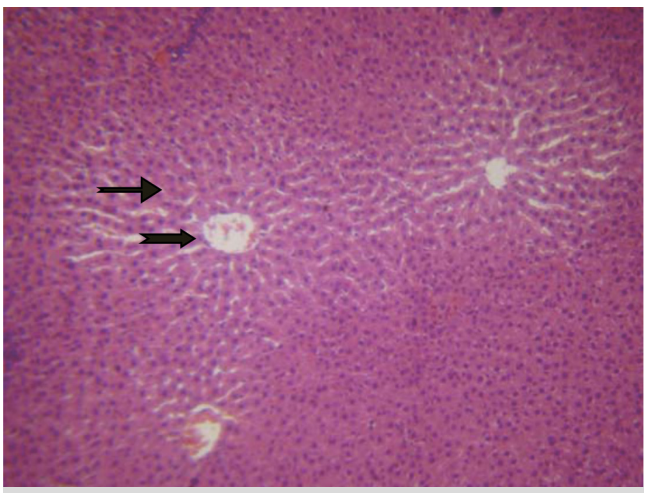

Figure-1. Control (Group -1) showing normal liver architecture, hepatocytes and terminal hepatic venule.

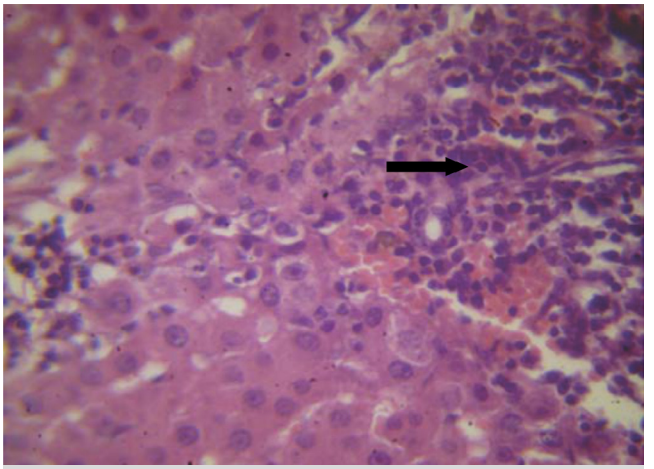

Figure-2. Acetaminophen induced intoxicated group (Group-2) showing dense chronic inflammatory cells.

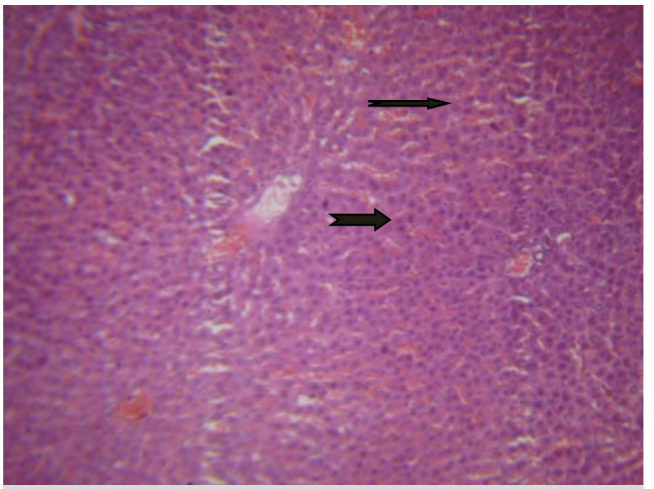

Figure-3. N-Acetylcysteine treated (Group-3) showing normal hepatocytes, mild to moderate infiltration of inflammatory cells and sinusoidal congestion.

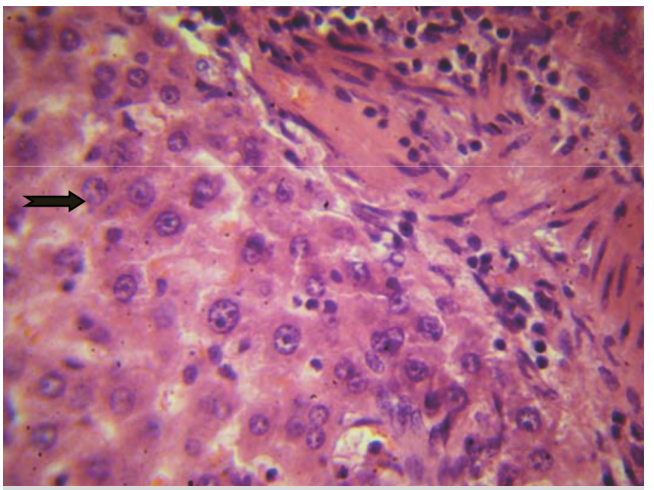

Figure-4. Tecomella treated (Group-4) showing normal looking hepatocytes and mild infiltration of chronic inflammatory cells.

DISCUSSION

Liver is the prime organ that maintains homeostasis of the body through metabolic processes.[18] In our study, liver damage was induced by giving Acetaminophen 500mg orally to experimental animals, and histopathological association of Tecomella bark extract was assessed in contrast with the Standard drug N-Acetylcysteine which is normally used as an antidote in acetaminophen intoxication.

Acetaminophen intoxication causes the formation of N-Acetyl P Benzoquinine (NAPQI). NAPQI is a highly toxic compound. It is converted by Glutathione to non-toxic compounds in liver. When higher doses of Acetaminophen are administered, the body exceeds its capacity to convert it into nontoxic compounds. Since NAPQI produces free radicals and they are highly reactive, therefore, they react with mitochondrial DNA. There is an opening of permeability pores of mitochondria which ultimately leads to increase in mitochondrial permeability. This results in a lack of ATP formation leading to hepatic tissue injury.[19]

Moreover, N-Acetylcysteine increases the Glutathione levels when administered in mice with hepatic injury induced by acetaminophen. Since, N-Acetylcysteine is the parent compound of Glutathione, its administration increases glutathione levels in mice.[20]

Currently, active researches are being conducted on plant based medicines worldwide. Plant based medicines have the advantage that they are economical and easily available and they have less side effects as compared to synthetic drugs. Moreover, there is limited availability of medicines for treating hepatic conditions due to their side effects. Furthermore, drug development also requires extensive resources.[21]

In our study, the Tecomella bark extract at the dose of 200mg/kg and N-Acetylcysteine (Standard Drug) was administered to experimental animals which showed presence of mild to moderate inflammatory cells with the absence of necrotic tissues on liver tissue histology in experimental group as compared to control and hepatotoxic

induced rats. This result is consistent with another study in which the Tecomella extract was given to experimental animals as compared to Silmyrin which revealed that the Tecomella extract maintained normal liver enzymes and liver histology.[22]

The histopathological findings of our study are in line with another study in which there was an improvement in liver architecture and other liver enzymes, when the Tecomella bark extract was given in hepatotoxicity induced rats. Furthermore, the study found profound effect of Tecomella plant extract on liver enzymes and hepatocytes which suggested that free radicals are damaged by various antioxidants present in this plant. Moreover, it was suggested that the Tecomella plant extract produces its hepatoprotective effect by increasing glutathione and catalase levels thus it decreases peroxidation of cellular membrane.[23]

The findings of our study are also in line with another study which revealed hepatoprotective effect of various plants including Tecomella Undulata in normalizing liver function test and histopathological parameters in hepatotoxicity induced by hepatotoxins including thioacetamide in rats.[24]

Our study assessed the effects of the Tecomella plant extract on liver histopathology. However, effects of the Tecomella plant bark extract on other functions of body and side effects were not evaluated. Hence, there is a crucial need to further evaluate its effects on a sample size which is much larger and all inclusive covering other parameters as well. This will help to generate more effective and robust evidence for the assessment of histopathological association of the Tecomella plant extract in acetaminophen induced hepatotoxicity in albino rats.

CONCLUSION

The Tecomella Bark extract improved histoarchitecture on acetaminophen induced hepatotoxicity in albino rats in comparison with N-Acetylcysteine.

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2	Muhammad Kamran	Data collection, Search of literature and revision of article.	
3	Shabih Zehra	Data analysis, Proof reading and final approval of article.	
4	Nighat Jamil	Histopathological interpretation of data, revision and final approval of article.	
5	Ijaz Hussain Zaidi	Conceptulization of study design.