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ORIGINAL ARTICLE

Hepatoprotective role of Azadirachta Indica and Vitamin E in acetaminophen induced liver toxicity in Wistar rats on the basis of liver enzyme

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ABSTRACT

Aim To study the comparative effect of acetaminophen with aqueous Neem leaf extract (Azadirachta Indica) and vitamin E mediated liver toxicity on the basis of liver enzymes.

Methods: A total of sixty (60) Wistar rats of either sex were divided equally into four groups. Each groupwas made up of 15 animals. Group A was the control group. Animals in Group B were treated with a single oral dose of 2 mg / kg b / w Paracetamol. Group C animals with 500 mg / kg b / w oral Neem extract for 15 days with oral administration of 2 mg / kg b / w oral Paracetamol. In Group D, animals received the same dose of Paracetamol and 100 mg / kg b / w intra-peritoneal vitamin E for 15 days, respectively. The liver enzymes ALT,AST, and ALP were then evaluated. Data was analyzed using SPSS Version 20.0 with level of significance being kept at p-value ≤0.05

Results: In the 4 groups, The ALT values were 22.8 (Group A), 100 (Group B), 29.11 (Group C), and 31.16 U/L (Group D). The AST values were 25 (Group A), 40 (Group B), 20 (Group C), and 15 (Group D) U/L. The ALP values were 220 (Group A), 445 (Group B), 242 (Group C), and 244 (Group D) U/L. There was significant increase in liver enzymes were found in Group B after induction of Paracetamol toxicity, however, hepatoprotective effects could be seen in the intervention Group C and D

Conclusion: Azadirachta Indica and Vitamin E showed hepatoprotective effects on the Wistar rats that were subjected to Paracetamol

Key words: Azadirachta Indicaleaf extract, Vitamin E, Paracetamol, Wistar rats.

INTRODUCTION

The most pivotal organ in the body that regulates many of the metabolic processes of our body is the liver. Located in the right hypochondrium of your abdomen, liver serves many functions in our body. From the mere synthesis of proteins, to the detoxification of waste, liver does all of this and more [1]. The liver is also active in drug absorption and detoxification and its undesirable compounds that can be hepatotoxic[2-3].

Hepatocytes may be fatally damaged by a number of items, including chemicals, alcohol intake and viral infections. The possible mechanism involved in chemical-induced hepatocyte injury is primarily by oxidation of fats and other different enzymes that carry out the process of oxidation. Extended alcohol consumption induces hepatic pathologies in the body, hypertriglyceridemia and eventually full out liver failure through variations in the free radical producing oxidant-antioxidant framework. Hepatic damage-induced drug pathogenesis typically occurs through the formation of various enzymes and free radical that are harmful. This consequently causes the lipid bilayer of the hepatocyte to oxidize, ultimately resulting in the necrosis of the hepatocytes, alterations in the fat content, and other noxious changes in the body[4-5].

Long and tiresome research and studies have gone onto implicate that more than a thousand drugs are responsible for causing impairment to the liver. Some of the drugs that are mentioned include halothane, various anti-epileptic agents, and Acetaminophen whose potential misuse causes serious fatality of the liver [6-7]. Paracetamol is metabolized by three step pathway in the liver: Glucuronidation, Sulfation (both account for 95 percent) and cytochrome p-450 (5%). A quantity of acetaminophen is transformed into a potentially hepatotoxic intermediate compound of Quinone by cytochrome p-450. The product is however, rapidly halted from causing any harm in the body. This is done by conjugating it with a substance called Glutathione. However, if somehow the amount of Glutathione is reduced in the body, the Quinine intermediate can then easily build up in the body causing havoc to the liver, ultimately resulting in damaged to the liver cells[8]

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Furthermore, studies have gone onto indicate cases of renal failure being observed in patients taken high doses of acetaminophen. Paracetamol over dose also affects the heart, resulting in ST segment defects, flattening of the T wave, pericarditis and myocardial infarcation[9-10].

As conventional medicine, killing of insects and antiseptic activities, the Neem leaves and its other components were used. In addition to these current studies, its antitumor, anticancer, anti-malarial and hypoglycemic activitieshave been recorded[11-12]. The effects of hepatic damage caused by Neem leaf extract in Paracetamol in rats have been studied. Utilizing extracts of Neem leaves led to a reduction of the hepatic enzyme to normal levels was found[13].

For hepatoprotective properties, many chemical agents are screened. Vitamin E is a popular compound, regarded widely because of its documented hepatoprotective effect[14]. Due to this special property, Vitamin E has received extensive attention. Vitamin E is an antioxidant which is normal and fat soluble and has been reported to have hepatoprotective properties. Vitamin E works as a scavenger, finding reactive oxygen species in the body, and detoxifying them so that they don't cause any harmful activities. The reduction of the effects caused by Paracetamol by the use of vitamin E and Azadirachta Indica remains to be validated. The goal of this research is to determine the hepatoprotective effect of vitamin E compared to Azadirachta Indica, in the overdose of Paracetamol toxicity.

METHODOLOGY

The experimental study was designed at the Department of Pharmacology, Shahida Islam Medical and Dental College, lodhran. Between the duration of April to September 2020. Total 32 numbers of male Wistar rats were randomized selected with weight of 150-250 Gms from the animal house after taken en ethical approval from the concerned authority. The animals were divided into four groups on the basis of therapy. Each group contains 8 rats and kept in separate cages with the maintenance of light and dark cycle. Group A (Control) given normal diet, Group B treated with paracetamol2gm / kg body weight orally at single dose, then they were observed for 24 hours and then blood samples were taken for biochemical parameters of I liver enzymes (AST, ALT,

ALP). Group C were given an oral Neem extract of 500 mg / kg body weight for 2 weeks days along with Paracetamol single dose. Group D were administered paracetamol+Neem extract+Vitamin E with 100mg/Kg/body weight for 15 days. The blood sample was taken from each group to compare the liver enzymes among the different treated groups. The leaves of Neem were taken from the local plant shop, after which they were thoroughly cleaned using tap water to remove debris. Leaves were finely cut in a beaker with double distilled water and boiled for half an hour. The extract was cooled and then filtered using a filter paper.The data was analyzed through SPSS version 20.0. To compare the Mean values of serum one way ANOVA followed by post hoc tukey's test applied and the level of significance was kept at P-value ≤0.05.

Compare the Mean values of liver Enzymes among different therapeutic groups

ALT	GP B vs A	GP B vs C	GP B vs D
	≤0.001	≤0.001	≤0.001

RESULTS

Figure 1 shows the Mean value of liver enzymes among different therapeutic groups (U/L). There was significant increase in liver enzymes were found in Group B after induction of Paracetamol toxicity.

Table 1 shows the comparison of Mean value between different therapeutic groups presented in the form of level of significance. The significant value considered to be P=<0.05.

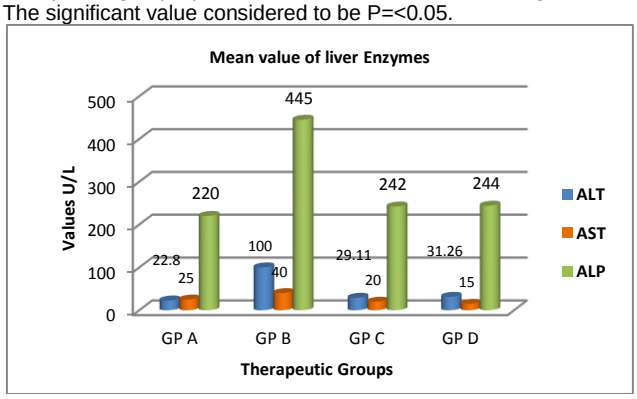

Table 1: Level of significance among different therapeutic groups

AST	≤0.001	≤0.001	≤0.001
ALT	≤0.001	≤0.001	≤0.001

One way ANOVA (post hic tukey's test) applied P=<0.05
ALT: ALANINE TRANSAMINAS, AST: ASPARTATE AMINOTRANSFERASE, ALP: ALKALINE PHOSPHATASE

DISCUSSION

The body's main centre for drug absorption and for breakdown of any toxic metabolic content is the liver. One issue that has been recognized regarding the liver is that toxic substances in our body such as drugs and other metabolic waste products can insult the liver [15]. Paracetamol is a very famous drug that is used to suppress the COX enzyme, exhibiting the properties anti-pyrexia, anti-inflammatory, and analgesia[16]. Although, Paracetamol is a widely used compound, it's most major side effect is hepatocellular necrosis. Cellular death of hepatic issue ensures due to the production of an intermediate compound Quinone, which is unable to be excreted by the kidney. Furthermore, depletion of Glutathione induces high levels of oxidative stress in the body leading to apoptosis of hepatocytes.

Vitamin E is a fat-soluble vitamin found also in the phospholipid bilayer of the cell membrane, where it plays a significant role in shielding the cells from various free radicals in the body that can cause oxidation of unsaturated fats. Vitamin E is well documented for exhibiting a hepatoprotective effect, and due to this property has received widespread attention in the world of medicine[17-18]. Studies have shown that the administration of Vitamin E has reduced the oxidative stress on the liver, by reducing MDA levels, restoring Glutathione, Superoxide Dismutase, and Catalase levels in the body. By regulating these important biochemicals in the body, the liver architecture remains preserved and repaired.

Neem Leave has shown to be very similar to Vitamin E, demonstrating a protective effect on the liver tissue, minimizing the damage caused by Paracetamol[13]. Both group C and D showed hepatoprotective effect with both having significant difference (P-value ≤0.05) when compared to group B. The protective nature of Neem Leaves was demonstrated in another study as well, on dogs that were induced with oxidative stress by Trypanosoma Brucei[19]. In another study the hepatoprotective effects of Azadirachta Indicaand methanolic extracts were evaluated on Wistar male rats with that study also indicating that Azadirachta Indica had a good potential to act as a hepatoprotective agent[20]. Neem leaves and Vitamin E both protected the liver and preventing liver damaged which can be seen by assessing the liver enzyme levels.

Group C and D both had very similar enzymes leaves which can also mean that adding Vitamin E didn't further amplify the protective effect on the hepatic parenchyma, meaning that giving Neem Leaves and Vitamin E in combination doesn't necessarily double the hepatoprotective effect. Future researches can be done to study the histology of liver parenchyma when they are subjected to Paracetamol, while also evaluating the protective effect of Neem leaves and Vitamin E as well as determining the exact mechanism of how Neem leaves show their protective nature.

CONCLUSION

Paracetamol is a harmful drug to the liver, inducing hepatic necrosis. However, Azadirachta Indicaand Vitamin E both are potent substance in protecting the liver from the noxious attack by Paracetamol,

Conflict of interest: Nil

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