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Effect of aqueous extract of *Azadirachta indica* on rifampicin- induced jaundice in rabbits

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Abstract

High rate of dependence on medicinal plants for maintenance of health care needs over the decades in communities all over the world especially in Africa and Asia needs adequate scientific verification and authentication. Liver related diseases have however become a global problem. Jaundice was established on 9 of a total population of 12 rabbits of mixed sex, weighing 1.50kg - 2.34kg using an overdose pretreatment of Rifampicin (300mg/ kg bw) P.O. After 3 days, the Animals were administered with various doses of aqueous extract of *Azadirachta indica* (600, 750, 900mg/kg bw) P.O for each group of rats – A, B, C and D (control), after which the animals were bled via marginal ear vein at zero hour, 7th and 14th days and the blood used for the following assays: Alkaline Phosphatase, Acid Phosphatase, Total Bilirubin, Conjugated Bilirubin and Alanine Transaminase. Experimental animals exhibited lack of appetite, reduced activity, scratching of the body, emesis, yellow coloration of the sclera, light-colored stool and a little dark urine over time. There was also increase in the levels of the biochemical parameters as the day increased. Aqueous extract of *Azadirachta indica* (Neem) exhibited increasing elevated levels of Alkaline Phosphatase (ALP), Acid Phosphatase (AP), Total Bilirubin (T/Bil), Conjugated Bilirubin (Conj/Bil), and slightly in Alanine Transaminase (ALT), thus exerted elevated hepatotoxicity on Rifampicin-induced Rabbits. Therefore, the traditional use of *Azadirachta indica* in management of jaundice should be discontinued.

Keywords: *Azadirachta indica*; Rabbit; Bilirubin; Jaundice; Rifampicin

1. Introduction

Herbs and foods may stimulate or assist the immune system to conquer sickness, injury, fatigue or a variety of maladies in human life. Many communities in Africa have much elaborated plant knowledge [1]. Medicinal plants are globally valuable sources of new drugs [2, 3, 4]. The term 'crude drugs of natural or biological origin' is used by pharmacists and pharmacologists to describe whole plants or parts of plants which have medicinal properties [5].

Azadirachta indica A. juss (AI; Family: *Meliaceae*) is a popular medicinal plant originally grown in India [6], but is now being cultivated in almost every part of the world including Nigeria [7].

Names for this plant in various languages include Hausa - Darbejiya, Dogonyaro, Bedi, Arabic - Neeb, Azad-darakhul-hind, Shajarat Alnnim, English - Margosa, Neem Tree, French - Azadirac de l'Inde, margosier, margousier, German - Indischerzedrach, Grossblaettigerzedrach [8]. *Azadirachta indica* is being used for various purposes. Neem is now used

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as an active ingredient in certain popular such as tooth paste in Germany and India since it prevents tooth decay and heals inflammation of gums, as chewing stick for toiletries [9], and in antidesertification [10, 11]. Very young leaves of *Azadirachta indica* and r. Juss. (Meliaceae – ‘Vembu’) are fried with *Carumnothum*Cl. (Apiaceae – ‘Omam’) salt, powdered and given in milk in order to cure jaundice [12]. The divine tree neem (*Azadirachta indica*) is mainly cultivated in the Indian subcontinent. Neem has been used extensively by humankind to treat various ailments before the availability of written records which recorded the beginning of history [13]. Jaundice is one of the most common medical condition affecting infants, children, and adults. Jaundice is not a disease but it is a visible sign and symptom of liver disease which occurs when there is an increase in the amount of bilirubin circulating in the blood due to the abnormal metabolism and excretion in the urine [14]. In spite of tremendous advances made in allopathic medical practices, herbs still play an important role in the management of various liver diseases. A large number of plants and formulations have been claimed to have hepato-protective activity [15]. Medicinal plants are being used long ago by our ancestors for the treatment of jaundice. At the present era, consumption of these herbal medicines is increasing at a high speed, due to its less or no side effect and cost-effectiveness as compared to synthetic medicines [14]. Rifampicin caused a significant increase in the total cholesterol, triglycerides and LDL-cholesterol, a significant decrease in HDL-cholesterol as well as significant increase in serum AST, ALT, bilirubin and urea levels in an investigation to evaluate the effect of two oral administered antibiotics (Rifampicin and Tetracycline) on some physiological parameters in the serum of male albino rats where histopathological alterations in liver and kidney examined [16]. Rifampicin is associated with frequent adverse reaction of which jaundice is most notable [17]. Serum bilirubin has also been proposed to be a useful marker to differentiate cardioembolic stroke from other subtypes of strokes [18]. Recently, high serum bilirubin levels were found to be significantly associated with the fatal outcome in patients infected with Ebola virus [19].

High rate of dependence on medicinal plants for maintenance of health care needs over the decades in communities all over the world especially in Africa and Asia need urgent attention as various claims on the use of plants by the communities differ while some are same even with the challenge of proper dosage, formulation and standardization before administration. Several works were reported on the utility of plants for the treatment of diabetes and jaundice [20, 21]. Continual and further studies on herbal preparations should always be in the front burner of medical and pharmaceutical research [22], thus further need to authenticate the use of *Azadirachta indica* in the treatment of jaundice. This study aimed to evaluate the effect of aqueous leave extract of *Azadirachta indica* on Rifampicin jaundice induced rabbits.



Figure 1 Neem tree [23]

2. Material and methods

2.1. Plant collection and extraction

Fresh leaves of *Azadirachta indica* was collected from Rayfield area of Jos- plateau state Nigeria. This was identified and authenticated at the Federal College of Forestry, Jos, Nigeria. The leaves were thoroughly washed with clean water and shredded. After this, 300g was weighed into 250ml of distilled water and grinded in an electric blender in order to

obtain a homogeneous mixture. This was then sieved with Whatman (size12) filter paper, while the filtrate was weighed and kept in the refrigerator until use.

2.2. Animal preparation and drug administration

12 rabbits of mixed sex, weighing between 1.50kg and 2.34kg were collected from the Animal House Unit of the Department of Pharmacology and Therapeutics, Bingham University, Jos, Nigeria. The animals were maintained with pelleted feed and water (*ad libitum*), optimum light and temperature in accordance with the principles and guidelines of handling laboratory animals according to [24]. The animals were divided into four groups of three animals each, kept in cages for 7 days, starved 24 hours prior to experiment for acclimatization and were administered with Rifampicin and aqueous extract of *Azadirachta indica*. Groups 1-3 were induced with Jaundice using Rifampicin at 300mg/ kg, P.O for 3 consecutive days in order to elevate the bilirubin (conjugated and total) and alkaline phosphates levels. On day 4, aqueous extract of *Azadirachta indica* (900mg/kg, P.O) was administered. However, group 4 served as control. Furthermore, fecal and urine appearances and animal behaviours were observed both at beginning and end of experiment.

2.3. Blood sample collection

The animals were all bled at zero hour and 3 days after Rifampicin administration as well as on day 7 and 14 post *Azadirachta Indica* extract administration. 5ml of blood sample was collected via the marginal ear vein of each animal into dry centrifuged tubes and were allowed to retract for one hour, the sera formed was centrifuged at 3000 rpm for 5 minutes to obtain a clear serum which was then transferred into plain EDTA bottles using pipette.

2.4. Biochemical Analysis

The following investigations were carried out to determine the serum concentrations of serum bilirubin according to [25], serum alkaline phosphatase and serum aminotransferases (AST and ALT) using commercial diagnostic kits by Randox UK.

2.5. Statistical analysis

Descriptive methods were used. All data were expressed as mean \pm SEM.

3. Results and discussion

Behavioural manifestations of the animals in the experimental group (A, B and C) starting from the first 5 days include refusal to eat, reduced activity thus became unusually reserved, robbing of body against the cage and scratching of the body. Others include Emesis, yellow coloration of the sclera, light-colored stool and a little dark urine within day. Group D animals however showed none of the mentioned signs and symptoms. The aforementioned portrayed that jaundice was established in the experimental animals. However, results increase level in of hepatotoxicity in the animals via the biochemical investigations as the number of days increased. This agrees with [26], who stated that Rifampicin is associated with frequent adverse reaction of which jaundice is most notable. Similarly, Rifampicin produces many metabolic and morphological aberrations in the liver due to the fact that the liver is the main detoxifying site for these anti-tubercular drugs [27, 26].

Having induced hepatotoxicity on the experimental animals, biochemical analysis revealed increased level of alkaline phosphatase in the presence of the aqueous extract of *Azadirachta indica* with time as seen in from baseline to week 2, with group C having the highest level followed by B and A respectively – Table 2; Figure 2. Same trend was observed in the Total Bilirubin level while the control group remained unchanged – Table 2; Figure 3 and that of the Conjugated Bilirubin level, but the animals had severe diarrhea while one mortality was recorded – Table 2; Figure 4.

Furthermore, there was a slight difference with the level of alanine Transaminase despite non-the-less, it followed same pattern of increasing levels of the biochemical parameters from baseline – week 2 - Table 2; Figure 5. The characteristic of the aqueous extract of *Azadirachta indica* in this case is in contrary with the study by [28] who reported that the ALT profile of rabbits treated with 25%, 50% and 75% *C. lemon* juice were extremely significantly different when compared to the control group at $P < 0.001$ and that of [29] who also reported *T. cardifolia* stem and leaves extract showed hepatoprotective action against lead-induced hepatotoxicity in mice due to the scavenging of free radicals generated by the lead toxicity. The foregoing is an indication of a detrimental effect on the biochemical functions of the liver of the experimental animals.

Table 1 Body weight and volume of drugs and extract administered per Animal

Group	Animal	Body weight(Kg)	Required dose(Mg/Kg)	Volume (ml)	Required dose(Mg/Kg)	Volume (ml)
A	I	1.80	300	1.8	600	3.6
	II	2.10	300	2.1	600	4.0
	III	1.90	300	1.9	600	3.8
B	I	1.85	300	1.9	750	4.6
	II	2.34	300	2.3	750	5.9
	III	2.0	300	2.0	750	5.0
C	I	1.50	300	1.5	900	4.5
	II	1.60	300	1.6	900	4.8
	III	2.01	300	2.0	900	6.0
D	I	1.85	1.8	-	-	-
	II	1.68	1.7	-	-	-
	III	2.01	2.0	-	-	-

Table 2 SEM of biochemical parameters of the blood samples of *Azadirachta indica* treated jaundiced Rabbits

Biochemical parameters	Group	Baseline	1 st Administration	Week 2 nd Administration	Week
Alkaline phosphatase	A	44.5 ±0.0	54.3 ±1.0	54.0±1.0	
	B	44.0±0.0	55.0 ±1.0	58.0 ±1.2	
	C	43.6±0.4	54.0 ±0.9	67.0±1.5	
	D	45.6±0.0	46.7±0.1	46.3±0.3	
Total bilirubin	A	5.1±0.0	8.6±0.7	10.2±0.4	
	B	5.1±0.0	8.7±0.2	15.3±0.8	
	C	5.1±0.0	8.±0.2	17.2±0.9	
	D	5.1±0.0	5.1±0.0	5.1±0.0	
Conjugated bilirubin	A	9.0±0.2	13.4±0.6	15.5±0.4	
	B	9.45±0.2	19.0±1.0	20.0±2.0	
	C	9.20±0.2	20.0±2.0	24.0±1.2	
	D	8.0±0.0	8.0±0.0	8.0±0.0	
Alanine transaminase	A	44.0±0.1	47.0±0.7	48.5±0.6	
	B	43.5±0.1	46.0±0.2	49.2±0.4	
	C	43.5 ±0.1	48±0.7	49.5±0.4	
	D	43±0.0	43±0.0	43±0.0	

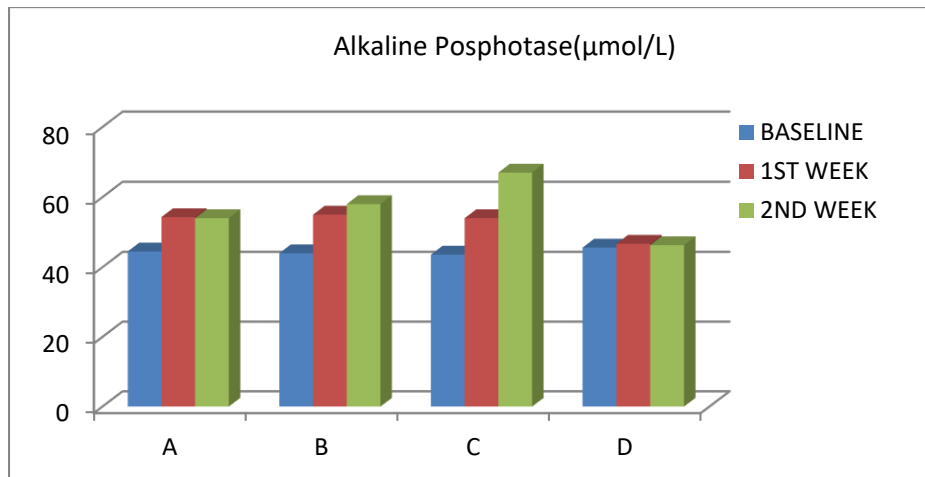


Figure 2 Alkaline phosphatase

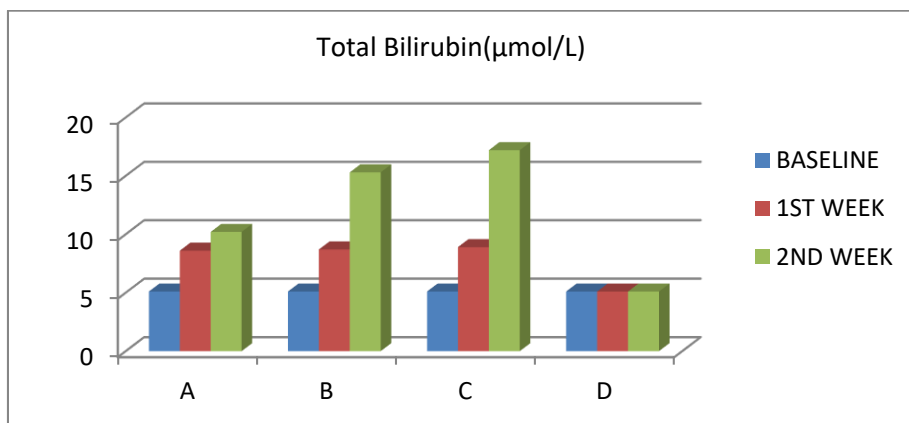


Figure 3 Total Bilirubin

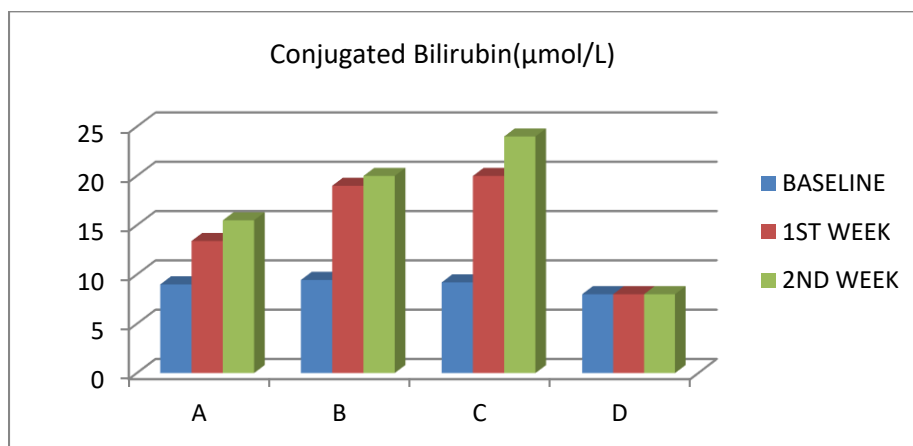


Figure 4 Conjugated bilirubin

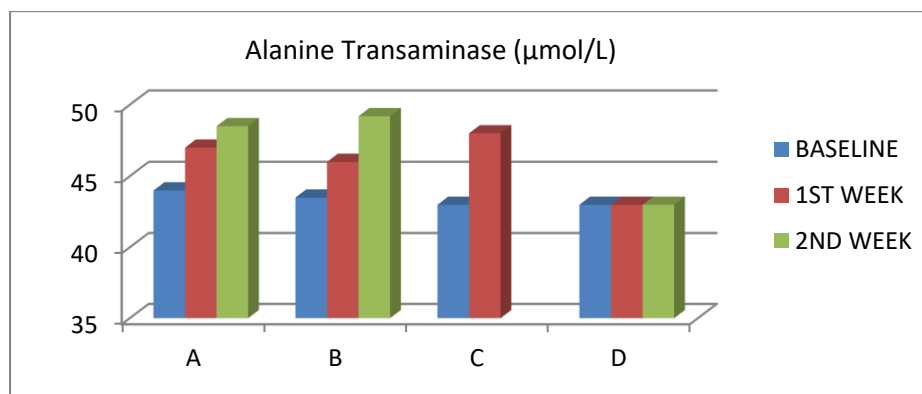


Figure 5 Alanine Transaminase

4. Conclusion

Aqueous extract of *Azadirachta indica* (Neem) exhibited increasing elevated levels of Alkaline Phosphatase (ALP), Acid Phosphatase (AP), Total Bilirubin (T/Bil), Conjugated Bilirubin (Conj/Bil), and slightly in Alanine Transaminase (ALT), thus exerted elevated hepatotoxicity on Rifampicin-induced Rabbits. Therefore, the traditional use of *Azadirachta indica* in management of jaundice should be discontinued.

Compliance with ethical standards

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Disclosure of conflict of interest

Authors declare no conflict of interest.

Statement of ethical approval

Authors are licensed to handle laboratory animals. Animal care research protocols were carried out based on the principles and guidelines in compliance with the guide for the care and use of laboratory animals as reported in Tuhin *et al.*, (2017) [30].

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