Original Article

Effects of Neem and N-Acetyleysteine on Damaged Liver

Comparative Study of Effects of Azadirachtaindica (Neem) Leaf Aqueous Extract and N-Acetylcysteine on Paracetamol Induced Liver Damage in Rats

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ABSTRACT

Objective: To study the comparative effects of aqueous Neem leaf extract with N-Acetylcysteine on the basis of liver enzymes (AST, ALT, ALP) and histopathological changes in paracetamol induced liver damage.

Study Design: Experimental / Interventional comparative study.

Place and Duration of Study: This study was conducted at the Pharmacology Department, Al-Tibri Medical College, Karachi from January 2015 to June 2015.

Materials and Methods: Total sixty (60) albino rats of either gender were equally divided into four (04) respective groups. Each group comprised of 15 animals. Animals of group A were considered as un-treated or control group. In group B animals were treated with a single dose 2mg/kg b/w of paracetamol orally. Group C animals with neem extract 500mg/kg b/w orally for 15 days along with oral administration of 2mg/kg b/w paracetamol. In group D, animals were treated with same dose of paracetamol and 140mg/kg b/w of N-Acetylcysteine intraperitoneal for 06 days.

Results: The results showed that the liver enzymes were markedly increased in paracetamol treated group of animals, but decreased when animals were treated with Neem and N-Acetylcesteine. The mean serum level of enzymes such as AST, ALT and ALP were found to be more i.e, 110.8, 40.00 and 444.33 respectively but the mean level decreased in the animals of group C such as 29.133, 20.00 and 240.33. However, liver enzymes were also reduced in group D but their levels were relatively lesser than animals of group C. Regarding histopathological review, the tissue sections showed necrotic hepatocytes, congestion in blood vessels in paracetamol treated group of animals. However, the changes were found significantly reversed in group C and group D, but marked changes were seen in animals of group C as compared to N-Acetylcysteine treated group of animals.

Conclusion: Paracetamol is a hepatotoxic drug causing histomorphological damage in liver along with alteration in the level of Liver enzymes. Azadirachta indica leaves have given better results compared to N-Acetylcysteine, on the basis of significant differences in biochemical parameters.

Key Words: Azadirachita indica leaf extract, N-Acetylcysteine, Paracetamol, Albino rats

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INTRODUCTION

Liver is the most important organ which plays a pivotal role in regulating various physiological processes in the body. It is involved in several vital functions such as metabolism, secretions, storage, regulation of serum glucose concentration, lipid metabolism and detoxification of various waste material[1]. The liver is also involved in the metabolism and detoxification of drugs and their unwanted substances which may be hepatotoxic other wise[2,3]

The variety of substances including chemicals, alcohol consumption and viral infections can cause lethal injury to hepatocytes. The probable mechanism involved in the injury to the hepatocytes induced by the chemicals is mainly through lipid per oxidation and other oxidative enzymes. Long term use of alcohol potentially causes liver diseases, hypertriglyceridemia and cirrhosis by changes in oxidant–antioxidant system by generating free radicals. The pathogenesis involved in the drugs induced hepatic damage is usually through production of toxic radicals and other metabolic enzymes, which in turn results in per oxidation of lipid

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bilayer of hepatocytes causing hepatocytes cell death, fatty change and other inflammatory changes[4,5].

An estimated 1000 drugs have been implicated causing liver diseases eg: Halothane and Anticonvulsants drugs etc[6]. Few of the drugs especially, Acetaminophen, if misused either intentionally or accidentally can cause significant liver damage[7,8]. Paracetamol is metabolized in liver via three pathways i-e: Glucoranidation, sulphation (both account 95%) and via cytochrome p-450 (5%). A small amount of Acetaminophen is converted by cytochrome p-450 to a potentially hepatotoxic quinone intermediate compound. In therapeutic doses, this compound is rapidly inactivated by conjugation with glutathione, but in case of hepatic glutathione depletion, this causes accumulation of quinone intermediate compound which results hepatic necrosis[9]. Acute renal toxicity (acute tubular necrosis) has also been seen with acetaminophen over dosage[10,11]. Paracetamol over dosage is also having effect on heart which results abnormalities in ST segment, T-wave flattening, pericarditis and myocardial necrosis[12,13].

N-Acetylcysteine (NAC) has been used as an antioxidant in the patients with acetaminophen over dose[14,15]. It induces the glutathione synthesis and due to this action it enhances the detoxification of free radicals in acetaminophen poisoning[16,17].

The Neem leaves and its other components have been used as traditional medicine, killing of insect and antiseptic activities. Beside this current studies have reported its antitumor, anticancer, antiviral, antimalarial and hypoglycemic activities[18-20]. The effects of neem leaves extract in paracetamol induced hepatic damage in rats have been studied. A significant reduction in the hepatic enzymes to the normal levels was found with the use of neem leaves extract[21].

Reducing the paracetamol induced effects by the use of aqueous neem leaves extract and N-Acetylcholine is yet to be validated. The objective of the present study is to identify the hepatoprotective effect of Aqueous Neem Leaf extract in comparison with N-Acetylcysteine (known Antidote) in paracetamol overdose induced liver toxicity on the basis of liver enzymes including AST, ALT, ALP and histopathological changes in liver.

MATERIALS AND METHODS

This is an experimental interventional comparative study conducted in Pharmacology Department, Al-Tibri Medical College and Hospital Karachi during Jan 2015 to June 2015. In this study a total of sixty adult albino rats of wistar strain of either gender having weight between 150-200 gms were included and were divided into four groups each containing fifteen animals.

Group A Healthy control (n=15) animals were given normal diet and water for 15 days. While in Group B (n=15) animals were treated with paracetamol 2gm/kg body weight orally single dose[21] and observed for 24 hours and then were sacrificed. Liver was exposed to see any macroscopic hemorrhage on it and sample was taken to confirm the hepatotoxicity through microscopic examination and blood sample was taken for biochemical parameters i-e Liver enzymes (AST, ALT, ALP).

Rats in Group C (n=15) were given aqueous neem extract extract 500mg/kg/day orally for 15 days and same group also received paracetamol 2gm/kg body weight orally (21). The hepatoprotective effects were seen by histopathology of liver and biochemical parameters through blood sample drawn from heart of the rats. In Group D (n=15) rats were given Paracetamol and N-Acetylcysteine at a dose 140mg/kg intraperitoneal for 06 days (22) and hepatic effect was seen by histopathology of liver and biochemical parameters through blood sample drawn from heart of the Rat.

RESULTS

In the present study the effects of paracetamol, neem compound and N-acetylcysteine were observed. The results revealed that the toxic effects of paracetamol were reversed by the use of neem extract and N-acetylcysteine and results further analyzed statistically.

Blood Serum (AST) Levels of Rats: Mean serum (AST) level of animals in group A, was 22.8U/L whereas in group B was 110.86U/L. The results showed loss of liver function in Group B when compared with Group A with significant p value of less than 0.001. Mean serum (AST) level of animals in group C was 20.133U/L. A non significant loss of liver function in Group C when compared with Group A with p value of less than 0.15 was observed. Mean serum (AST) level of animals in group D was 31.26U/L. While comparing the serum AST levels in Group A with Group D, the p value was found to be <0.03 as shown in the Table No-1.

Table No.1: Serum AST level in different group of animals

	Mean	Standard Deviation	Standard Error of Mean
Group A	22.80	7.55	1.94
Group B	110.86	12.17	3.14
Group C	29.13	5.06	1.30
Group D	31.26	5.95	1.53

Blood Serum (ALT) Levels of Rats: Mean serum (ALT) level of animals in group A was 33.86U/L whereas in group B was 110.20U/L. The serum ALT levels were elevated showing significant loss of liver function in Group B when compared with Group A. The p value was found to be <0.001. Mean serum (ALT) level of animals in group C was 35.33U/L. A non significant loss of liver function in Group C when compared with Group A with p value of less <0.08 was

observed. Mean serum (ALT) level of animals in group D was 37.73U/L. While comparing the serum ALT levels in Group A with Group D, the p value was found to be <0.01 as shown in the Table No-2.

Table No. 2: Serum ALT levels in different group of animals

	Mean	Standard Deviation	Standard Error of Mean
Group A	25.00	7.11	1.83
Group B	40.00	13.00	3.35
Group C	20.00	6.26	1.61
Group D	15.00	4.77	1.23

Blood Serum (ALP) Levels of Rats: Mean serum (ALP) level of animals in group A was 220U/L whereas in group B was 444.33U/L. The serum ALP levels were elevated showing significant loss of liver function in Group B when compared with Group A with p value of <0.001. Mean serum (ALP) level of animals in group C was 240.33U/L. A non significant loss of liver function in Group C when compared with Group A with p value of <0.06 was observed. Mean serum (ALP) level of animals in group D was 244.33U/L. While comparing the serum ALP levels in Group D with Group A, the p value was found to be <0.01 as shown in the Table No-3.

Table No.3: Serum ALP level in different group of animals

	Mean	Standard Deviation	Standard Error of Mean
Group A	220.00	17.92	4.62
Group B	444.33	32.23	8.32
Group C	240.33	18.36	4.74
Group D	244.66	16.08	4.15

Histo-Pathological Observations in Group A (control): The biopsy specimens of liver from control Albino rats were observed for morphological and histological structure following the staining with routine Hemotoxyline and Eosin (H&E) stain.

Histopathological Observations of Group B:

- Normal parenchyma distorted.
- Dilated and engorged central vein.
- Congestion in hepatic sinusoids.
- Marked necrotic hepatocytes seen.

Histopathological Observations of Group C: Regenerating hepatocytes with reduced necrotic cells and retrained hepatic architecture seen. Few inflammatory cells and dilated sinusoids indicate recovery and resolution.

Histopathological Observations of Group D:

- Mild congestion in central vein.
- Mild to moderate inflammation near hepatic cords.
- Few necrotic hepatocytes.

DISCUSSION

Liver plays an important role in metabolism of drugs and detoxification in the body. Liver injury caused by toxic chemicals and certain drugs has been recognized as one of the toxicological problems[23]. Acetaminophen has antipyretic, analgesic and weak anti-inflammatory effects because of weak ability to inhibit COX on inflammatory site due to the presence of peroxides[24]. Hepatic necrosis is a severe adverse effect of paracetamol over dosage. The process by which it causes the hepatocellular injury and then death is by the conversion into intermediate quinone compound which is not excreted by kidney, and due to depletion of glutathione which causes oxidative stress that lead to apoptosis of highly susceptible hepatocytes[25].

N-Acetylcysteine has shown its hepatoprotective effect by increasing the synthesis of glutathione with marked improvement in Liver enzyme (ALT, AST, ALP) as well as on histopathology of the organ. In various studies it has been proved that synthetic drugs being used in the treatment of hepatotoxicity are having serious adverse effects[26]. In view of this, it is prudent to look for an alternative like medicinal plants since few or no side effects have been reported for neem extract and also to evaluate on scientific basis for their efficacy which has been claimed to possess or having hepatoprotective effects.

Many herbal plants like Parkia Biglobosa stem bark have hepatoprotective effect on paracetamol induced Liver damage[27]. In present this study alkaline phosphotase level was not significantly reduced as compared to Parkia Biglobosa plant. Omega-3 has three essential fatty acids which protect the liver from the paracetamol induced liver damage among the swiss albino rats. This effect occurs only because of antioxidant action and it markedly decreased the level of liver enzymes like ALT, AST and ALP[28]. Another study also showed the hepatoprotective effect of neem leaf in diabetic albino rats induced by the Alloxon. Leaf extract of neem was also used for the hepatoprotective activity against the administration of CCL4 (75mg/k s/c) in albino rats[29].

In another study hepatoprotective effect was compared between Neem leaves and Silymarin in Albino rats which concluded that both herbal medicine is having same hepatoprotective effect, and having no significant difference in biochemical parameters[21]. In the present study effects of aqueous neem extract on paracetamol induced liver toxicity was compared with N-Acetylcysteine, which is known antidote widely used to prevent hepatic toxicity since very long time. This was confirmed from our study on the basis of significance difference of biochemical parameters and histological

slides. Neem leaves extract is having better hepatoprotective effect with least side effects.

Neem leaves extract has anti-lipoprotective property because it is rich in flavonoid content, which is well known antioxidant and similar findings was also confirmed by another study[30]. Decreased glutathione levels influenced by paracetamol over dose results in oxidant antioxidant imbalance and programmed cell death of hepatocytes. In this study neem leaves aqueous extract has reversed the hepatic injury. The possible suggested mechanism is through anti-oxidant and anti-apoptotic activity of Neem leaves extract, similar findings were also confirmed in another study[31].

CONCLUSION

Paracetamol is a hepatotoxic drug causing histomorphological damage in liver along with alteration in the level of Liver enzymes. Azadirachta indica leaves have given better results compared to N-Acetylcysteine, on the basis of significant differences in biochemical parameters.

Conflict of Interest: The study has no conflict of interest to declare by any author.

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