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Toxicological Effects of Crude Saponin Extracted from the Leaves of *Azadirachta Indica* on The Tissues of Albino Rats

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Abstract- ***Background: Azadirachta indica A.Juss (Neem), belonging to the Meliaceae family, is renowned for its versatility and medicinal properties widely used in Nigeria, India, and globally. It is considered that Azadirachta indica shows therapeutic role due to the rich source of antioxidant and other bioactive compounds. The study aims to evaluate the efficacy, safety and toxicity of the effects of crude saponin extract from the leaves of Azadirachta indica on albino rat. The effect of methanolic crude saponin extract from Azadirachta indica (CSEAI) leaves on tissues of albino rats was evaluated by measuring the tissues and serum biochemical parameters.***
Methods: Twenty-five adult's albino rats were randomly assigned to one of five experimental groups; Group 2-5 were orally administered with 200-500mg/kg of the crude saponin extract respectively for 21 days while group 1 was the control. Serum and tissues (liver, heart, and kidney) biochemical profile were estimated using standard methods after day 21.
Results: Results indicated no significant alterations in body or organ weights. Tissue enzymic activities of Alanine Transaminase (ALT), Alkaline Phosphatase (ALP), and Creatinine Kinase (CK) increased significantly ($p<0.05$), with a concomitant decrease in their serum levels. Conversely, tissue activities of Aspartate Transaminase (AST), Acid Phosphatase (ACP), and Lactate Dehydrogenase (LDH) showed no significant variation. Serum levels of Blood Urea Nitrogen and Creatinine remained unchanged. The 500mg/kg dose elicited the most pronounced tissue enzyme elevation with corresponding serum reduction.
Conclusion: Conclusively, this study may suggest that CSEAI on rat is presumably safe. However, further researches are required to validate these findings.

Keywords: Crude saponin, Azadirachta indica, Tissues, Methanolic Extract, Medicinal plant.

I. INTRODUCTION

Medicinal plants have been part of human society to prevent and treat disease from the dawn of civilization. Many people in local African communities use plant-derived remedies without sufficient knowledge about their efficacy or potential risks. Scientific evaluation of medicinal plants is important to discovery of novel drugs and also helps to assess toxicity risk associated with the use of either herb preparation of conventional drugs of plant origin. Herbal products can cause unwanted effects due to the intricate combinations of compounds in plant extracts, potentially harming key organs (Arunsi et al., 2020). To ensure safe and effective use, it's essential to conduct scientific research to validate the benefits and properties of medicinal plants, providing reliable guidance for users of natural products (Rajput et al., 2020).

Azadirachta indica is an unusual, drought-resistant tree with rapid growth. The tree is widely recognized by several names, including nim tree, neem, and Indian lilac. In Nigeria, it's commonly referred to as "Dongoyaro" particularly in the Hausa language, for the Neem tree (Azadirachta indica). It is also widely used in other parts of the country. (Enwerem and Amos, 2017). Dongoyaro trees can grow up to 20-23 meters tall with a straight trunk and compound leaves, producing green fruits that turn golden yellow when ripe. Its leaves, bark, seeds, and other parts yield a range of bioactive compounds with various medicinal properties, including anticancer, antimalarial, antibacterial, and anti-inflammatory effects (Fernandes et al., 2019; Ahmed and Raniya, 2021). Due to its extensive therapeutic benefits, Dongoyaro tree is often called a "village pharmacy"

in India and has been honoured globally by the United Nations as the "Tree of the 21st century" (Braga et al., 2021).

Saponins are naturally occurring compounds found in many plants, characterized by their soap-like foaming properties in water (Góral and Wojciechowski, 2020). They are composed of a sugar moiety linked to a triterpene molecules, giving them unique properties (Lemine et al., 2022). The multifaceted benefits of saponins make them important in food production, traditional medicine, and pharmaceutical applications. Research has highlighted the diverse medicinal properties of saponin, including, anti-inflammatory (Passos et al., 2022), antiviral (Sharma et al., 2021), anticancer (Zhou et al., 2021), cytotoxic and molluscicidal properties (Oleszek et al., 2019; El Hazzam et al., 2020), making them valuable compounds.

Little is known about the toxicological evaluation of the crude saponin extract of A. indica. This present study aims to assess the potential toxicity of the effects of crude saponin extract from the leaves of Azadirachta indica on rat, as a systematic approach to evaluate its efficacy and safety profile. This research will help determine the potential risks associated with using these plant extracts ensuring their safe use in traditional medicine or other applications.

II. MATERIALS AND METHODS

Plant Materials

Fresh leaves of Azadirachta indica were obtained from Ekiti State University campus. The fresh A. indica was identified in the Department of Plant Science and Forestry Herbarium, Ekiti State University, Ado Ekiti, Nigeria and a voucher number UHAE 2025050 were assigned.

Preparation of Methanolic Extract

Fresh matured Dongoyaro leaves were cleaned, air-dried, and grounded to obtain a powder (1500g) which was extracted in methanol at 65oC using soxhlet extractor. With the aid of rotatory evaporator the methanolic extract was distilled to give off methanol. The extract was partitioned in 4:1 litre of absolute butanol and distilled water and was allowed to partition into two layers in a separating funnel. The upper part of the extract was then distilled to concentrate and then precipitated with absolute diethylether to obtain the crude saponin extract which was then stored at room temperature until used.

Experimental Animals

Wistar rats (Rattus novegicus) weighing between 140g and 165g were sourced from the University of Ilorin's Biochemistry Department animal house in Kwara State. The animals were housed in controlled conditions with regulated light and temperature, fed standard pellet diets, and had unlimited access to water throughout the study.

Experimental Design

Twenty five (25) R. novegicus used in this study were randomly divided into five groups of five rats each. Group I was the control and has unlimited access to only drinking water while group II, III, IV and V were orally exposed once daily for 21 days to graded doses of 200 mg/kg, 300 mg/kg, 400 mg/kg and 500mg/kg crude saponin extract respectively.

Sample Preparation

Following the 21 days oral administration period, the rats were weighed and humanely sacrificed under anaesthesia. Blood was collected into a well labelled sample bottle and allows to clot. The serum was removed with the aid of pasteur pipette into a centrifuge tube and spun in a centrifuge at 3000 revolutions per minute for 5 minutes. The processed serum was stored at -20oC in a refrigerator until when needed for analysis. The liver, kidney, and heart were also quickly excised, rinsed in ice-cold 0.25M sucrose solution, and weighed. The organs were homogenized in cold sucrose solution of 1:4w/v and the homogenates were centrifuged at 1,500 for 10 minutes to obtain the supernatant used in the determination of enzyme activity.

Biochemical Analysis

The levels of Aspartate aminotransferase, Alanine aminotransferase, Alkaline phosphatase and Acid phosphatase activity using commercial kits (Randox Ltd. U.K) as described by Reitman and Frankel method; while serum metabolites of creatinine, blood urea nitrogen were evaluated using methods of (Fossati et al.,1980).

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Statistical Analysis

The data was subjected to statistical analysis via ANOVA and further evaluated using Duncan's Multiple Range Test.

III. RESULTS

Body and internal organs weight (g)

Table1: Effects of crude saponin extract from A. indica leaves on the body weight of R. novegicus and internal organs

Dose (mg/kg body weight)	Initial body weight	Final body weight	Liver	Heart	Kidney
Control	102.34 ± 1.21	105.36 ± 3.81	6.74 ± 0.40	3	0.86 ± 0.01
200	122.78 ± 2.99	124.42 ± 3.04	7.71 ± 0.37	0.48 ± 0.03	0.89 ± 0.03
300	113.42 ± 4.55	117.64 ± 5.89	6.19 ± 0.09	0.40 ± 0.01	0.81 ± 0.01
400	130.42 ± 13.95	135.86 ± 18.49	5.89 ± 0.27	0.47 ± 0.00	0.58 ± 0.04
500	163.42 ± 3.32	166.00 ± 3.58	7.55 ± 0.16	0.57 ± 0.01	1.09 ± 0.09

Expressed values as , n=5

Aspartate Aminotransferase (AST) assay

Table2: Effect of crude saponin extract from A. indica leaves on Aspartate Aminotransferase enzyme activity in the serum and tissues of R. novegicus

Organ/Enzyme activity (U/L)

Dose (mg/kg body weight)	Serum	Liver	Heart	Kidney
Control	127.00 ± 18.73^{a}	206.25 ± 21.13^{a}	223.75 ± 30.64^{a}	165.75 ± 49.54^{a}
200	135.75 ± 21.51^{a}	188.25 ± 34.72^{a}	195.50 ± 14.98^{a}	140.00 ± 27.81^{a}
300	138.00 ± 11.97^{a}	230.50 ± 29.69^{a}	201.25 ± 18.59^{a}	162.25 ± 16.38^{a}
400	111.00 ± 24.40^{ab}	195.25 ± 22.41^{a}	188.75 ± 26.20^{a}	152.50 ± 25.21^{a}
500	95.75 ± 29.90^{b}	221.50 ± 35.30^{a}	192.25 ± 15.93^{a}	161.50 ± 18.55^{a}

Expressed results are presented as , n=5. Means with identical letter(s) are not different ($p < 0.05$) by DMRT

Table 2 shows that there was no significant differences ($P<0.05$) in the serum level and the tissues homogenate level of AST relative to the control. The serum level was significantly decreased ($p<0.05$) at a dose of 500mg/kg relative to the control.

Alanine Aminotransferase (ALT) assay

Table 3: Effect of crude saponin extract from A. indica leaves on Alanine Aminotransferase enzyme activity in serum, and tissues of R. novegicus

Dose (mg/kg body weight)	Serum (U/L)	Liver (U/L)	Heart (U/L)	Kidney (U/L)
Control	251.25 ± 34.10^{a}	887.00 ± 25.98^{a}	557.75 ± 25.99^{b}	497.50 ± 31.07^{c}
200	215.25 ± 16.89^{a}	923.25 ± 21.58^{c}	479.75 ± 29.82^{a}	381.00 ± 06.47^{c}
300	230.75 ± 05.59^{a}	875.75 ± 15.46^{a}	453.00 ± 08.45^{a}	465.00 ± 23.68^{b}

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400	147.25 ± 20.84 [b]	1005.00 ± 24.96 [b]	504.75 ± 17.35 [b]	446.75 ± 17.42[b]
500	123.50 ± 10.16 [b]	1119.50 ± 16.78 [b]	537.00 ± 19.57 [b]	502.25 ± 22.89[a]

Expressed results are presented as , n=5. Means with identical letter(s) are not different (p< 0.05) by DMRT.

The crude saponin extract rose significantly (p<0.05) in liver ALT activity versus control. However, no significant difference was observed in the heart and kidney ALT activity. Additionally, extract lowered serum levels (p<0.05), particularly at the 500mg/kg dose, as shown in table 3.

Alkaline Phosphatase (ALP) assay

Table 4: Effect of saponin extract from A. indica leaves on alkaline phosphatase enzyme activity in the serum and tissues of R. novegicus

Organ/ Enzyme activity (U/L)

Dose (mg/kg body weight)	Serum	Liver	Heart	Kidney
Control	259.35 ± 27.12[c]	193.14 ± 57.16[b]	319.24 ± 66.37[b]	662.22 ± 18.31[b]
200	181.75 ± 12.32[a]	206.15 ± 57.79[ab]	406.48 ± 27.73[a]	455.63 ± 06.17[a]
300	143.11 ± 13.40[b]	189.00 ± 10.95[b]	464.16 ± 70.63[a]	579.44 ± 29.69[b]
400	86.46 ± 22.51[d]	298.87 ± 61.53 [a]	495.19 ± 22.66 [a]	689.85 ± 31.33[b]
500	138.11 ± 13.36 [b]	268.81 ± 24.43 [a]	389.80 ± 32.28 [b]	654.99 ± 10.27[b]

Expressed results are presented as , n=5. Means with identical letter(s) are not different (p< 0.05) by DMRT Crude saponin extract rose significantly (p< 0.05) in tissue ALP activity versus control, while significantly decreasing serum ALP levels (p<0.05).Notably, the 400 mg/kg dose caused marked decrease in serum ALP and a corresponding increase in tissue ALP.

Acid Phosphatase (ACP) assay

Table 5: Effect of crude saponin extract from A. indica leaves on Acid Phosphatase enzyme activity in the serum and tissues of R. novegicus

Organ/ Enzyme activity (U/L)

Dose (mg/kg body weight)	Serum	Liver	Heart	Kidney
Control	67.73 ± 15.34[a]	288.64 ± 28.10[b]	194.61 ± 10.43[a]	301.73 ± 26.83[a]
200	64.67 ± 18.60[a]	221.33 ± 24.14[a]	101.12 ± 13.27[b]	264.53 ± 18.82[a]
300	85.35 ± 24.65[a]	231.03 ± 19.33[a]	164.39 ± 19.05[a]	282.67 ± 27.44[a]
400	81.16 ± 20.06[a]	243.31 ± 15.01[a]	114.63 ± 03.59[b]	251.43 ± 13.75[a]
500	53.66 ± 12.77[b]	294.82 ± 18.03[b]	189.94 ± 15.17[a]	312.96 ± 21.62[a]

Expressed results are presented as , n=5. Means with identical letter(s) are not different (p< 0.05) by DMRT.

No notable changes (p>0.05) observed in kidney ACP activity across all groups compared to control. However, serum ACP levels showed a significant

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reduction ($p< 0.05$) only at the 500mg/kg dose, while other doses had no significant effect compared to control.

Creatine Kinase (CK) assay

Table 6: Effect of crude saponin extract from A. indica leaves on Creatine Kinase enzyme activity in the serum and tissues of R. novegicus

Organ/ Enzyme activity (U/L)				
Dose (mg/kg body weight)	Serum	Liver	Heart	Kidney
Control	90.44 ± 27.15^{a}	267.40 ± 13.05^{b}	567.50 ± 28.04^{c}	2.17^{a}
200	118.04 ± 11.07^{b}	112.30 ± 10.85^{a}	329.90 ± 09.40^{a}	145.22 ± 36.31^{a}
300	129.22 ± 11.08^{b}	246.30 ± 22.04^{b}	316.80 ± 20.46^{a}	153.31 ± 22.09^{a}
400	90.60 ± 21.12^{a}	317.70 ± 18.09^{c}	378.50 ± 11.09^{a}	182.99 ± 14.35^{b}
500	81.22 ± 15.42^{a}	321.10 ± 18.11^{c}	426.60 ± 26.11^{b}	174.37 ± 18.34^{b}

Expressed results are presented as , n=5. Means with identical letter(s) are not different ($p< 0.05$) by DMRT.

Table 6 shows that higher doses (400 and 500 mg/kg) of the treatment notably increased ($p<0.05$) CK activity in the liver and kidney versus control group. However, serum CK levels remained unaffected ($p>0.05$) at these doses.

Lactate Dehydrogenase (LDH) assay

Table 7: Effect of crude saponin extract of A. indica on Lactate Dehydrogenase enzyme activity in the serum and tissues of R. novegicus.

Organ/ Enzyme activity (U/L)				
Dose (mg/kg body weight)	Serum	Liver	Heart	Kidney
Control	135.22 ± 25.11^{a}	1115.70 ± 45.20^{b}	1855.60 ± 28.08^{b}	7.59^{b}
200	129.07 ± 18.32^{a}	463.40 ± 51.14^{a}	1267.30 ± 46.31^{a}	368.70 ± 07.90^{a}
300	112.72 ± 15.30^{b}	551.10 ± 22.28^{a}	1381.50 ± 13.62^{a}	492.20 ± 31.31^{b}
400	144.40 ± 19.11^{a}	569.80 ± 19.22^{a}	1779.10 ± 38.49^{b}	373.80 ± 18.20^{a}
500	93.18 ± 08.51^{b}	988.50 ± 34.77^{b}	1895.60 ± 37.19^{b}	502.50 ± 22.46^{b}

Expressed results are presented as , n=5. Means with identical letter(s) are not different ($p< 0.05$) by DMRT.

Table 7 shows a notable decrease ($p<0.05$) in serum LDH levels at 500 mg/kg relative to control.

Blood Urea Nitrogen assay

Table 8: Effects of crude saponin extract from A. indica leaves on Blood Urea Nitrogen in the serum of R. novegicus.

Dose (mg/kg body weight)	Serum (mg/dl)
Control	6.55 ± 0.34^{a}
200	6.10 ± 0.10^{a}
300	6.41 ± 0.46^{a}
400	4.64 ± 0.33^{b}
500	6.25 ± 0.17^{a}

Expressed results are presented as , n=5. Means with identical letter(s) are not different ($p< 0.05$) by DMRT

Creatinine

Table 9: Effects of crude saponin extract from A. indica leaves on Creatinine in the serum of R. novegicus

Dose (mg/kg body weight)	Serum (mg/dl)
Control	77.34 ± 1.71^{a}
200	80.47 ± 9.90^{a}
300	68.87 ± 7.13^{a}
400	18.75 ± 3.53^{b}
500	85.47 ± 11.26^{a}

Expressed results are presented as , n=5. Means with identical letter(s) are not different ($p< 0.05$) by DMRT

IV. DISCUSSION

Neem (Azadirachta indica), belonging to the Meliaceae family, is recognized for its health benefits due to its high antioxidant content. Research has validated its role in neutralizing free radicals and preventing disease progression, highlighting the potential benefits of neem and its constituents in maintaining overall well-being. It is considered as safe medicinal plants and modulates the numerous biological processes due to the bioactive compounds found in neem extracts' leaf without any adverse effect (Innocent et al., 2021). The results of the phytochemical screening of neem plants reveals that saponin was one of the most phytochemical components present in aqueous extract of A. indica (Innocent et al., 2021). Saponins are plant derived bioactive compounds. Saponins are natural glycosides known for their diverse biological and medicinal properties, including antioxidant activity, with minimal side effects. Characterized by their soap-like foaming ability, they produce foam when mixed with water (Goral and Wojciechowski, 2020). Medicinal plants have been reported to have antioxidant activity (Salehi et al.,2023).Findings have documented that crude neem extracts hold potential as a natural antioxidant source (Hossain et al., 2013;Islas et al.,2020). Antioxidants stabilize or deactivate free radicals, preventing cellular damage and support antioxidative enzymes that control oxidative stress (Ayoka et al., 2022). Studies by Mallick et al., (2013); Seriana et al.,(2021) found that neem leaf extract showed no toxic effects on rat liver and kidney even at high doses beyond the effective level. A study on neem tree extracts found that aqueous leaf extract and ethanolic flower and stem bark extracts exhibited strong antioxidant activity, achieving 50% free radical scavenging efficacy (Sithisarn et al., 2005; Abdulaziz Rabiu et al., 2017). The present study has evaluated the effect of crude saponin extract from the leaves of Azadirachta indica where the level of these liver marker enzymes; ALT, AST, ALP, ACP, LDH, CK, as well as BUN, and Creatinine in the liver, heart, kidney and serum were determined.

Rats administered with crude saponin extract from A. indica leaves showed no notable clinical or behavioural changes. The extract had no impact on body weight, with normal weight gain observed across all groups (Table 1). Additionally, organ weights remained unaffected, with no significant differences ($p<0.05$) as extract concentration increased. These findings agreed with the earlier studies of Ashafa (2012) and Braga et al., (2021) that rats showed no increase in body weight when fed with 50% of the extract. AST was significantly low ($p<0.05$) in the serum at 500mg/kg relative to the control group. There was no significant difference in enzyme activity in the tissues when compared with control. Most enzymes present in the human body are synthesized intracellularlly and they carry out their functions within the cells in which they are formed. Enzymes are retained within their cells of origin by plasma membrane surrounding the cell (Stockham and Scott, 2002).

ALT in the liver recorded a significant highest value because it is present in the liver in high concentration and to a lower extent in skeletal muscle, kidney and heart. Liver AST levels were elevated ($p<0.05$), while heart and kidney levels remained comparable to controls. Notably, serum AST levels decreased significantly at the 500mg/kg dose compared to controls. The probable reason for the increase in ALT liver in the tissues might be due to increased denovo synthesis of enzyme in response to the administration of crude saponin extracts that is, activation of the enzymes in situ (Zingue et al.,2019; Seriana et al.,2021). The levels of the activity of the enzymes showed no adverse liver damage, as the liver synthesizes these enzymes if there was liver damage

by the administration of crude saponin extract, the enzymes will leak out from the liver cells.

ALP, a marker enzyme for endoplasmic reticulum and plasma membrane (Wright and Plummer, 1974), showed highest activity in kidney and lungs, and lowest in liver. This aligns with previous findings (Peters et al., 2017) that organs with high ALP activity, such as kidney proximal tubules and intestinal mucosa are involved in active transport mechanisms. ALP activity increased significantly ($p<0.05$) in all tissues at 400mg/kg, while serum activity decreased correspondingly, compared to controls. ALP is found in high concentration in the tissues with a total absence or very low level in the serum (Santos et al., 2022).

Tissues ACP activity was comparable to controls, while serum ACP levels were unaffected except at 500mg/kg, where a notable decrease occurred. However, this indicated that these organs were not affected by the extract perhaps as a result of the defensive role exhibited by the extract due to its antioxidant activity that prevented an alteration in the lysosomal membrane; hence less of free radicals occur in the system. Acid phosphatase has been shown to be lysosomal in origin (Collins and Lewis, 1971) and is a 'marker' enzyme for the lysosomal membrane (de Duve et al, 1962).

The activity of CK was significantly high in the heart compared to other tissues studied. Creatine kinase is an enzyme chiefly found in the brain, skeletal muscle and heart. Higher doses (400 and 500 mg/kg) significantly increased CK activity in liver and kidney tissues ($p<0.05$), while serum CK levels remained unchanged compared to controls (Table 6).

Lactate Dehydrogenase is an enzyme found in almost all body tissues cells. It plays an important role in cellular respiration. Serum LDH levels decreased significantly ($p<0.05$) at 500mg/kg compared to controls, whereas tissue LDH activity remained unchanged. From this present study, the serum contents of BUN and Creatinine as shown in tables 8 and 4 respectively indicated that the serum contents of both BUN and Creatinine were not significantly different ($p<0.05$) when compared with the control but a significant reduction was observed at 400mg/kg dose when compared to control.

V. CONCLUSION

This study suggest that oral administration of A. indica leaf crude saponin extract is non-toxic, with no significant impact on liver marker enzymes, creatinine, and BUN levels. Although some enzymes activities increased at high doses (500mg/kg), the extracts overall safety profile is promising. Given its potential to enhance enzymatic antioxidants, further research is warranted to explore the molecular mechanisms underlying its biochemical and pharmacological effects.

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