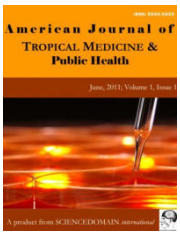

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Some Protective Effects of Aqueous Leaf Extract of *Azadirachta indica* on Paracetamol-induced Hepatotoxicity in Adult Wistar Rats

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Research Article

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ABSTRACT

Background*: Azadirachta indica* is a plant used widely in the Indian system of medicine for its diverse medicinal properties. Its extracts have a vast pharmacological activity and are used as raw materials for pesticide, medicine and other commodities. Each part of this plant have its own therapeutic importance and uses which include: antifertility antipyretic, anti-inflamatory,antimalaria, anti-rheumatic and others. The present study investigated some effects of aqueous extract of *Azadirachta indica* on paracetamol-induced hepatotoxicity in adult wistar rats.
Place and Duration of Study: Department of Anatomy, Faculty of Basic Medical Sciences, LAUTECH,Nigeria between November 2009 and July 2010.
Methodology: A total of twenty-four (24) rats of wistar strain of both sexes weighing 150g-232g were divided into four (4) groups of six (6) rats each. Groups A served as normal control given only distilled water for seven days orally. Group B received only paracetamol at a dose of 3g/kg body weight orally, on the fifth day of seven days administration, group C received a pretreatment of *Azadirachta indica* aqueous extract at a dose (400mg/kg b.w orally) for seven days while paracetamol (3g/kg .orally) was given on the fifth day of the administration and group D only received the aqueous extract of *Azadirachta indica* (400mg/kg/ b.w.) orally for seven days.
At the end of the experimental period, all animals were sacrificed using cervical dislocation method, blood was obtained for assay for the following of hepatic marker enzymes Alanine

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amino transaminase (ALT), Aspartate amino transaminase (AST) and Alkaline phosphatase (ALP) respectively. Liver tissue was removed, fixed in 10% formol saline and processed for histopathological studies using Heamatoxylin and Eosin (H and E) staining technique.

Results: The results obtained from body weight parameter showed that paracetamol treated group (group B) showed a significant reduction in weight compared to groups A, C and D. Histopathological observations revealed well preserved histoarchitecture, without portal inflammation, cytoplasmic inclusion, binucleation or necrosis in group A, C and D. However, group B showed portal inflammation, moderate to mild cytoplasmic inclusion, sinusoid congestion and marked tissue necrosis in most of the group compared with group C in which the leaf extract of *Azadirachta indica* inhibited paracetamol induced hepatic damage. Biochemical analysis of hepatic enzymes also showed an increased in serum marker enzymes of hepatic damage (AST, ALT and ALP) after paracetamol administration in group B. *Azadirachta indica* pretreatment stabilized the serum levels of these enzymes in group C and D respectively.

Conclusion: The study concluded that aqueous leaf extract of *Azadirachta indica* offers protection to the hepatocytes against paracetamol induced hepatotoxicity in wistar rats.

Keywords: Liver; hepatotoxicity; paracetamol; Azadirachta indica; enzyme; hepatic damage;

1. INTRODUCTION

Neem (*Azadirachta indica*) is a member of the meliaceae family. It is a native plant to south Asia and Southeast Asia, which grows well in the hot river valley areas. The Indian system medicine claims various medicinal properties for it (Chopra et al., 1956). It is reported to have hypoglycemic (Khosla et al., 2000), anti-inflammatory (Chattopadhyay, 1998), immuno-modulatory (Ray et al., 1996) and adaptogenic (Sen et al., 1992) properties. Neem is regarded as “The Wonder Tree” and “Nature’s Drug store”, because its extracts have a vast pharmacological activities and are used as raw materials for pesticide, medicine and other commodities. Neem is considered to be “one of the most promising of all plants and the fact that it may eventually benefit every person on this planet”. Probably no other plant yields as many strange and varied neem-products or has as many exploitable by-products. In addition, neem has also been shown to possess spermicidal and antifertility effects (Joshi and Ahmed, 1996) and has been reported to be effective in wound healing, skin diseases and cardiovascular disorders (Chopra et al., 1956; Dhawan and Patnnaik, 1993). Paracetamol (called acetaminophen in the USA) is one of the most commonly used non-narcotic analgesic and antipyretic agents. It has relatively weak anti-inflammatory activity. Paracetamol is reported to be selective inhibitor of Cox 3 (cyclooxygenase). Although some reported evidence show that paracetamol has significant anti-inflammatory action (Ray et al., 1996). Paracetamol toxicity is one of the most common causes of poisoning worldwide. In the United States and United Kingdom it is the most common cause of acute liver failure (Wikipedia, free encyclopedia, 2010).

Paracetamol was the fourth most common cause of death following self-poisoning in the United Kingdom in 1989; (Rang et al., 2003), yet it is still one of the most common analgesic and antipyretic drugs often used around the world to treat pains and mild feverish conditions. As far as this is true, it is also one of the major causes of liver damage such as liver necrosis. Traditionally, a number of herbal medicines have been used in ameliorating this

problem of hepatotoxicity such as fresh garlic; (Christian et al., 2009), ethanolic extract of neem; (Chattopadhyay et al., 1992). Toxic doses of paracetamol cause a serious potencially fatal hepatotoxicity. These toxic effects occur when the liver enzymes catalyzing the normal conjugation reactions are saturated, causing the drug to be metabolized by the mixed function oxidases. The resulting toxic metabolized, N-acetyl-p-benzoquinone imine (NAPQI), is inactivated by conjugation with glutathione, but when glutathione is depleted the toxic intermediate accumulates and reacts with nucleophilic constituents in the cell. This causes necrosis in the liver and also in the kidney tubules. In addition to these earlier research findings, this study investigated the possible herbal protective activity of *Azadirachta indica* (neem), against paracetamol-induced hepatic damage in adult Wistar rats.

2. MATERIALS AND METHODS

Twenty four adult Wistar rats of both sexes weighing 150-232g were used for the experiment. The rats were housed in colony cage and acclimatized them for 3 months due to their smaller weights as at the time of purchased at the ambient temperature of 25±20ºC and 45-55% relative humidity with a 12h light/dark cycle. The animals had free access to standard pellet chow and drinking water *ad libitum*. A total of 24 rats were used for the study and they were separated into four groups of 6 rats each, and they received the following treatment schedule.

Group A: Normal control (received only distilled water)
Group B: Paracetamol treated control (3g/kg. body weight, orally)
Group C: Paracetamol (3g/kg,body weight, orally) + *Azadirachta indica*
Group D: *azadirachta indica* leaf aqueous extract (400mg/kg, body weight, orally).

2.1 Administration of Extract and Paracetamol

A fixed dose of *Azadirachta indica* extract (400mg/kg b.w., orally) was used as working dose (Chattopadhyay et al., 1992; Chattopadhyay, 1998).The normal control (Group A) received only distilled water for seven days, Group B received a single dose of paracetamol (3g/kg b.w., orally) on the fifth day(Asha and Pushpangadan,1997) of the administration while Group C received pretreatment of the aqueous leaf exract of *Azadirachta indica* (400mg/ kg b.w., orally) for seven days and paracetamol (3g/kg b.w., orally) given on the fifth day of the seven days administration. Group D served as treated control of the extract. The rats in this group received the extract for seven days. Group C received paracetamol at 3g/kg body weight orally on the fifth day (Asha and Pushpangadan, 1997). Group D animals received only *Azadirachta indica* juice, at 400mg/kg body weight, orally for seven days.

At the end of the experiment (48 hours after paracetamol administration) all the rats were sacrificed using cervical dislocation method. Their abdomens were cut open and the liver samples were removed and fixed in 10% formal saline for light microscopy following the method of Carleton (1967) and blood samples were also collected in EDTA bottles for analysis of hepatic marker enzymes: AST, ALT, ALP.

Digital photomicrography of the liver sections were obtained using a trinocular light microscope with digital camera attached to one of the eyepieces to show the possible morphological changes that occurred in the treated groups (B, C and D) compared to the control group (A).

2.2 Enzyme Assay

The serum levels of Alanine aminotransaminase (ALT), Alkaline phosphatase (ALP) and Aspartate aminotransaminase (AST) were determined by colorimetric method (Varley et al., 1980) using Randox diagnostic kits (USA). The assay for Aspartate aminotransaminase (AST) followed a modification of the colorimetric method reported by Varley et al. (1980). Pyruvate solutions of varied millimolar concentrations were used to prepare a standard curve from which AST activities were computed as described by Varley et al., (1980). Alanine aminotransaminase (ALT) assay was carried out as described for AST except that 200mM DL- alanine replaced L-aspartate on the procedures. Alkaline phosphatase (ALP) activity determination followed the procedure reported by Varley et al. (1980).

2.3 Statistical Analysis

All data were presented as Mean ± S.E.M. Statistical analysis of the data of comparisons between the control and treated groups in this study were carried out using analysis of variance and significance of group data difference was tested for using student's t-test. $P < 0.05$ was considered statistically significant.

3. RESULTS

The record of the body weights obtained before and after the administration period, indicated that high doses of paracetamol affected the body weights of the rats slightly but not at a significant level compared with other groups (A, C and D) as indicated in Table 2 . To some extent, the aqueous extract of *Azadirachta indica* has no significant effect on the body weight compared with the control group (A).

It could be inferred from this result that the leaf aqueous extract of *Azadirachta indica* reduced the effect of paracetamol overdose on the body weight of the rats (Table 1).

Table 1. Mean ±S.E.M of the body weights of the rats before and after treatment of animals

Groups	Initial (Mean ± SEM) g	Final (Mean ± SEM) g
A	153.78 ± 1.82	157.55 ± 1.92
B	181.36 ± 3.74	178.27 ± 3.97
C	165.25 ± 0.99	166.00 ± 1.03
D	230.13 ± 3.67	230.78 ± 3.89

All values are expressed as Mean ± S.E.M for each group.
Group = A – normal control; Group = B – Paracetamol – treated; Group = C – Paracetamol + A.I.; Group = D - A.I treated control

Table 2: Difference and % difference between the initial and final body weights

Parameter	Group A	Group B	Group C	Group D
Difference between final-initial weight (grams)	3.77	-3.09	0.75	0.65
% difference in weight	2.45%	-1.7%	0.45%	0.28%

All values are expressed as Mean ± S.E.M for each group.
Group = A – normal control; Group = B – Paracetamol – treated; Group = C – Paracetamol + A.I.; Group = D - A.I treated control

The result in Table 3 shows a significant increase ($P<0.05$) in the levels of the hepatic enzymes in group B paracetamol-treated rats compared with control groups A , C and D which received extract A.I treatment.

Hepatic damage induced by paracetamol has been observed in this study (Figure 2). There was a significant rise ($P<0.05$) in the marker hepatic enzymes; ALP, ALT and AST in paracetamol treated rats (Table 2). Administration of paracetamol resulted in changes of degeneration, fibrosis and necrosis on histological observation of the rat liver of paracetomol -treated rat (Figure 2). The pre-treatment of the rats in group C with aqueous leaf extract of AI preserved the histological features of the liver by evidently reducing the levels of hepatic enzyme markers of injury and preventing histopathological changes of degeneration and necrosis with evidence of regeneration (Figure 3). The administration of AI aqueous leaf extract before paracetamol exposure in group C rats significantly reversed or prevented all the biochemical and histological alterations caused by paracetamol exposure in group B paracetamol- treated rats.

Table 3. Effect of *Azadirachta indica* and paracetamol on hepatic enzymes in adult Wistar rats (n=6)

Enzyme	Group A	Group B	Group C	Group D
ALP (U/L)	33.12 ± 2.05^{a}	65 ± 2.8^{ab}	24.37 ± 2.05^{ab}	29.68 ± 1.63^{b}
ALT (U/L)	17 ± 1.36^{a}	67.3 ± 5.5^{ab}	30.5 ± 1.17^{abc}	19.5 ± 1.2^{bc}
AST(U/L)	54.25 ± 2.04^{a}	87 ± 6.5^{ab}	65 ± 2.02^{ab}	56 ± 3.02^{b}

Subscripts of the same alphabets show significance at the level of P<0.05;
All values are expressed as Mean ± S.E.M for each group.
Group = A – normal control; Group = B – Paracetamol – treated; Group = C – Paracetamol + A.I.;
Group = D - A.I treated control

The histoarchitecture of the liver was well preserved in group D rats following exposure to aqueous leaf extract of AI (Figure 4). The histological features of the liver section in group D AI-tread rats appeared to be normal relative to the group A control rats. Similarly, the values of hepatic enzymes were also found to be normal compared with the values obtained from the control rats (Table 3). There was no significant difference between group A (control) and group D (A.I – treated) rats histologically and biochemically as shown in (Figure 4 and Table 3).

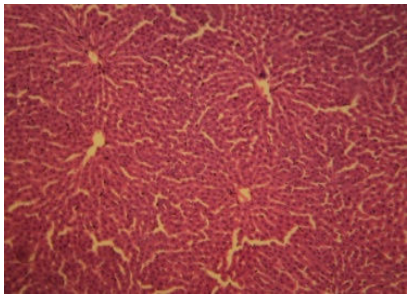

Figure 1 Group A: The control group shows normal lobular architecture and normal hepatic cells with well preserved cytoplasm and well-central vein, with no fatty changes, no sinusoid congestion, no portal inflammation and no tissue necrosis.

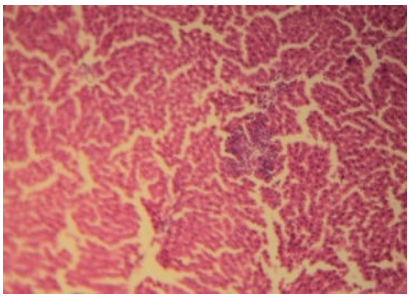

Figure 2 Group B: The paracetamol treated group shows moderate to mild lobular architecture, with cyto plasmic inclusions, mild to normal portal inflammation, binucleation of some hepatocytes, increased intercellular space and tissue necrosis.

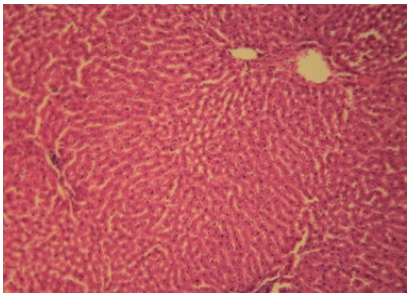

Figure 3 Group C: Pretreated group shows preservation of lobular architecture and less prominent hepatic damage without cytoplasmic inclusion, portal inflammation, no sinusoid congestion and fatty changes. There is also no tissue necrosis observed.

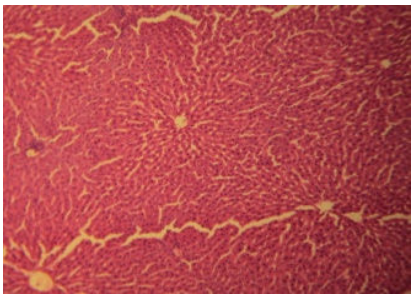

Figure 4 Group D: Treated group with *Azadirachta indica* aqueous extract shows well preserved lobular architecture and well defined nucleus and central vein without any cytoplasmic inclusion and no portal inflammation, sinusoid congestion, fatty change and no tissue necrosis.

4. DISCUSSION

Liver disorders have raised a great concern in health delivery system, although Liver protective drugs may not be readily available but medicinal plants play important role in the management of liver disorders (Pingale , 2010). *Azadirachta indica*, a plant used widely in traditional medicine due to its anti-inflammatory, immunomodulatory and adaptogenic properties. The hepatoprotective role of fresh juice of tender leaves of *Azadirachta indica* (200 mg/kg body wt. p.o.) inhibited paracetamol (2 g/kg body wt. p.o.)-induced lipid

peroxidation and prevented depletion of sulfhydryl groups in liver cells. Besides, there was an increase in serum marker enzymes of hepatic damage (aspartate transaminase, alanine transaminase and alkaline phosphatase) after paracetamol administration (Yanpallewar et al., 2003). Chattopadhayay (1992) had reported the effect of *Azadirachta indica* leaf extract on serum enzyme levels (glutamate oxaloacetate transaminase, glutamate pyruvate transaminase, acid phosphatase and alkaline phosphatase) which revealed elevated serum enzyme levels in paracetamol induced animals compared with those receiving a combination of paracetamol and leaf extract. The most serious adverse effect of acute overdose of acetaminophen is dose-dependent, potentially fatal hepatic necrosis (Thomas, 1993).

Research findings previously have shown that the extract possesses antiulcer effects (Garg et al., 1993), immunomodulatory and anti-inflammatory properties (Ray et al., 1996; Chattopadhayay, 1998). Devasagayam and Sainis (2002) have suggested that agents possessing these properties can be used as antioxidant beneficial effect of *Azadirachta indica* if any on paracetamol induced hepatotoxicity in rats.

Hazai et al. (2002) reported that the mechanism of hepatotoxicity of paracetamol has been studied extensively. The toxicity occurs because of its reactive metabolite, N-acetyl-p-benzoquinone imine (NAPQI). NAPQI exerts its toxicity mainly through its oxidative effect on cellular proteins. Sulfhydryl compounds are among the most important endogenous anti oxidants. Glutathione (GSH) is the main intracellular non protein sulfhydryl and it plays an important role in the maintenance of cellular proteins and lipids in their functional slates. NAPQI binds to GSH, forming a conjugate which results in conversion of GSH to GSSG (oxidized form of glutathione).

When GSH is lowered, the toxic effects of oxidative insult are well pronounced, resulting in increased membrane and cell damage. At this point others protein and non-protein sulfhydryl groups present in the cell provide an important alternate protection (Genet et al., 2000). Sinclair et al. (1991) reported that any oxidative insult to a cell induces lipid peroxidation of cell membrane lipids. The toxic peroxidative products cause widespread damage of macromolcules.

The antioxidant activity of *Azadirachta indica* seed extract has also been demonstrated in vivo during horse grain germination, which is associated with low levels of lipoxygenase activity and lipid peroxides (Rao et al., 1969). The chemical investigation of the leaves of *Azadirachta indica* resulted in the isolation of quercetin with an antioxidant potential comparable to that of vitamin E (Bramley and Pridham, 1995).

Results of previous research findings have shown that *Azadirachta indica* most likely exerted its hepatoprotective effect by acting as an antioxidant inhibiting lipid peoxidation.

The liver sections of the rats given only *Azadirachta indica* aqueous extract (Figure 4) extract showed no significant morphological changes as compared to the rats in the control group (Figure 1). Liver section from the control group showed normal lobular architecture and normal hepatic cells with a well-preserved nucleus. It also showed no cytoplasmic inclusion, portal inflammation nor any morphol ogical changes which is consistent with the reports of Chattopadhyay et al. (1992), Chattopadhyay (2003) and Yanpallewar et al. (2003).

Liver sections from the rats given paracetamol (Figure 2) only showed marked hepatic cell damage in form of portal inflammation, binucleation and nuclear form of enlargement cytoplasmic inclusion fibrosis, degeneration and tissue necrosis, compared to control and

Azadirachta indica treated groups (Figure 1 and 4). These morphological changes in the paracetamol-treated rats might be due to oxidative stress resulting from toxicity of paracetamol on the hepatic cells. Pretreament with *Azadirachta indica* (group C) showed normal lobular structure with no evidence of hepatic damage in paracetamol challenged rats (Figure 3) compared with the control group (Figure 1). The reversal of all the biochemical enzymatic parameters and histological alterations observed in the paracetamol-treated section in Figure 2 following pretreatment with aqueous leaf extract as shown in Figure 3 might be due to antioxidant property of the extract which preserved the hepatic tissue from possible paracetamol – induced oxidative stress which could lead to hepatic damage as observed in Figure 2. The anti-oxidant property of *Azadirachta indica* has been reported by Olabinri et al. (2009). The hepatic protective property of AI aqueous leaf extract may also be because of its other properties such as anti-inflammatory property which may inflammatory hepatic damage and anti-oxidant property thereby reducing the oxidative stress imposed by the drug. This antioxidant mechanism seems to be important as AI aqueous leaf extract has been shown to reduce oxidative stress (Arivazhagan et al., 2000).

The activities of enzymes AST, ALT and ALP in serum are used routinely to assess the functional status of the liver in both clinical and experimental settings. They are used as serum markers of hepatic damage. Elevated levels of these enzymes in serum of paracetamol- treated group (group B) indicate liver dysfunction compared with Groups A and D. Pretreated group with *Azadirachta indica* (A.I) aqueous leaf extract has a reduced level of these enzymes significantly compared with paracetamol-exposure group showing that AI also maintains the functional capacity of the liver (Figure 3). The beneficial effect of AI on biochemical parameters is also indicated by histopathological observations earlier reported. The regenerative activity seen in liver cells of rats treated with paracetamol signifies the compensatory changes to cellular insult. Preservation of lobular architecture and less prominent regenerative activity in liver sections of rats pretreated with A.I (Figure 3) showed that the extract helps to maintain the structural integrity of liver against paracetamol induced damage. The high levels of hepatic enzymes in paracetamol-treated hepatic tissue which became significantly reduced in *Azadirachta indica* pretreated group (Table 3) might be due to the antioxidant property of the extract administered. The antioxidant property of AI has been reported by Olabinri et al. (2009). It could be stipulated that the extract treated group was protected from hepatic cell damage caused by paracetamol induction. The findings were further confirmed by histopathological study of liver. The results obtained from this research showed that the aqueous leaf extract of *Azadirachta indica* has protective function against paracetamol- induced hepatic damage which is in agreement with the work of Bhanwra et al. (2000) and Chattopadhyay et al. (1992). Based on this research work, it can be concluded that *Azadirachta indica* aqueous leaf extract has a protective potential on the liver against paracetamol induced hepatotoxicity. The aqueous extract of neem leaf was found to offer protection against paracetamol induced liver necrosis in rats. Bhanwra et al. (2000). The elevated levels of serum aspartate amino transaminase (AST), alanine amino transaminase (ALT) and gamma glutamyl transpetidase (GGT) indicative of liver damage, were found to be significantly reduced on administration of the neem leaf aqueous extract, indicating its antioxidant activity over paracetamol-induced hepatotoxicity.

Administration of *Azadirachta indica* leaf extract significantly enhanced the hepatic level of glutathione dependent enzymes and superoxide dismutase and catalase activity suggesting that the hepatoprotective effect of the extract on paracetamol induced hepototoxicity may be due to its antioxidant activity. Also, it is well documented that the compounds quercetin, rutin, vitamin C and E are strong antioxidant. It is presumed that the quercetin and rutin

compounds of *Azadiracta indica* leaf extract may be responsible for its hepatoprotective activity (Chattopadhyay and Bandyopadhyay, 2003).

5. CONCLUSION

This study concluded that high doses of paracetamol predisposes to hepatic damage. However, aqueous leaf extract of *Azadirachta indica* has the potential of protecting the liver cells from paracetamol induced hepatotoxicity due to its antioxidant property, However, further studies involving the extracts and/or its phytochemicals are needed with the aim of corroborating these findings**.**

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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