



EFFECT OF ORAL ADMINISTRATION OF *ALBIZIA LEBBECK* AQUEOUS STEM BARK EXTRACT ON SOME LIVER FUNCTION INDICES IN RATS

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ABSTRACT

Background: *Albizia Lebbeck* is also used in traditional system and folk medicine as well as to treat several inflammatory conditions such as asthma, arthritis, allergic rhinitis, bronchitis, helminthes infections and burns. **Objective:** this study is undertaken to ascertain the effect of oral administration of *Albizia Lebbeck* aqueous stem bark extract on some liver function indices in albino rats. **Materials and Methods:** The effect of *Albizia lebbeck* aqueous stem bark extract administered orally for twenty four days (24) was studied in albino rats. The research work was undertaken to ascertain serum concentrations or activities of some liver enzymes; Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) and Alkaline phosphatase (ALP) and also the levels of Total bilirubin (TBIL). During this study, sixteen (16) albino rats were used and divided into four (4) groups. Group I served as control. However, Groups II, III and IV were considered to be test groups and were administered with *Albizia Lebbeck* aqueous stem bark extract of 100, 300 and 500mg/kg body weight dosage respectively. The mean serum activities of enzymes AST, ALT, were determined using the method of Reitman ad Frankel, 1957, ALP According to the Method of Klein, Read and Babson (1960) however, that of TBIL levels by the method of Jendrassik and Grof, 1938. Mean serum levels of AST, ALT, ALP and TBIL for each dose of 100, 300 and 500mg/kg body weight were found to be; [22.18±03.10U/L, 25.00±04.22U/L, 150.85±30.99U/L and 11.97±02.42mm/L], [27.38±02.74U/L, 35.70±05.02U/L, 175.35±34.83U/L and 13.26±04.50mm/L] and [45.40±07.18U/L, 55.45±09.69U/L, 218.83±56.35U/L and 15.39±10.34mm/L] respectively. However, control results for the above parameters (AST, ALT, ALP and TBIL) of; [15.01±02.15U/L, 13.90±01.76U/L, 57.53±08.67U/L and 11.97±05.76mm/L] were also obtained respectively. Therefore, the results of this work indicated a significant increases ($p < 0.05$) in the AST, ALT and ALP activities of test groups in comparison with control. Hence, the level of TBIL in the test groups revealed no significant increases ($p > 0.05$) in comparison with control. **Conclusion:** We revealed that daily oral administration of aqueous stem bark extract of *Albizia lebbeck* for 24 days induced significantly hepato-toxicity in rats at the administered doses with elevated levels of AST, ALT, ALP and TBIL concentrations.

Keywords: *Aspartate aminotransferase, Alanine aminotransferase, Akaline phosphatase, Total bilirubin, Serum, control*

1.INTRODUCTION

Medicinal plants have been used in treating human diseases for thousands of years. It was estimated that 64% of the world's population use medicinal plants to treat illnesses and relieve pains [1]. They have been used by all cultures throughout history and thus, herbal medicine is the oldest form of health care known to mankind, it was an integral part of the development of modern civilization [2]. Medicinal plants are huge reservoir of various chemical substances with potential therapeutic properties and are being increasingly utilized to treat a wide variety of clinical diseases [3]. The therapeutic properties of medicinal plants used by traditional medical practitioners may be due to one or more of the many compounds of the plant materials. These plant materials called phytochemicals include complex carbohydrates, lipids, alkaloids, glycopeptides, terpenoids, tannins, saponins, peptides and amines, steroids, flavonoids, coumarins, sulphur compounds and inorganic ions among numerous others. The primary benefit of using plant derived-medicine is that; they are relatively safer than synthetic alternatives, offering profound therapeutic benefits and affordable treatment [4].

Albizia lebbeck (L) Benth, is native to deciduous and semi deciduous forest in Asia from eastern Pakistan through India and Sri Lanka to Burma. The tree has been introduced as an ornamental and plantation tree throughout the tropics and northern subtropics, including the Greater and Lesser Antilles, Central America, Colombia, Venezuela, and Brazil [5]. It is a fast-growing, medium-sized deciduous tree with a spreading umbrella-shaped crown of thin foliage and smooth, finely fissured, grayish-brown bark [6]

Albizia Lebbeck is also used in traditional system and folk medicine as well as to treat several inflammatory conditions such as asthma, arthritis, allergic rhinitis, bronchitis, helminthes infections and burns. Stem bark extract have documented anti-inflammatory properties, blood tonic, sedative, analgesic and antihypertensive [7]. Leaves are reported to be good for ophthalmic diseases, night blindness, syphilis ulcer, cold, cough, and other respiratory disorders. Flowers are also useful for the treatment of spermatorrhea; however, seeds are utilized as brain tonic as well as for treating gonorrhoea, cure piles, and scrofulous swellings.

Liver is the second largest organ of the body and can be referred to as the metabolic "engine-room of the body". Almost all the drugs, foods and water constituents are metabolized and detoxified in the liver, and as such it is often exposed to maladies resulting in a number of clinical syndromes [8]. Many chemicals, foods, drugs and infections (parasitic, bacterial,

viral or fungal) can cause variety of liver diseases such as hepatitis, jaundice, cirrhosis, liver cancer, etc. Liver also has a pivotal role in regulation of physiological processes. Other functions of liver include; inter-conversion and synthesis of protein, carbohydrate and lipid metabolism [9]. Liver stores various compounds that are essential for metabolic processes, for example, glycogen. Also, Kupffer cells of the liver, which are specialized macrophages/phagocytes, are involved in the immunological activities performed by the reticuloendothelial system. Thus, the liver contributes to the body immune system [10]. Metabolism and excretion of bilirubin, production of red blood cells in the first trimester of fetus occurs in the liver.

It is believed and accepted that plants contains biological active compounds which seems to protect against degenerative diseases [11]. But, it must be noted that not all medicinal plants are safe for consumption in the crude form. Some levels of toxicity may arise as a result of potential toxic compounds they contains and pesticide application during cultivation [12]. Moreover, insufficient literacy on dosage prescription and combination also contributed. Thus, looking at the medicinal significant of *Albizia Lebbeck* and vital functions of the liver in the body, it is extremely significant to determine any change in liver function on administration of *Albizia Lebbeck*. Therefore, this study is undertaken to ascertain the effect of oral administration of *Albizia Lebbeck* aqueous stem bark extract on some liver function indices in albino rats.

The methods selected are accurate, convenient and within the range of daily routine analytical methods. They are neither tedious nor cumbersome. Also, the indices (parameters) to be analyzed in this research work are among the primary indicators or makers for liver function test.

2. MATERIAL AND METHODS

2.1 Animals

Sixteen (16) wister albino rats of both sexes, body weight ranging from 120-150g, were obtained from Animal House, Biological Sciences Department, Bayero University, Kano-Nigeria. They were allowed free access to water and normal feeds (grower's palletized feeds from agrovet animal feeds, Ikeja, Lagos- Nigeria). They were also allowed to acclimatize for one week prior to the commencement of the experiment.

2.2 Sample collection (Plant material): The stem bark of *Albizia Lebbeck* was obtained from Gano Local Government Area, Kano-Nigeria. The sample was authenticated at Botany Unit of Biological Sciences Department, Bayero University, Kano-Nigeria. It was shade dried, grounded into powder and sieved.

2.3 Preparation of aqueous stem bark extract: The sieved powder of *Albizia lebbeck* stem bark was weight and suspended in 150ml of distilled water and allowed to stay overnight (24hours).The suspension was then filtered using a Whatman No.1 filter paper. Filtrate was allowed to evaporate in an oven and the fine powder finally obtained was reconstituted using distilled water so as to obtain a desired concentration for administration to the rats. The extract prepared was kept in refrigerator at 25°C, and refresh after each three (3) days.

2.4 Experimental design: A total of sixteen (16) Wister albino rats were utilized, and the animals were randomly divided into four (4) groups of four (4) rats each. Group I served as control, group II, III and IV were the test groups.

2.4.1 Extract administration: Extract of *Albizia Lebbeck* aqueous stem bark of 100 300 and 500mg/kg were administered orally to groups; II, III and IV respectively, Group I (control group) was only given 1ml of water. The administration of the extract lasted for 24 days.

2.5 Collection of the sample (serum): At the end of three (3) weeks daily oral administration of *Albizia Lebbeck* aqueous stem bark extract, the rats were sacrificed using a razor blade and blood samples were collected into a clean and dried centrifuge tubes. The blood samples were allowed to clot at room temperature for about 30mins, after which application stick was used to carefully loosen the blood. They were then centrifuged at 2,500rpm for about 15minutes. A clean Pasteur pipette was used to carefully collect the serum and dispensed into a clean labelled specimen bottle. Sera samples been collected were analyzed for activities of AST, ALT, ALP and TBIL.

2.6 Methods of analysis

2.6.1 Determination of serum Aspartate aminotransferase (AST) activity by the Method of [13]

Principle: Aspartate aminotransferase catalyses the transfer of amino group from Aspartate to 2-oxoglutarate, forming oxaloacetate and glutamate. The oxaloacetate reacts with 2, 4 - dinitrophenyl hydrazine (DNPH) to form 2, 4 - dinitrophenyl hydrazone, which in alkaline medium gives a coloured complex. The intensity of the colour produced is proportional to AST activity in the sample.

Procedure: To each test- tube labelled sample blank and sample, Reagent 1 (0.5ml) was introduced. Serum (0.1ml) was added to the test tube s labelled sample. The content of each test – tube was mixed and incubated for exactly 30mins at 30°C. Reagent 2 (0.5ml) was then added to each of the test – tubes. Serum (0.1ml) was added to the test tube labelled sample. The tubes were mixed again and allowed to stand for exactly 20mins at 20-25°C. Sodium hydroxide (5.0ml) was then added to each of the test tubes.

The content of the test tubes were mixed again after addition of sodium hydroxide and absorbance of test samples were read against sample blank, after five minutes calorimetrically at 505nm. The activity of Aspartate aminotransferase (AST) in each test sample was obtained using the reference table provided in the randox test kit.

2.6.2 Determination of serum Alanine aminotransferase (ALT) Activity the Method of; [13]

Principle: Alanine aminotransferase (ALT) catalyses the transfer of amino group from Alanine to 2- oxoglutarate, forming pyruvate and glutamate. The pyruvate reacts with 2, 4 – dinitrophenyl hydrazine, which forms a coloured complex in alkaline solution. The intensity of the colour produced is proportional to (ALT) activity in the sample.

Procedure: To each test- tube labelled sample blank and sample, Reagent 1 (0.5ml) was added. Serum (0.1ml) was added to the test- tubes labelled sample. The content of each test – tube was mixed and incubated for exactly 30mins at 37°C. Reagent 2 (0.5ml) was added to each test tube. Serum (0.1ml) was added to the test tube labelled sample. The contents of the test tubes were mixed again and allowed to stand for twenty (20) minutes at 20- 25°C. Sodium hydroxide (5.0ml) was then added to each of the tube.

The content of the test tubes were mixed after addition of sodium hydroxide and absorbance of test samples were read against sample blank, after 5mins, calorimetrically at 505nm. The activity of Alanine aminotransferase (ALT) in each test sample was obtained using the reference table provided in the randox test kit.

2.6.3 Determination of serum Alkaline phosphatase (ALP) Activity according to [14]

Principle: Alkaline phosphatase catalyses the hydrolysis of p – nitro-phenyl phosphate to phosphate and p – nitro-phenol. The p- nitro-phenol (a coloured compound) can be measured calorimetrically. The absorbance of the p- nitro-phenol is proportional to ALP activity in the sample.

$P - \text{Nitro-phenyl phosphate} + H_2O \rightarrow p - \text{nitrO-phenol} + \text{phosphate}$

Procedure: The ALP reagent was brought to 37°C before the commencement of the test. Serum (0.5ml) was introduced in to a clean, dry test tube and working reagent (3.0ml) was added and mixed. The absorbance was then read twice (initial absorbance measured immediately and second absorbance measured after two minutes at 450nm. Change in absorbance $\Delta A = A_1 - A_2$ is ALP concentration.

Calculation: Alkaline phosphatase (ALP) activity in each sample was calculated using; $\Delta A/\text{min} \times \text{factor} = 2757$.

2.6.4 Determination of serum Total Bilirubin (TBIL) [15]

Total bilirubin is determined in the presence of caffeine, which release albumin bound bilirubin by the reaction with diazotized sulphanilic acid to yield a purple solution.

Procedure: Two test–tubes were labelled sample blank and sample respectively. Reagent 1 (0.2ml) was added to each labelled test- tubes above. Reagent 2 (0.5ml) was introduced in to the test-tubes labelled sample. Reagent 3 (1.0ml) was added to each test-tube, followed by serum (0.2ml) to each. The contents of the test –tubes were allowed to stand for 10mins at 25°C. Finally, Reagent 4 (1.0ml) was added to each test –tube. The test – tubes were mixed again, and allowed to stand for 15mins at 25°C. The absorbance of the sample was read against sample blank (A_{TB}) at 578nm.

Where; TB = Total bilirubin, A = absorbance.

Total bilirubin (mmol/L) = $17.1 \times A_{TB}$

2.7 Statistical analysis: Data were presented as mean+standard deviation of duplicate measurement. The significant difference between the mean groups was assessed by student T-test [16].

3.RESULTS

The results of AST, ALT, ALP activities and TBIL concentrations in the control and test groups administered with 100, 300 and 500mg/kg of *Albizia lebbbeck* stem bark aqueous extract orally for four (4) weeks indicated significant increases ($P < 0.05$)

in the activities of the enzymes in test groups. However, no significant increases ($P>0.05$) was observed in the concentrations of total TBIL in test groups in comparison with control.

Table 1: Effect of Oral Administration of Aqueous Stem Bark Extract of *Albizia lebbbeck* on the Activities of Enzymes and Levels of Total bilirubin.

Dosage	AST (U/L)	ALT (U/L)	ALP (U/L)	T-BIL (mmol/L)
Control	15.01±02.15	13.90±01.76	57.53±08.67	11.97±05.76
100mg/kg(<i>Albizia lebbbeck</i> aqueous stem bark extract)	22.18±03.10 ^A	25.00±04.22 ^A	150.85±30.99 ^A	11.97±02.42*
300mg/kg(<i>Albizia lebbbeck</i> aqueous stem bark extract)	27.38±02.74 ^A	35.70±05.02 ^A	175.35±34.83 ^A	13.26±04.50*
500mg/kg(<i>Albizia lebbbeck</i> aqueous stem bark extract)	45.40±07.18 ^A	55.45±09.69 ^A	218.83±56.35 ^A	15.39±10.34*

Values are presented as mean ± standard deviation; Number of rats per group = 4; ^A = Significant increase in the test groups indices compared with that of the normal control group ($P<0.05$); * = No significant increase in the test group indices compared with that of control group ($P>0.05$).

4. DISCUSSION

The ALT, AST, ALP activities and TBIL levels in the control rats were found to be (13.90±01.76U/L), (15.10±02.15U/L), (57.53±08.67U/L) and (11.97±05.76mmol/L) respectively. For the 100mg/kg *Albizia Lebbbeck* stem bark extract oral administration, the serum concentrations of the above indices were observed to be (25.00±04.22U/L), (22.18±03.10U/L), (150.85±30.99U/L) and (11.97±02.42mmol/L) respectively. Also, for 300mg/kg of the extract administration revealed the activities of AST, ALT, ALP and TBIL to be; (35.70±05.02U/L), (27.38±02.74U/L), (175.35±34.83U/L) and (13.26±04.50mmol/L) respectively. However, the levels of the above indices for 500mg/kg of the extract administration were found to be; (55.45±09.69U/L), (45.40±07.18U/L), (218.83±56.35U/L) and (15.39±10.34mmol/L) respectively (Table1).

The results of this work indicated that; daily oral administration of *Albizia lebbbeck* aqueous stem bark resulted in a significant increases ($P<0.05$) in the activities of AST, ALT and ALP in the test groups compared with that of the control group. However, TBIL levels indicated no significant increases ($P>0.05$) for all dosages compared with that of control.

Therefore, these increases in the enzymes activities might be due to the fact that the daily dosages of 100, 300 and 500mg/kg of the extract administered for four (4) weeks have induced toxic effects, since higher AST and ALP activities are indicative parameters (indices) of liver damage or disorder [17]. The plant is said to have hepato-toxic effect for all dosages administered, hence, dose in depended.

The elevated levels of the above parameters (especially AST and ALT) can also be used in assessing and monitoring liver damage [18]. Their presence in serum may give information of organ dysfunction [19]. But, they are poor prognosticators for severity of liver injury or acute liver failure. The ability to predict necrosis both at the molecular and cellular levels, extend of the damage and the source of the insult is currently a challenge using classical toxicological assays, parameter or biomarkers [20].

5. CONCLUSION AND RECOMMENDATIONS

The results from this study have revealed that daily oral administration of aqueous stem bark extract of *Albizia lebbbeck* for twenty four (24) days have induced significantly hepato-toxicity in rats at the administered doses. Although, numerous health benefits can be derived from the plant in general as discussed in literature, but, care needs to be taken on the dosage and also the liver function indices needs to be monitored on administration of the plant extract, especially the stem bark. Further study should be carried out on the isolation, purification and characterization of the bioactive compounds present in this plant that is responsible for the elevated levels of AST, ALT, ALP and TBIL concentrations. Histopathological analysis of the liver should also be examined to determine any hepato-cellular damage or injury.

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