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Sodium Benzoate Mediated Hepatorenal Toxicity in Wistar Rat: Modulatory Effects of *Azadirachta indica* (Neem) Leaf

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Research Article

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ABSTRACT

Aims: Morbidity and mortality from kidney and liver diseases is rapidly increasing worldwide due to exposure of these organs to many kinds of xenobiotics. Medicinal herbs have been used widely to treat these disorders as there is no specific treatment in modern medicine to counter the menace. The study was carried out to investigate the protective role of *Azadirachita indica* (neem) leaf on kidney and liver damage caused by subchronic administration of sodium benzoate in rat.
Study design: Experimental animal study
Place and Duration of Study: Department of Biochemistry, College of Science, Engineering and Technology, Osun State University, Osogbo, Nigeria between January and June 2011.
Methodology: 200mg/kg bw of sodium benzoate was administered to rats in test groups (B, C and D) every 4 days while control group (A) received distilled water. Group C and D were treated with daily administration of 200mg/kg bw and 500mg/kg bw methanolic leaf extract of *Azadirachta indica* respectively for 14 days while group B were not treated.
Results: Sodium benzoate caused growth depression in rats as well as alteration in hepatic and renal functions revealed by significant elevation in serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), urea, uric acid and creatinine. Administration of *Azadirachta indica* leaf extract tend to ameliorate the adverse effect of sodium benzoate toxicity in rat tissue as it bring the affected biochemical parameters close to normal in a dose dependent manner.
Conclusion: These results suggest that *Azadirachta indica* leaf has modulatory effect on sodium benzoate induced toxicity in rats.

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Keywords: Azadirachta indica; sodium benzoate; serum; marker enzymes; renal and hepatic functions.

1. INTRODUCTION

Sodium benzoate is a common preservative used in many foods including salads, carbonated drinks, jams and fruit juices as well as in pharmaceutical industries for preservation of liquid medicines (Chipley, 1983). This compound which has been reported to cause serious adverse effect in the body is indirectly consumed widely by the human population as food additives (Kubota and Ishizaki, 1991).

Benzoate is metabolized in the liver by conjugation with glycine, resulting in the formation of hippuric acid (US FDA 1973). The utilization of glycine in the detoxification of benzoate results in depletion in glycine level of the body which can affect metabolic process in which glycine is involved. Low glycine level in the body can cause reduction in creatinine, glutamine, urea and uric acid levels (Kubota and Ishizaki, 1991). Studies also revealed that sodium benzoate can be metabolized in the body under irradiation to form benzine, a derivative capable of damaging mitochondrial DNA which has also been implicated in kidney and liver injury (Nair, 2001; Maki and Suzuki, 1985).

Medicinal plants play an important role in the lives of rural people, particularly in remote parts of developing countries with poor health facilities. Products derived from plants are not only useful for traditional medicine, but also often have a considerable market value. Neem is a popular medicinal plant originally grown in Indian but now being cultivated in almost every parts of the world including Nigeria (Das et al., 2002; Sonibare et al., 2006).

Neem have been used medicinally over thousands of years and has established a reputation as a useful cure for various ailments. The leaf has been used for the treatment of rheumatism, chronic syphilitic sores, ulcer and various skin infections (Biswas et al., 2002). Combination of the bark, leaf, root, flower and fruit has been used to cure blood morbidity, biliary infections, itching, skin ulcers and burning sensation (Schmutterer, 2002). Studies about bioactivities of neem leaf have provided evidence for its hypoglycaemic, antioxidant, antimalarial, antibacterial and immunostimulative properties (Singh et al., 1996). Neem leaf is recently being claimed to be useful in the treatment of jaundice and renal dysfunction by traditional healers in Nigeria but there are no approved reports stating its therapeutic efficacy. This study investigates the protective potential of neem leaf on liver and kidney damage caused by sodium benzoate in rats.

2. MATERIALS AND METHODS

2.1 Drugs/Chemicals

Sodium benzoate salt is a product of May and Baker Ltd. Dagenham, England. All other chemicals which are products of Sigma Chemical Company, St Louis, USA were of analytical grade and were prepared in the laboratory in double glass distilled water.

2.2 Collection and Treatment of Plant Materials

Azadirachta indica leaves were collected in Osogbo town, Nigeria and air-dried. It was then ground into powder with an electric blender. The powdered leaf was soaked in 6 volumes 80% methanol for 14 days after which the mixture was filtered followed by removal of the solvent on a water bath to give a dark-brown crude extract.

2.3 Experimental Animals

Twenty (20) albino rats (*Rattus novegicus*, average weight 148g), divided into four groups of five rats each were used for the study. They were obtained and raised at the Biochemistry Animal House, Osun State University, Osogbo. They were kept under laboratory conditions in cages cleaned of waste daily and allowed to acclimatize for three days before the experiment. The animals were exposed to 12hr daylight and darkness and fed rat pellet and water ad libitum. Group A serves as the control and administered with distilled water. Hepatorenal damage was induced in group B, C and D with oral administration of subchronic dose of sodium benzoate (200mg/kg bw at every 4 days). Group C and D were treated daily with 200mg/kg bw and 500mg/kg bw, respectively of methanolic leaf extract of *Azadirachta indica* for 14 days while group B rats were not treated. Administration was done with the use of oral intubator. Animal growth in each group was monitored by taken their average body weight every other day.

2.4 Preparation of Serum

Rats were anaesthetized in a jar containing cotton wool soaked in diethyl ether vapour after which they were sacrificed by cutting through the jugular vein and blood sample collected into clean, dry centrifuge tube. The blood was left for 20 min at room temperature to clot and then centrifuged at 3,000g in an MSC (Essex, UK) bench centrifuge. Serum was collected into clean, dry sample bottles and then frozen.

2.5 Preparation of Tissue Homogenate

The animals were quickly dissected at the end of experimental period and the tissues (liver, kidney, heart and brain) removed. They were cleaned of blood and weighed before keeping in 5 volumes ice-cold 0.25M sucrose solution to maintain cell integrity. The tissues were then homogenized using a Teflon homogenizer and kept frozen overnight before analyses.

2.6 Determination of Biochemical Parameters

Protein concentration was determined in the serum by the method of Lowry et al. (1951) using bovine serum albumin as standard. The bromocresol green method described by Cheesbrough (1991) was used to determine albumin concentration. Serum globulin was estimated using the procedure of Mokady et al. (1989). AST and ALT activities were determined in the serum and tissues using Randox diagnostic kits based on the principle described by Reitman and Frankel (1957). Serum creatinine and urea were determined as described by Cheesbrough (2005).

Uric acid was estimated in the serum according to the method of Cheesbrough (1991). All measurements were done using Spectronic 21 digital spectrophotometer attached to a recorder (Bausch and Lomb, Rochester, New York).

All values were expressed as mean ±SD. Comparison was done using one-way analysis of variance (ANOVA). P values <0.05 were considered statistically significant.

3. RESULTS AND DISCUSSION

3.1 Results

The growth pattern of experimental rats compared with the control is illustrated in Figure 1. Rats in the control group and those treated with *Azadirachta indica* leaf extract recorded normal growth while rats administered with sodium benzoate but not treated with the extract showed significant growth depression starting from the second day till the end of the experimental period. Relative organ weight of rats in the control and test groups as shown in Table 1 reveal no significant difference indicating no organ enlargement.

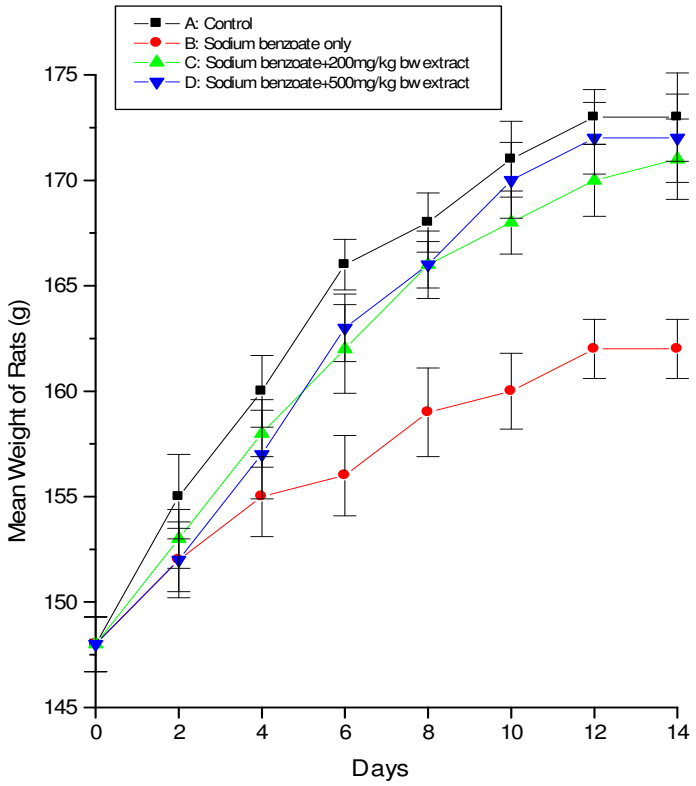

Fig. 1: Growth pattern of rats administered with sodium benzoate and *Azadirachta indica* leaf extract

Tables 2 and 3 show the serum and tissue concentrations of ALT and AST, respectively. There was significant elevation of ALT and AST in the serum accompanied with significant reduction of the enzymes in the liver of rats administered with sodium benzoate. This alteration in AST and ALT levels were however significantly reversed close to normal in rats treated with the extract. The levels of these enzymes in the kidney, heart and brain in the

four groups were not significantly different from each other. Serum concentrations of some metabolites in the experimental animals are shown in Table 4.

There was significant elevation in the levels of creatinine, urea and uric acid in the serum of rats administered with sodium benzoate compared with the control which was reversed with administration of *Azadirachta indica* leaf extract. There was no significant difference in the concentrations of albumin, globulin and total protein between the test groups and the control.

Table 1: Relative organ weight (g/100g body weight) of rats administered with sodium benzoate (SB) and leaf extracts of *Azadirachta indica*

Tissue	Control	SB Only	SB + extract 200mg/kg bw	SB + extract 500mg/kg bw
Liver	3.41±0.22	3.39±0.30	3.46±0.28	3.52±0.31
Kidney	1.34±0.14	1.40±0.16	1.46±0.18	1.38±0.14
Heart	0.47±0.03	0.49±0.02	0.46±0.04	0.48±0.03
Brain	1.87±0.12	1.79±0.11	1.96±0.10	1.86±0.22

Values are expressed as mean of 5 rats ±SEM. All values are not significantly different at P<0.05

Table 2: Serum and tissue ALT (IU/L) in rats administered with sodium benzoate (SB) and leaf extracts of *Azadirachta indica*

Tissue	Control	SB Only	SB + extract 200mg/kg bw	SB + extract 500mg/kg bw
Serum	48.49±2.12^a	61.16±3.25^b	50.23±2.22^a	49.67±2.14^a
Liver	389.14±8.35^a	305.21±7.40^b	365.41±8.69^a	374.43±9.55^a
Kidney	258.52±7.23^a	247.95±6.82^a	249.68±7.22^a	261.34±5.36^a
Heart	229.25±5.79^a	237.46±6.21^a	233.81±5.72^a	243.48±4.93^a
Brain	135.05±4.84^a	129.55±6.26^a	142.35±4.70^a	138.24±5.22^a

Values are mean of 5 rats ±SEM. Values with different alphabetical superscript (a, b) along a row are significantly different at P<0.05

Table 3: Serum and tissue AST (IU/L) in rats administered with sodium benzoate (SB) and leaf extracts of *Azadirachta indica*

Tissue	Control	SB Only	SB + extract 200mg/kg bw	SB + extract 500mg/kg bw
Serum	85.98±3.23^a	129.38±4.54^b	92.10±2.56^a	88.71±2.60^a
Liver	683.20±9.84^a	432.66±9.23^b	676.62±9.47^a	669.45±8.85^a
Kidney	326.38±6.72^a	308.75±7.34^a	297.55±5.88^a	318.41±6.80^a
Heart	343.72±5.39^a	322.28±6.22^a	318.84±5.79^a	338.43±6.33^a
Brain	256.90±4.68^a	262.42±3.66^a	259.77±4.39^a	260.84±2.96^a

Values are mean of 5 rats ±SEM. Values with different alphabetical superscript (a, b) along a row are significantly different at P<0.05

Table 4: Serum metabolites of rats administered with sodium benzoate (SB) and leaf extracts of *Azadirachta indica*

Parameters	Control	SB Only	SB + extract 200mg/kg bw	SB + extract 500mg/kg bw
Urea (mg/dl)	38.11±2.34[a]	58.43±2.64[b]	36.16±2.28[a]	39.52±2.52[a]
Uric acid (mg/dl)	16.43±1.79[a]	27.55±2.42[b]	17.58±2.02[a]	18.33±1.54[a]
Creatinine (mg/dl)	12.30±1.23[a]	28.26±1.46[b]	13.51±1.44[a]	13.53±1.33[a]
Albumin (g/dl)	4.05±0.36[a]	4.11±0.44[a]	3.98±0.50[a]	3.98±0.23[a]
Globulin (g/dl)	5.46±0.38[a]	5.38±0.42[a]	5.10±0.31[a]	5.29±0.57[a]
Total protein (g/dl)	10.50±1.23[a]	10.41±2.10[a]	9.94±1.28[a]	10.27±1.36[a]

Values are mean of 5 rats ±SEM. Values with different alphabetical superscript (a, b) along a row are significantly different at P<0.05

3.2 Discussion

The significant growth depression observed in rats administered with sodium benzoate might be due to loss of appetite or malabsorption. Drugs that cause loss of appetite and gastrointestinal irritation in animals initiate poor absorption of essential nutrients leading to malnutrition and growth depression (Robbins et al., 1984). Sodium benzoate caused derangement of liver function as revealed by significant elevation of serum ALT and AST as well as significant reduction of these enzymes in the liver. Determination of AST and ALT in the serum is largely used in the assessment of liver damage (Moss and Rosalki, 1996). Membrane damage to the liver releases the enzymes into circulation and hence can be measured in the serum. High level of serum AST and ALT indicate liver damage (Wang and Srivastava, 2002).

The significant elevation of serum urea, creatinine and uric acid by sodium benzoate are indicative of damage to renal function (Cameron and Greger, 1998). Urea, uric acid and creatinine are waste products of metabolism. They are found in the liver and conveyed through the blood to the kidney for excretion. Healthy kidneys remove these compounds from the blood to be excreted in the urine. Accumulation of these metabolites in the blood is an indication of defective kidney function (Cotran et al., 2005).

The fact that there was no significant change in serum albumin, globulin and total protein in the rats shows that toxicity of the drug was not significant enough to inhibit protein synthesis in the liver. The observed non alteration in the concentration of these proteins is an indication that the gut function in the rats was not affected. If gut absorption is affected, not enough amino acid is absorbed and protein synthesis is compromised. Albumin and globulin are globular proteins found in the serum and they are synthesized by the liver. Serum levels of these proteins are markers of the liver's ability to synthesis proteins (Smith and Lunn, 1984). Low levels of these proteins indicate liver damage. Malnutrition and dehydration can also cause depletion of these proteins in the serum.

The observed biochemical alterations of sodium benzoate in this study agree with that of Fujitani (1993) who reported reduced weight as well as changes in serum and liver parameters after a short term oral administration of sodium benzoate. The result however contrasted the view of Fanelli and Halliday (1963) who observed that toxicity of oral sodium benzoate is not to be expected due to its rapid absorption and metabolism as well as quick excretion of its metabolites in the body.

Azadirachta indica extract appear to have protective role on sodium benzoate induced liver and kidney toxicity in this study as it normalized the levels of ALT, AST urea, uric acid and creatinine in the serum and tissue close to that obtained in the control. These protective properties of the extract may not be far fetch from the fact that the leaf is very rich in phytochemical ingredients with antioxidant properties (Singh et al., 1986). Neem has been reported to contain mainly four triterpenoids with antioxidant properties namely azadirachtin, nimbin, nimbidin and limonoids (Schmutterer, 2002). The efficacy of any poison antidote depends on its capacity to either reduce the harmful effect or restoring the normal physiology caused by the poison. Most hepatoprotective drugs act by inhibiting the aromatase activity of cytochrome P450 thereby favouring liver regeneration (Brent and Rumack, 1993). The observed restoration of normal functions to the damaged liver and kidney by *Azadirachta indica* indicates its protective roles on the structural integrity of the organs.

4. CONCLUSION

The results of the present investigation reveal that leaf extract of neem (*Azadirachta indica*) has protective effect on kidney and liver damage in rat as it significantly reversed the observed disruption in cellular integrity caused by sodium benzoate. These findings corroborate the benefit of *Azadirachta indica* leaf as a traditional remedy for the treatment of liver and kidney diseases.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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