Original Article

HISTOPATHOLOGICAL EVALUATION OF PROTECTIVE EFFECT OF HONEY AGAINST ACETAMINOPHEN-INDUCED HEPATOTOXICITY IN WISTAR ALBINO RATS

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ABSTRACT

Objective: The morphological evaluation of the protective effects of honey against acetaminophen induced hepatotoxicity and to estimate the effects of honey on serum hepatic biomarkers in controls and honey treated Wistar albino rats.

Material and Methods: This experimental study was carried out for 6 months at animal house; Isra University, Hyderabad and Department of Animal Husbandry and Veterinary Sciences Sindh Agriculture University Tando Jam. A total of 60 albino Wistar healthy rats from either sex, 200-300g weight were divided into 05 groups, each comprising of 12 rats (the groups were from A to E namely Negative control group, Positive control group, Pre-treatment group, treatment group and Post-treatment group respectively, in whom doses of paracetamol and honey were given at different time). At the end of 8th week, rat's blood samples were drawn through cardiac puncture for biochemical analysis and liver tissue samples were preserved in 10% formalin, followed by processing for histopathological examination.

Results: The present study showed significant rise in serum bilirubin, ALT and ALP levels in the positive control (Group B) as compared to the negative control group. However, administration of honey significantly decreased the serum bilirubin levels in groups C, D and E. Liver sections from the acetaminophen treated group revealed wide areas of centrilobular coagulative necrosis, inflammatory cell infiltrates and severe congestion and dilatation in both central and portal vein in 83.33 %, 100 % and 100 % of the cases respectively. Honey treated groups given before, after and simultaneously showed improvement with restoration of histologic changes with statistically significant p-value (0.001).

Conclusion: The results of present study have demonstrated satisfactory protection by honey against acetaminophen induced toxicity in liver of rats. It recovers the normal cellular structure along with improvement in the biochemical alterations

Key Words: Acetaminophen, Hepatotoxicity, Serum bilirubin.

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INTRODUCTION

The management of chronic pain is challenging because it may result in severe distress, depression, physical debility, social deprivation, greater exploitation of health service and is a chief cause of expenses to the economy, health systems and others [1]. In common practice analgesics like opioids and acetaminophen are utilized for an indefinite time period with or without prescription at the expense of acquiring their hazardous effects and often taking its overdose [2]. Acetaminophen (N-acetyl-p- aminophenol; APAP) is a globally utilized drug which is available without prescription to alleviate pain and fever. At recommended doses it is considered to be harmless. However, an overdose (>140mg/ kg) or chronic use may result into life threatening hepatotoxicity (acute liver failure) [3]. At recommended dose; its larger amount is conjugated in liver through glucuronidation, sulfation and a small quantity is metabolized via cytochrome P-450 enzyme system to form an electrophile named N-acetyl-p- benzoquinoimine (NAPQI), which is swiftly detoxified by glutathione sulfhydryl (GSH) [4]. On the other hand, supra-therapeutic dose leads to formation of excessive amount of NAPQI which causes exhaustion and depletion of GSH. The adduct bind with organelle membranes to cause lipid peroxidation, mitochondrial membrane permeability transition and mitochondrial respiration failure. All these cascades end into hepatocyte necrosis and acute liver failure [5].

At present, acetaminophen toxicity is the top most source of acute liver failure in United States and many Western countries. The antidote N-acetylcysteine (NAC) was introduced in 1970s. Since then it is the single existing antidote with narrow

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range of efficiency; mostly useful for those who present early [6].

Researchers are now delving into antioxidant potentials of various natural products (Complementary and alternative medicine) to alleviate the toxic effects of acetaminophen to benefit not just at early presentation but also at late presentation or after its chronic use besides being low on budget. Honey is one of those natural products [7]. Based on its physicochemical properties, it is well documented that honey possesses anticancer, antibacterial, anti-inflammatory and antioxidant properties [8]. The antioxidant potential is ascribed to its polyphenolic components (phenolic acids and flavonoids) and colours [9]. Numerous researchers validated that honey serves as a basis of natural medicine, which is helpful in plummeting the peril of heart disease, carcinomas, immune disorders, cataracts, diverse inflammatory processes etc [10]. Keeping all these facts in mind, this study was conducted with objectives to evaluate morphologically, the protective effects of honey against acetaminophen induced hepatotoxicity in wistar albino rats and to estimate the effects of honey on serum hepatic biomarkers in controls and honey treated wistar albino rats.

MATERIAL AND METHODS

This experimental study was carried out for 6 months at animal house; Isra University, Hyderabad and Department of Animal Husbandry and Veterinary Sciences Sindh Agriculture University Tando Jam. Honey (Langnese Honey, Germany's strongest brand) was purchased from local pharmacy, orally administered at the dose of 20gms/kg/body. Acetaminophen (Paracetamol manufactured by WERRICK PHARMACEUTICALS) was purchased from local pharmacy and administered intra orally as 300mg/kg/day [11].

A total of 60 albino Wistar healthy rats from either sex, 200-300g weight were obtained from the animal house of the Sindh Agriculture University Tando Jam. Before starting the experiment, 2-3 ml blood was drawn from retrobulbar area of rats and transferred into plain tube [12]. The samples were carried to Isra University Hospital Laboratory for measuring the serum total bilirubin, ALP, ALT for exclusion of any liver disorder. The tests were performed on HITACHI ANALYZER 902.The rats were housed in stainless steel cages under the hygienic, well-ventilated environment, controlled temperature and 12 hours day night cycle. The rats were fed on standard chow diet and water ad libitum having a scientifically approved composition as per instructions of veterinary experts following NIH guide for the care and use of laboratory animals [13]. The chow was given as raw food. Animals were tagged, weighed and kept in separate cages as control and experimental groups. Animals with normal lab reports were divided into 05 groups each comprising of 12 albino rats. The group A (Negative control group) was given normal saline for 8 weeks. Group B (Positive control group) was given normal saline for 4 weeks followed by paracetamol 300mg/kg/day for 4weeks along with Normal saline. Group C (Pre-treatment group) was given honey 20gms/kg/day for 4 weeks followed by paracetamol 300mg/kg/day for 4 weeks along with honey at the same dose. Group D (treatment group) received same doses of paracetamol admixed with honey for 8 weeks. The animals in group E (Post-treatment group) were given paracetamol 300 mg/kg/day for 4 weeks followed by 20gms/kg/day of honey along with same dose of paracetamol for next 04 weeks.

At the end of 8th week rats were kept fasting for 18 hours and then sacrificed by cervical dislocation. Blood samples were drawn from cardiac puncture, kept in plain tube and liver samples were preserved in 10% formalin and embedded in paraffin for histopathological examination. The specimens were cut into sections measuring 5μm in thickness, followed by staining with hematoxylin-eosin for examination under light microscope (MZ3000 Micros, Austria). All sections were examined for characteristic histological changes like congestion, inflammation, loss of lobular architecture and necrosis. Laboratory and histopathological findings were recorded in proformas. Data analysis was done on SPSS version 22.0 (IBM, incorporation, USA). Serum liver function test were analyzed by using ANOVA. Histological findings were analyzed by Fisher's Exact Test. Statistical significance was taken at $p \leq 0.05$.

RESULTS

The present experimental study showed the protective effects of honey in acetaminophen induced hepatotoxicity in Wistar albino rat models. The mean ± SD of serum bilirubin in the groups A, B, C, D and E was noted as 0.42±0.16, 1.68±0.88, 0.48±0.49, 0.49±0.52 and 0.51±0.32 mg/dl respectively (F-value 9.468, P-value=0.001). A significant (p-value=0.001) rise in serum bilirubin levels was noted in the positive control (Group B) as compared to the negative control group. However, administration of honey significantly (p-value=0.001) decreased the serum bilirubin levels in groups C (Pre-treatment), D (treatment) and E

(post-treatment). Hence it is revealed that honey given not only before and along with toxic dose of acetaminophen shows promising results but it is also equally effective when given after acetaminophen toxicity had occurred.

The mean ± SD of serum ALT in the groups A, B, C, D and E was 23.13±6.91, 81.57±32.27, 25.01±11.08, 25.40±10.21 and 29.15±19.26 mg/dl respectively (F-value 19.79, P-value=0.001). Significant rise in serum ALT levels was noted in the positive control (Group B). Pre-treatment rats (group C) and treatment group rats (group D) responded well to honey treatment. Whereas substantial decrease in its level were seen with honey treatment in group E.

The mean ± SD of Serum ALP in the groups A, B, C, D and E was noted as 85.32 ± 25.32, 180.02 ± 16.00, 98.74 ± 28.02, 97.83 ± 30.78 and 100.02 ± 40.08 U\L respectively (F-value 6.46, P-value=0.01). As compared to normal rats, acetaminophen treated rats (group B) showed significant rise in serum ALP levels. While honey treatment showed a considerable decreased serum ALP level in groups C and D given afore and after acetaminophen dosage respectively. However, post treatment group (group E) also showed equally significant decreased serum ALP level receiving honey after acetaminophen toxicity. Liver sections from the untreated rats (negative control group A) exhibited normal lobular architecture and hepatocytes with acidophilic cytoplasm and single central rounded vesicular nuclei and some bi-nucleated cells. The central veins, portal tracts and sinusoids were of normal appearance.

Acetaminophen treated group (positive control Group B) revealed loss of lobular architecture, wide areas of centrilobular coagulative necrosis, inflammation and severe congestion and dilatation in both central and portal vein in most of the cases as shown in photomicrograph 01-03 (A). On the other hand, honey treatment given prophylactically, concurrently and after prolonged acetaminophen induced toxicity (group C-E) significantly preserved the liver histology evident by absence of necrosis (in 83.33%, 83.33%, 66.66% of cases respectively) and waning of inflammation with presence of normal hepatic cells (in 100%, 75%, 75% of cases respectively) as shown in Photomicrographs 02 (B-D). Effects of honey were also obvious on congested and dilated central and portal vein as most of the cases in all the three honey treated groups (group C-E) exhibited substantial (p=0.001) defense against acetaminophen induced damage and there was little evidence of central vein congestion and dilatation observed in the liver section of these rats (in 83.33%, 75%, 75% of cases respectively) shown in Photomicrograph 03 (B-D).

Table-1: Necrosis in liver among various groups (n=60).

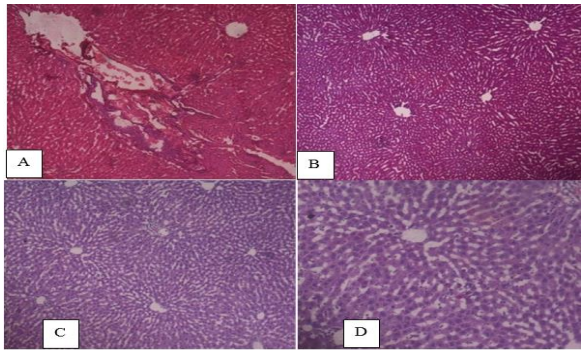
Groups	Necrosis Present	Necrosis Absent	Fisher's value	P value
Group (A) (negative controls)	00	12		
Group (B) (positive controls)	10	02	23.50	0.001
Group (C) (pre-treatment group)	02	10		
Group (D) (treatment group)	02	10		
Group € (Post-treatment group)	04	08		

Table-2: Necrosis in liver among various groups (n=60).

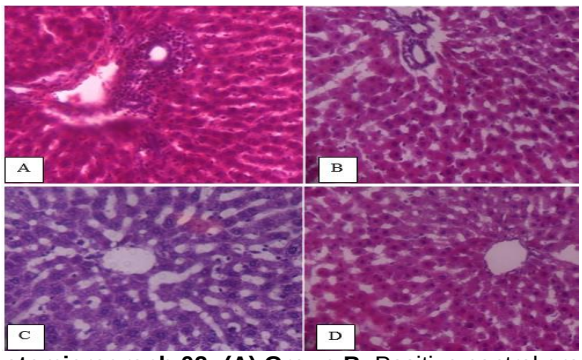
Groups	Necrosis Present	Necrosis Absent	Fisher's value	P value
Group (A) (negative controls)	00	12		
Group (B) (positive controls)	10	00	23.50	0.001
Group (C) (pre-treatment group)	02	10		
Group (D) (treatment group)	03	09		
Group (E) (Post-treatment group)	03	09		

Table-3: Central vein congestion in liver among various groups (n=60).

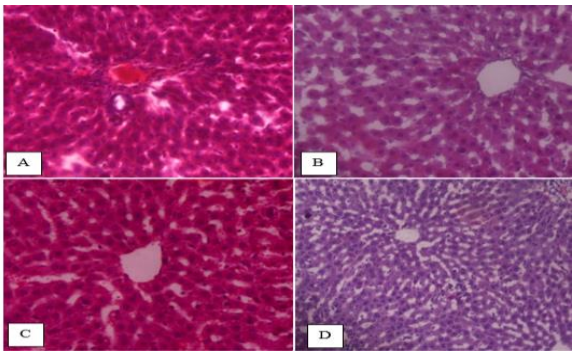
Groups	Congestion Present	Congestion Absent	Fisher's value	P value
Group (A) (Negative Controls)	0	12		
Group (B) (Positive Controls)	12	0		
Group (C) (Pre-treatment group)	2	10	23.79	0.001
Group (D) (Treatment group)	3	9		
Group € (Post-treatment group)	3	9		



Photomicrograph 01: (A) Group B, positive control group showing loss of liver architecture H & E × 100.
(B) Group C, pre-treatment group showing restoration of liver architecture H & E ×100.
(C) Group D, treatment group showing restoration of liver architecture H & E × 100.
(D) Group E, post-treatment group showing restoration of liver architecture H & E ×200.



Photomicrograph 02: (A) Group B, Positive control group showing necrosis & inflammation. H & E × 200.
(B) Group C, Prê-treatment group showing absence of inflammation. H&E × 200.
(C) Group D, treatment group showing absence of inflammation. H& E × 200.
(D) Group E, post-treatment group showing absence of inflammation. H & E × 200.



Photomicrograph 03: (A) Group B, positive control group showing central vein dilation, congestion & inflammation H & E × 200.
(B) Group C, pre-treatment group showing normal looking central vein. (Congestion & inflammation not present). H & E × 200.
(C) Group D, treatment group showing normal looking central vein. (Congestion & inflammation not present).H & E × 200.
(D) Group E, post-treatment group showing normal looking central vein. (Congestion & inflammation not present). H & E × 200.

DISCUSSION

The hepatotoxicity induced by a number of drugs lead us to delve into protective effects of various compounds used in allopathic and herbal medicines. Acetaminophen (Paracetamol) is the most commonly used over the counter analgesic and antipyretic used in Pakistan without knowing its possible hazardous effects [14]. Although it is non-toxic in therapeutic amount but it can cause lethal damage to liver in humans and animals. Therefore, in experiments, it is utilized as a potent hepatotoxicant which can be demonstrated by significant increase of liver enzymes in serum and distorted liver architecture [15]. Honey has been traditionally used to alleviate many diseases since ancient medicine. Lately, various studies have been conducted to see the protective effects of honey. This study was intended to investigate the effects of honey on acetaminophen prompted hepatic injury in Wistar albino rats. To the best of knowledge, previously none of the studies has examined the effects of honey after chronic utilization of acetaminophen.

The current data undeniably confirmed that honey counterbalances APAP induced assessment such as total bilirubin, AST, ALT, and ALP. Elevation of these enzymes in blood after acetaminophen ingestion indicates injury to liver with hypofunction [16]. The present study showed that the hepatic enzymes (serum bilirubin, ALT and ALP) were markedly raised in positive control groups following acetaminophen ingestion representing considerable waning of the hepatic functions owing to cellular damage which is ascribed to the progression of hepatotoxicity. These ratios remained normal in pre-treatment and treatment groups. A significant preventive role of honey can be demonstrated in the pre-treatment groups in which the enzyme levels had remained closer to those in negative control groups. Remarkably, in post treatment groups, the level of hepatic enzymes started declining towards normal ranges. Thus, we can conclude from these results that honey can protect and restore liver even after the damage has been done by APAP. These results are corroborated by Wang Y *et al* who suggested that honey possibly stabilizes the cell boundaries of hepatocytes and kindles their regeneration. In that way it gives the idea that honey relieves the acetaminophen induced intoxication [17]. Erejuwa *et al* reported that honey considerably decreases the raised-up levels of AST, ALT and ALP hence employs hepatoprotective outcome by reducing oxidative stress [18].

The damage by oxidative stress was also confirmed by some other researchers such as Zhou *et al*(2015), Simeonova *et al*. (2013) etc who have stated that acetaminophen produces excessive amount of reactive oxygen radicals (ROS) in conjunction with enfeebled antioxidant scavenging system of body which is illustrated as significantly declined levels of antioxidant enzymes superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) [19] [20]. Wang Y *et al* reported that pre-treatment with honey also offsets the drop in these antioxidant enzymes and successively restrained the oxidative stress [17].

The histopathological results of present study are not different from the biochemical results. The signs of APAP induced hepatotoxicity in positive control group observed in this study include loss of liver architecture, central vein congestion and dilation, necrosis and Inflammation. The use of honey prior to APAP or in conjunction revealed preservation of cellular structure with very little amount of damage which principally validated the protective effect of honey. These results are in similarity with those testified by others [21]. Korkmaz and Kolankaya (2009) witnessed that there was no hepatocyte congestion and inflammation in rats treated with honey before Nethylmaleimide (NEM). They also stated that pre-treatment with honey virtually entirely thwarts the NEM-provoked inflammatory changes in the hepatocytes of rats via non- protein sulfhydryl groups in a number of amino acids plus other constituents of honey [22]. Present study further confirmed the role of honey in post-treatment groups and observed the healing and repairing effects of honey by reducing inflammation and necrosis with restoration of normal cellular architecture in liver. Furthermore, Omar NA *et al*[23] and El Denshary *et al*[11] confirmed a significant defensive outcome of honey in combination with other remedies such as ginseng. They also found a potent antioxidant and anti- inflammatory role of honey in carbon tetrachloride-induced hepatotoxicity. Hence honey can be cogitated a novel biological antioxidant remedy which acts on various cellular pathways and molecular targets and exhibits multiple beneficial effects against inflammation, oxidative stress, altered immune system, diabetes, cardiovascular diseases, various neoplasia and mutations and infertility [24].

Though, further analysis is indispensable to illuminate the precise mechanism of how honey protects against such hepatotoxicity in humans and to recognize the varieties of antioxidants present in honey. This is supported by numerous earlier researches which confirmed that mice deficient of antioxidant enzymes such as Superoxide Dismutase (SOD) or those treated with substances that diminish antioxidants promptly could not withstand acetaminophen-induced hepatotoxicity [25]. On the contrary, animals showing over expression of antioxidants comprising SOD1 and plasma Glutathione peroxidase (GPx) or GSH synthesis enhancing enzymes such as glutamate cysteine ligase are resilient to its toxicity [26].

CONCLUSION

As the researchers are looking forward to modernizing drug manufacturing from natural resources with minimum side effects, the use of honey can be highlighted for the prevention and treatment of several ailments. The results of present study demonstrated satisfactory protection by honey against acetaminophen induced toxicity in liver of rats. It recovers the normal cellular structure along with improvement in the biochemical alterations. Hence, honey can be used as a low-priced substitute/curative agent which can be taken daily to counter regulate hepatic and renal toxicity mediated by acetaminophen not only prior to taking APAP but also benefits those who did not have honey earlier.

AUTHORS CONTRIBUTION

Shumaila Khowaja: Conception and design, acquisition of data

Farkhunda Nadeem: Analysis and interpretation of data, methodology

Shomail Siddiqui: Acquisition of data, literature review

Amin Fahim: Statistical Analysis, drafting the article

Abdul Majid: Methodology**,** critical revision

Ghulam Shah Nizamani: Literature review, proof reading

Shankar Lal Rathi: Supervision, final version of the manuscript

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