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ORIGINAL ARTICLE

Anti-oxidative Role of Azadirachta Indica and Vitamin E in Restoration of Histopathological changes in Acetaminophen induced liver toxicity: a comparative animal study

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ABSTRACT

Aim: To study the comparative effect of Acetaminophen induced liver toxicity with aqueous extract of Azadirachta Indica (Neem) and vitamin E on the basis of liver Histopathology.

Methods: Sixty Wistar Rats of both sexes were split into four groups. Each group contained 15 animals. The control group was group A, Group B was treated orally with single dose of Paracetamol 2 mg / kg by weight, Group C was administrated orally with aqueous Neem extract 500 mg/kg +2 mg/Kg by weight oral Paracetamol, and Group D was given orally paracetamol+Neem extract+Vitamin E with 100mg/Kg/body weight for 15 days. Rats from all groups were decapitated, the liver was sliced, and liver tissues were taken for histological examination. Tissue samples were fixed in 10% formaldehyde, embedding in paraffin followed by Hematoxylin and Eosin dye (H&E) and observed under 400x magnification with a digital microscope.

Results: On Histopathological examination of the rat`s liver in we found that the control group had a normal appearance, colour, and uniform surface without any necrosis. Group B showed severe necrosis and haemorrhagic patches. In comparison, Group C revealed normal appearance, colour, and smooth surface with no necrotic alterations. Livers from the group D looked virtually normal in terms of colour, undersurfaces, and organ weight. However, hepatoprotective effects were observed in the Group C and D. Therefore, we can conclude that Azadirachta indica and Vitamin E could serve as a good medication for defence against liver injury.

Conclusion: Our findings showed thatextract of Azadirachta Indica and Vitamin E exhibited hepatoprotective effects on the Wistar rats that were subjected to Acetaminophen.

Key words: Azadirachta Indica leaf extract, Vitamin E, hepatoprotective Paracetamol, Wistar rats.

INTRODUCTION

AzadirachtaIndica (Neem plant), is the most effective and extensively used medicinal plant throughout the world[1]. The United State National Academy of Sciences has issued a report with the title, “Neem-a tree for solving global problem”, which aimed to review all aspects of Neem tree including biological activities, pharmacological actions, clinical trials, and possible medicinal application of neem compounds or extracts together with their safetyvaluation. The leaves of the this tree have beneficial effects and are traditionally used in preparations of a lot ofmedicines havingimmunomodulatory, anti-hepatotoxicity, anti-oxidative stress, anti-inflammatory, anti-hyperglycemic, anti-ulcer, anti-malarial, anti-fungal, anti-bacterial, anti-viral, anti-mutagenic, and anti-carcancereffects[2,9]. AzadirachtaIndica has an anti-oxidant and chemoprotective effect which is due to the presence of several active compounds like quercetin, nimbin, nimbidiol, azadirachtin, and nimbidin.Mallicket al. hasconfirmed that neem leaves extract has non-toxic effect on liver and kidney of rats, even in higher doses[8].

lot of *in vivo* study has been documented on use of Neem extract for the treatment of hepatotoxicity in rats.Isolation of Quercetin and β-sitosterol from the leaves of Azadirachtaindica has been documented[4]. Quercetin is categorized into flavonol (subclass of flavonoid compounds), which has an active antioxidant potential as Vitamin E[5]. β- sitosterol is a type of plant sterol (phytosterols) which is potent Anti-inflammatory and immunomodulator[6]. Yanpallewar et al. reported that the components found in neem leavesare responsible for lipid peroxidation inhibition. Moreover he also found that neem extract more likely to protect the liver from paracetamol induced hepatic damage by acting as a potent antioxidant[3].

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Vitamin E is lipid soluble antioxidant found in liver which can effectively preserve membrane integrity of liver damaged by toxicant or carcinogen that produce ROS (Reactive oxygen species), responsible for oxidative destruction by trapping reactive oxy-radicals[7].The free radicals are responsible for lipid peroxidation which is involved in the pathogenesis of numerous diseases. Vitamin E prevents lipid peroxidation *in vivo* as well as *in vitro*. These findings propose that vitamin E reduces the risk and it is useful in the treatment of liver diseases mediated by free radicals[15].

The most commonly used analgesic in the world is Acetaminophen (Paracetamol), discovered in 1889, which is an active metabolite of phenacetin and is the first line of treatment of cancer pain recommended by WHO. Its excessive use lead to hepatic necrosis, liver cirrhosis, acute renal toxicity, liver failure, and over dose even can lead to death[10,11].

Over the past few eras, there have been several cases reported against acetaminophen induced liver toxicity and studies involving the association between therapeutic doses of acetaminophen and liver damage[12]. However, it is referred to as non-toxic when administrated in recommended therapeutic doses which is not more than 4 g per day. High dose of paracetamol may associate with acute liver failure, hepatic necrosis, hypoglycaemia, renal tubular necrosis[13,14].

The present study aimed to assess the hepatoprotective effect of aqueous extract of Azadirachta Indica and Vitamin E, if any, on Acetaminophen-induced hepatotoxicity.

MATERIALS AND METHODS

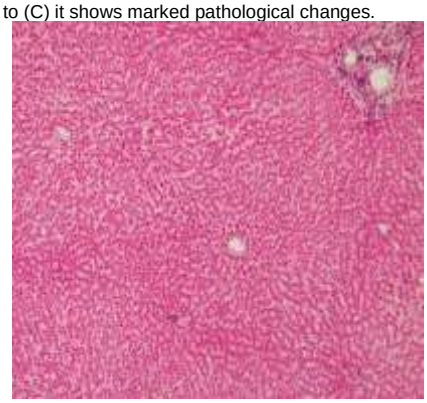
The present study was designed at the Department of Pharmacology, Indus Medical College and Hospital, Tando Mohammad Khan, between the duration of February 2021 to July 2021. The experiment was performed after taken an ethical approval from the concerned authority.

Animals and Experimental Design: Total 32 wistar male rats of 150-250 gram were selected randomly from Animal house of Indus Medical College. On the basis of different therapy the animals were distributed into four groups. Each group comprises of 8 rats kept in separate cages. The groupings were made as follows:
Group A was our control group and received normal diet.
Group B wastreated with paracetamo l2gm / kg per body weight orally.
Group C was served with the extract of Azadirachta Indica 500 mg / kg per body weight along with Paracetamol single dose for about 2 weeks.
Group D was administered with a combined formula of paracetamol + Neem extract + Vitamin-E 100mg/Kg/body weight for 2 weeks.
The animals in cages were free to move, chow standard pellet and drinking water.
Preparation for Neem Extract: The Fresh tender green leaves of Azadirachta Indica were bought from the local plant shop; they were thoroughly cleaned with tap water to eliminate any detritus present on leaves. Leaves were separated, chopped and boiled for half an hour in a beaker with double distilled water. After boiling for half an hour we got the neem extract finally. The extract was cooled at room temperature and then filtered through a filter paper. The filtrate was stored at cool place.
Experimental Procedure: For histopathological examination, rats from of all groups were sacrificed by decapitation and liver was dissected out and liver samples were collected. Tissue samples were sliced into small pieces and fixed immediately in 10% phosphate buffered formaldehyde for 24-48 hours. The piece of tissue was go through a number of process including dehydration with 70%, 80% and 95% ethyl alcohol for one an hour each, clearing with xylene for one an hour, infiltration with molten paraplast at 58 ^{0}C followed by casting (block embedding), longitudinal sectioning of about 3 µm thickness, mounting, and staining with haematoxylin and eosin stain and then photographed. The slides were observed under 400 x magnifications with a digital microscope.

RESULTS

The sections of liver were taken from each group and were observed for the normal histological and morphological structures. Photomicrograph of liver sections were stained with haematoxylin and eosin dye (H&E). The slides were taken to compare the portal areas of liver which are very important for histomorphological study in control and tested groups at 100x magnification.
Histopathological Morphology in Control Group A: In our control group, polygonal prism shaped lobules of liver was preserved with no change in cross section of each lobule, and each lobule presenting normal hepatocytes arranged into hepatic cords separated through sinusoids as showed in figure 1.
Histopathological Morphology in Group B: Figure 2 showed acetaminophen induced liver injury in albino rats. Normal parenchyma distorted, central vein is dilated and filled with haemorrhages, moreover, congestion in blood vessels with massive necrotic hepatocytes were observed in this group.
Histopathological Morphology in Group: This group was treated to observe the hepatoprotective effects of Neem extractin Albino rats. Figure 3 indicates regeneration of hepatocytes with removal of necrotic tissues from hepatic parenchyma. Furthermore, few inflammatory cells and dilated sinusoids were also observed which indicates recovery and resolution.
Histopathological Morphology in Group D: Treatment of rats with a combination of paracetamol + Neem extract + Vitamin-E mainly obstruct the acetaminophen-induced histopathological changes in the liver, which can be observed in figure 4 as specified by a decrease inhepatocytic injuries and inflammatory cellular infiltration. All findings were reconfirmed by the Department of Histopathology, AL-Tibri Medical College Karachi.
Vitamin E

Figure 4: Liver section of group (B) showing mildly distorted hepatic architecture of lobule. Hepatocytes damaged and mild infiltration of immune cells (Thick arrows). Dilated sinusoids (Thin arrows) and portal vein (PV). Considerable numbers of cytoplasmic clear vacuoles (Arrow heads) are visible. In comparison to group (A) figure is histologically better but in comparison to (C) it shows marked pathological changes.



DISCUSSION

Therapies designed in accordance with contemporary medicine are frequently ineffective, carry the potential of adverse effects, and are often prohibitively expensive, particularly in underdeveloped countries. As a result, treating liver illnesses with natural substances that are readily available and do not require time-consuming pharmaceutical synthesis appears to be very promising. The liver the major drug metabolizing and detoxifying organ in the body, and is subject to potential damage from the many of the widely used therapeutic drugs, including remedies, synthetic medicines, chemicals, and antibiotics. More than 600 medicines are documented as being capable of creating hepatic damages[16]. For this reason, an efficient anti-oxidant defense system found in our liver that becomes overload under oxidative stress[7,20].

In various studies it has been proved that drugs administrated for the treatment of hepatotoxicity having deleterious effects. For this purpose, our aim of study is to investigate the effect of most beneficial medicinal plants Neem and Vitamin E and to check their hepatoprotective effects on liver damage that's occurs due to high dose of paracetamol[19]. There are several other herbal plants like ParkiaBiglobosa stem bark which also have hepatoprotective effects[17].

According to Oyagbemi et al. Vitamin E should be advantageous to individuals who are suffering from oxidative stress. In addition,hepatoprotective effect of Azadirachtaindica was also shown to be comparable to that of vitamin E in this investigation. As a result, when Azadirachtaindica or vitamin E was used as a pretreatment in a corresponding dose for a period of time, hepatocyte damage was prevented[2].

Previous study explained that the hepatoprotective effect of Azadirachtaindica was found to be equivalent to that of vitamin E. As a result, pretreatment with Azadirachtaindica or vitamin E decreased hepatocyte damage, which could be related to anti-oxidative and lipid peroxidation inhibition property of Azadirachtaindica. These properties of Neem leaves play a fundamental role in fight against free radicals and other oxidant storms[2,18]. Histopathological findings from different resources showed that AzadirachtaIndica and Vitamin E has also a positive influence on biochemical parameters. The defensive alterations to cellular injury are reflected in the healing activity found in liver cells of animals treated with paracetamol[21,22].

Histopathological examination of the rat`s liver in the control group had a normal appearance, red colour, smooth and uniform surface with no signs of haemorrhage or necrosis. On the other hand, Group B (paracetamol-treated group), revealed severe centrilobular necrosis with sinusoids congestion, and tiny lipid globules in the liver. In contrast, Group C (AzadirachtaIndica

leaves aqueous extract treated group) revealed virtually normal lobular architecture with moderate centrilobular degeneration of hepatocytes with no necrotic alterations. Livers from the group D (AzadirachtaIndica leaves aqueous extract + Vitamin E+ paracetamol) looked virtually normal in terms of colour, undersurfaces, and organ weight.

CONCLUSION

Macroscopic and histological examination of our data revealed that aqueous extract of AzadirachtaIndica and Vitamin E plays a major role in reducing paracetamol induced liver damage and produce hepatoprotective effect in Albino rats.

Conflict of interest: None

REFERENCE

1. Dkhil MA, Al-Quraishy S, Aref AM, Othman MS, El-Deib KM, Abdel Moneim AE. The potential role of Azadirachtaindica treatment on cisplatin-induced hepatotoxicity and oxidative stress in female rats. Oxidative Medicine and Cellular Longevity. 2013 Dec 4;2013.
2. Ajibola M, Olugbemi O, Stephanie A, Joseph D, Denen A. Hepatoprotective effect of Parkiabiglobosa stem bark methanolic extract on paracetamol induced liver damage in wistar rats. Am. J. Biomed. Life Sci. 2013;1:75-8.
3. Yanpallewar SU, Sen S, Tapas S, Kumar M, Raju SS, Acharya SB. Effect of Azadirachtaindica on paracetamol-induced hepatic damage in albino rats. Phytomedicine. 2003 Jan 1;10(5):391-6.
4. Pandey G, Verma KK, Singh M. Evaluation of phytochemical, antibacterial and free radical scavenging properties of Azadirachtaindica (neem) leaves. Int J Pharm Pharm Sci. 2014;6(2):444-7..
5. Li Y, Yao J, Han C, Yang J, Chaudhry MT, Wang S, Liu H, Yin Y. Quercetin, inflammation and immunity. Nutrients. 2016 Mar;8(3):167.
6. Trivedi A, Fatima N, Husain I, Misra A. An update on the therapeutic potential of neem and its active constituents: A panacea for all diseases. Era's Journal of Medical Research. 2019;6(1):110-7.
7. Naziroğlu M, Çay M. Protective role of intraperitoneally administered vitamin E and selenium on the antioxidativedefense mechanisms in rats with diabetes induced by streptozotocin. Biological trace element research. 2001 Feb;79(2):149-59.
8. Mallick A, Ghosh S, Banerjee S, Majumder S, Das A, Mondal B, Barik S, Goswami KK, Pal S, Laskar S, Sarkar K. Neem leaf glycoprotein is nontoxic to physiological functions of Swiss mice and Sprague Dawley rats: histological, biochemical and immunological perspectives. International immunopharmacology. 2013 Jan 1;15(1):73-83..
9. Chattopadhyay R. Possible mechanism of hepatoprotective activity of Azadirachtaindica leaf extract: part II. Journal of ethnopharmacology. 2003 Dec 1;89(2-3):217-9.
10. Ibrahim MA, Wani FA, Rahiman S. Hepatoprotective effect of olive oil and camel milk on acetaminophen-induced liver toxicity in mice. International Journal of Medical Science and Public Health. 2017 Jan 1;6(1):186-94.
11. Roberts E, Nunes VD, Buckner S, Latchem S, Constanti M, Miller P, Doherty M, Zhang W, Birrell F, Porcheret M, Dziedzic K. Paracetamol: not as safe as we thought? A systematic literature review of observational studies. Annals of the rheumatic diseases. 2016 Mar 1;75(3):552-9.
12. Prescott LF. Paracetamol, alcohol and the liver. British journal of clinical pharmacology. 2000 Apr;49(4):291-301.
13. Tittarelli R, Pellegrini M, Scarpellini MG, Marinelli E, Bruti V, Di Luca NM, Busardò FP, Zaami S. Hepatotoxicity of paracetamol and related fatalities. Eur Rev Med Pharmacol Sci. 2017 Mar 1;21(1 Suppl):95-101.
14. McEvoy, G. K. AHFS Drug Information 2007. Bethesda, MD: American Society of Health-System Pharmacists. Inc, 2007.
15. Niki E. Evidence for beneficial effects of vitamin E. The Korean journal of internal medicine. 2015 Sep; 30(5):571.
16. Bhanwra S, Singh J, Khosla P. Effect of Azadirachtaindica (Neem) leaf aqueous extract on paracetamol-induced liver damage in rats. Indian journal of physiology and pharmacology. 2000 Jan 1;44(1):64-8.
17. Oyagbemi AA, Omobowale TO, Ola-Davies OE, Adejumobi OA, Asenuga ER, Adeniji FK, Adedapo AA, Yakubu MA. Protective effect of Azadirachtaindica and vitamin E against arsenic acid-induced genotoxicity and apoptosis in rats. Journal of dietary supplements. 2018 May 4;15(3):251-68.
18. Islam MT, Quispe C, Mubarak MS, Salehi B, Reiner Ž, Martorell M, Sharifi-Rad J, Setzer WN. Protective Effects of Natural Products and Their Derivatives on Genetic Material: A Critical Review. RECORDS OF NATURAL PRODUCTS. 2021 Nov 1;15(6):433-62.
19. Vietmeyer ND. Neem: a tree for solving global problems. Report of an ad hoc panel of the Board on Science and Technology for International Development, National Research Council. National Academy Press; 1992.
20. Bhanwra S, Singh J, Khosla P. Effect of Azadirachtaindica (Neem) leaf aqueous extract on paracetamol-induced liver damage in rats. Indian journal of physiology and pharmacology. 2000 Jan 1;44(1):64-8.
21. Nwobodo EI, Nwosu DC, Ogbodo SO, Ugwuene FO, Ihim AC, Ani NO, Nnodim JK, Ani O. Effects of Azadirachtaindica leaf aqeous extract on the antioxidant enzymes in paracetamol-induced hepatotoxicity in Wistar rats. International journal of biological and chemical sciences. 2018;12(1):1-0.
22. Khalique-ur-Rehman, Khan H, Soomro SH, Jiskani AR, Faisal R, Hameed F. Restoration of spermatozoa: Antioxidant effects of virgin coconut oil and corn oil in Phenytoin induced toxicity of seminiferous tubules in rats. The Professional Medical Journal. 2020 Dec 10;27(12):2564-8.