**Evaluation of the potential risks and benefits of ethanolic leaf extract of *Anacardium occidentale* on liver enzymes and hematological indices in male albino rats.**

**ABSTRACT**

**Aim:** Investigation of the potential risks and benefits of ethanolic leaf extract of *Anacardium occidentale* on liver and haematological biomarkers of male Albino rats. **Methods:** The experimental animals were orally administered the leaf extracts for 14 days. **Major results:** The phytochemical analysis revealed the presence of flavonoids, saponins, phenolic compounds, phytosterols, terpenoids, and triterpenoids, with alkaloids and cardiac glycosides absent. While Macro-nutritional analysis revealed moderate levels of proteins and amino acids, and low levels of carbohydrates and reducing sugars. Acute toxicity tests indicated that the extract is safe at 5000 mg/kg body weight. Liver function tests showed no significant changes in aspartate transaminase, alanine aminotransferase , and alkaline phosphatase concentrations across the groups, while the result of the hematology showed a significant increase for the group that received the highest dose of the extract at 800mg/kg body weight when compared to the control group. **Conclusion/recommendation:** The study showed no critical toxicological effect on the hepatocytes and hematocytes at the tested doses. Thus these safe doses may be recommended for therapeutic purposes.

**Keywords:***Anacardium occidentale*; toxicity; phytochemical; liver function; hematology; macro-nutrient

**1.0 INTRODUCTION**

1.1 Background of the Study

In the quest to combating human health issues, especially in developing countries (Africa). Plants have been one the major source of treatment and the use of indigenous plant is also actively in use. The use of complementary and alternative medicine (CAM) by individuals in the developing nation often include herbal remedies (Tédong *et al.,* 2007).The liver plays a crucial role in detoxification, metabolism, and maintaining homeostasis within the body (Akpinar, 2020). However, exposure to various toxins, including chemicals from natural sources, can disrupt its normal functioning and lead to liver damage (Blann, 2014). *Anacardium occidentale*, commonly known as cashew, is a plant with various traditional medicinal uses, including its application as an herbal remedy for different ailments; its nut is widely consumed as snacks and culinary ingredient.

Several studies have explored the pharmacological properties of *Anacardium Occidentale*, highlighting its antioxidant, anti-inflammatory, and hepatoprotective effects (Adedamola *et al.*, 2023). However, despite its potential benefits, concerns have been raised regarding its safety. Ethanol is commonly used as a solvent for extracting bioactive compounds from plant materials, but it can also exert hepatotoxic effects when consumed in excessive amounts as against the extraction purpose it was made for.

**2.0 REVIEW OF RELATED LITERATURE**

**2.1 The resource plant: *Anacardium occidentale***

*Anacardium occidentale*, commonly known referred to as cashew tree, is a tall evergreen tree indigenous to South America, and categorized within the *Anacardiaceae* family (Lyare *et al* 2017). The tree bears both the renowned cashew nut, widely consumed as snacks and culinary ingredient and the apple, which is considered a secondary fruits.

**2.2 Common names of the plant**

The cashew tree boast a diverse range of common names across its geographical distribution .the most widely recognized common name is cashew (Akiniyi and Adebayo, 2012; Souza *et al.*2013).

In brazil and other Portuguese speaking countries it is known as caju/Acaju (Pereira *et al.,* 2020). In addition, in India, one of the major producers of cashew, it is called Kaju (Asare and Banful, 2016). Also in Spanish, particularly in Latin American countries it is known as Cajueiro (Akiniyi and Adebayo, 2012).

Furthermore, in Nigerian *Anacardium occidentale* has it various local common names for example in Igbo it is known as Kashu, in Yoruba and Hausa it is known as Kaju.

**2.3 Biological Classification of the Plant.**

Taxonomically, *Anacardium occidentale* belongs to the *Anacardiaceae* family which includes various genera and species with economic importance and diverse characteristic e.g. Mangos and pistachios (Adriano *et al.,* 2023).

The plant belong kingdom plantae (Adriano *et al.,* 2023). Subkingdom Tracheobionta that is vascular plant (Ganesh, *et al.,* 2015). Super division: Spermertophyta (seedplant), Division: Magnoliophyta (flowering plant), Class Magnoliopsida (Dicotyledons) (Cabi, 2022) . In addition cashew belong to the subclass :Rodidae, Oder:Sapindales, Family of Anacardiaceae (cashew family) (Palei,2020). Furthermore its genus Anacardium L.Cashew, and Species; *Anacardium occidentale L. (*cashew tree) (Leite *et al.,* 2016).

**2.4 Toxicology**

The origins of poison usage trace back to ancient times. Early humans likely observed the toxic effects of various substances in nature, often by chance. They recognized the harmful or deadly outcomes resulting from the consumption of certain plants or animal products, which may have led to the intentional extraction and utilization of these substances for hunting or warfare purposes. Historical records from around 1500 BC document the use of poisons such as hemlock, opium, arrow poisons, and certain metals for purposes of enemy poisoning or state-sanctioned executions. Over time, poison usage became more widespread and sophisticated (Langman and Kapur, 2006).

In the past, toxicology was simply called "the science of poisons." It focused on the properties of these poisons, how they affected living things, ways to detect them, and how to treat people who were poisoned. The important thing to remember is that a poison is only harmful if you get enough of it in your system (Langman and Kapur, 2006).

**2.5 Mechanism of Toxicity**

Understanding the mechanisms through which toxic substances exert their effects on living organisms is crucial for assessing and managing risks associated with chemical exposures (Michalaki *et al.,* 2022). Some of mechanism includes;

 Direct Interaction with Biomolecules: Toxicants can directly interact with Biomolecules such as proteins and enzymes, altering their structure and function (Guengerich, 2020).For example, certain pesticides inhibit the activity of acetylcholinesterase, leading to neurotoxic effects (Casida and Durkin, 2019).

Oxidative Stress and Reactive Oxygen Species (ROS): Chemicals can induce oxidative stress by generating reactive oxygen species (ROS), resulting in cellular damage and dysfunction (Lushchak, 2021).Antioxidant defences, including enzymes like superoxide dismutase, help mitigate oxidative damage (Sies, 2020).

Disruption of Cellular Signalling Pathways: Toxicants may also interfere with cellular signalling pathways, disrupting essential processes such as cell growth and apoptosis (Davies and Roberts, 2020).Endocrine-disrupting chemicals can mimic or block hormone receptors, leading to hormonal imbalances (Gore *et al.*, 2021).

Epigenetic Modifications: Emerging evidence suggests that certain toxicants can induce epigenetic changes, altering gene expression without modifying the DNA sequence (Rusyn *et al*., 2018).Epigenetic mechanisms such as DNA methylation and histone modifications play a role in mediating toxicant-induced effects.

**3.0 Method**

**3.1 Collection of the Plant Materials and Authentication**

*Anacardium occidentale* (cashew) leaves were harvested from the trees within Clifford university compound Ihie campus, Isialangwa North Local Government Area of Abia State, Nigeria and transported to Biochemistry Laboratory, Clifford University, Owerinta, Ihie Campus, Abia State, Nigeria. The plant was then sent for authentication at the department of Plant Science and Biotechnology, Abia State University Uturu by a taxonomist and voucher samples deposited in the Departmental herbarium.

**3.2 Preparation of Plant Extract**

The extract was prepared using the method described by Daniel *et al*., (2012) with slight modifications.

**3.4 Phytochemical Screening (Qualitative Test)**

The phytochemical screening was done according to the AOAC, 2000 method with slight modifications in some of the test.

**3.5 Macro-nutritional Analysis (qualitative test)**

3.5.1 Carbohydrates (Seliwanoff’s Test)

3.6 Liver Function Test

3.6.1 Determination of Aspartate Aminotransferase (AST) Activity (Reitman and Frankel, 1957)

3.6.3 Determination of alkaline phosphatase (ALP) activity (Ochei and Kolhatkar, 2008)

3.6.4 Determination of Total protein (Weichselbaum, 1946)

3.6.5 Serum Albumin (ALB) Concentration

The serum ALB concentration was determined using Randox kit (Randox Laboratories Limited UK) based on the method described by Douman *et al*., (1971).

3.6.6 Determination of Total Bilirubin was determined by the colorimetric method described by Jendrassik and Grof (1981).

3.7 Determination of Packed Cell Volume (PCV)

This was determined using standard hematological procedure as described by Ochei and Kolhatkar (2008).

3.8 Determination of Red Blood Cells

This was determined using standard hematological procedure as described by Ochei and Kolhatkar (2008).

3.9 Determination of white blood cells (Total White Blood Cell Count)

This was determined using standard hematological procedure as described by Ochei and Kolhatkar (2008).

Determination of Hemoglobin: Using the heme-analyser by the method of (van Kampen and Zijlstra, 1961).

**4.0 RESULTS AND DISCUSSIONS**

**4.1 RESULTS**

**Table 1: Phytochemical composition of ethanolic leaves extracts of *Anacardium occidentale***

|  |  |  |  |
| --- | --- | --- | --- |
| S/NO | PHYTOCHEMICALS | TEST | INFERENCE |
|  | Cardiac glycosides | Keller-killani test | - |
|  | Alkaloids  | Iodine test  | - |
|  | Flavonoids  | Ferric chloride Test | ++ |
|  | Tannins  | 10% naoh test | + |
|  | Saponins  | Olive oil test | +++ |
|  | Phenolic compounds | Lead acetate test | + |
|  | Phytosterols | Salkowski’s test | + |
|  | Terpenoids | Chloroform test | + |
|  | Triterpenoids | (Salkowski’s test | ++ |

Table 1: Shows the qualitative phytochemical screening of *Anacardium occidentale*. Phenolic compounds, Phytosterols, and Terpenoids were present at low proportion, Flavonoids and Triterpenoids were present at moderate proportion, and Saponins was present at high proportion, while Alkaloid and cardiac glycosides were absent.

Key

**+**: Present at low proportion

**++**: Present at moderate proportion

**+++**: Present at high proportion

**-**: Absent

**Table 2: qualitative nutrient composition of ethanolic leaf extract of *Anacardium occidentale***

|  |  |
| --- | --- |
| Nutrient | Inference |
| Carbohydrate | **+** |
| Reducing Sugar | **+** |
| Protein/Amino Acids | **++** |

Table 2 above indicates qualitative nutritional composition of ethanolic leaf extract *Anacardium occidental*. Protein/Amino acids are present at moderate proportion while carbohydrate and reducing sugars are present at low proportion respectively.

**Key**

**+**: Present at low proportion

**++**: Present at moderate proportion

**Table 3: Showing the effects of *ethanolic* leaf extract *of Anacardium occidentale* on the liver biomarkers of male albino rats**

|  |  |  |  |
| --- | --- | --- | --- |
| **Parameters** | **Control** | **(LD) 400mg/kg b.w** | **(HD) 800mg/kg b.w** |
| **Total protein (g/dl)** | 6.35±0.04a | 6.79±0.13b | 7.22±0.07c |
| **Albumin (g/dl)** | 3.23±0.06a | 3.39±0.03b | 3.62±0.04c |
| **Total Bil. (mg/dl)** | 0.53±0.03a | 0.46±0.05a | 0.43±0.02b |
| **AST (u/l)** | 43.67±1.53a | 41.33±1.55a | 40.00±3.00a |
| **ALT (u/l)** | 30.33±0.58a | 28.00±2.64a | 28.67±1.15a |
| **ALP (u/l)** | 63.80±2.60a | 59.80±1.04a | 62.17±2.53a |

Values are mean ± SD. Values across the row bearing the same letter of alphabets are not significantly different (P>0.05) while values with different subscript are significantly different to each other at (P<0.05).

Keys:

AST: Aspartateaminotransferase

ALT: AlanineAminotransferase

ALP: Alkaline phosphatase

From the table above Total protein and Albumin had a significant increase (P<0.05) in the treatment groups when compared to the control group. Whereas Total Bil. decreased significantly p<0.05 in the group that received the high dose, AST, ALT and ALP had non- significant reductions (P>0.05) in the treatment groups when compared to the control.

**Table 4: Showing the effects of *Anacardium occidentale* ethanolic leaf extract on Hematological parameters in male albino rats**

|  |  |  |  |
| --- | --- | --- | --- |
| Parameters | **Control** | **(LD) 400mg/kg b.w** | **(HD) 800mg/kg b.w** |
| RBC (x106/mm3) | 6.78±0.35a | 7.07±0.15a | 7.50±0.17b |
| PCV (%) | 42.00±2.00a | 44.33±1.54a | 46.67±1.53b |
| Hb (g/dl) | 15.83±0.55a | 16.70±0.30a | 17.50±0.50b |
| WBC (x103/mm3) | 9.93±0.09a | 9.18±0.25b | 11.06±0.49c |
| PLT (x103/mm3) | 260.33±3.28a | 251.33±8.74a | 246.33±7.02a |

Values are mean ± SD. Values across the row bearing the same letter of alphabets are not significantly different *(P>0.05)* while values with different subscript are significantly different to each other at *(P<0.05)*.

**Keys:**

RBC: Red blood cell, PCV: Packed cell volume, Hb: Hemoglobin, WBC: White blood cell, Plt: Platelet

From the table above, RBC, PCV, Hb and WBC all had a significant increase for the group that received the highest dose of the extract at 800mg/kg body weight when compared to the control group. Whereas blood platelets had a non significant reduction across the groups when compared to the control.

**4.2 DISCUSSION**

The phytochemical analysis of ethanolic leaf extract of *Anacardium occidentale* revealed the presence of flavonoids and triterpenoids at moderate while phenolic compounds, phytosterol and terpenoids were present at low levels. This is in line with the studies conducted by Tédong *et al.,* (2007) while saponins were discovered to be abundant. These compounds are known for their various health benefits, including antioxidant, anti-inflammatory, and antimicrobial effects. Meanwhile, alkaloids and cardiac glycosides were found to be absent in the plant extract which is not in line with the studies conducted by Ngozika *et.al.*(2020) where they found the compounds to be present. This variation may be due to the location of the plant, method and solvent used for the extraction. This absence suggests a lower risk of certain toxic effects associated with the compounds. However, there is an evidence that very high levels of phytosterols might contribute to liver fibrosis (Fernandez *et al.,* 2022). The presence of phytosterol at low concentration in this study indicates potential benefits of ethanolic extract of *Anacardium occidentale* without significant risk. This study also revealed that the ethanolic leaf extract of *Anacardium occidentale* contains proteins and amino acids in moderate proportions, carbohydrates and reducing sugars in low proportions. This nutrient composition highlights several potential health benefits. The moderate presence of proteins and amino acids in *Anacardium occidentale* as observed in the present study suggests that it could significantly contribute to dietary protein intake, supporting muscle development, immune function, and cellular repair (Lemos *et al.,* 2017; Nweke *et al*., 2019). The data indicates a dose-dependent increase in total protein and albumin levels, alongside a significant decrease in total bilirubin at higher doses. Specifically, total protein increased from 6.35±0.04 g/dl in the control group to 7.22±0.07 g/dl at a high dose (800 mg/kg body weight), while albumin levels rose from 3.23±0.06 g/dl to 3.62±0.04 g/dl. Conversely, Total bilirubin decreased from 0.53±0.03 mg/dl to 0.43±0.02 mg/dl in the high-dose group. There were no significant changes observed in AST, ALT, and ALP levels across the groups.

The increase in the levels of TP (Total protein) suggests that the ethanolic leaf extract of *Anacardium occidentale* enhanced protein synthesis and improved liver function. This aligns with the findings of Santos *et al.* (2016), who demonstrated that cashew nut extracts could enhance protein synthesis, thereby improving overall protein levels in the body. Similarly, Nweke *et al.* (2019) reported that Anacardium occidentale extracts exhibit hepatoprotective effects, which correlate with the slight increase in albumin levels observed in this study. Furthermore, the significant reduction in Total bilirubin is consistent with the hepatoprotective effects noted by Silva *et al*. (2018), indicating improved liver function and reduced haemolysis.

AST (Aspartate aminotransferase), ALT (Alanine transaminas), and ALP (Alkaline Phosphatase) are enzymes used to evaluate liver health. Elevated levels of these enzymes typically indicate liver damage or inflammation. In this study, there was no significant change observed in the levels of AST, ALT, and ALP across the groups, suggesting that the ethanolic leaf extract of *Anacardium occidentale* does not cause liver damage at the administered doses. Specifically, AST levels slightly decreased from 43.67±1.53 u/l in the control group to 40.00±3.00 u/l in the high-dose group, ALT levels decreased from 30.33±0.58 u/l to 28.67±1.15 u/l, and ALP levels showed a minor reduction from 63.80±2.60 u/l to 62.17±2.53 u/l. These non-significant changes imply stability in liver enzyme levels. This is in line with Wattanathorn *et al.* (2019) and Nwozo, *et al.* (2016) who also observed a similar trend in their studies.

However, these findings contrast with some previous studies. Silva *et al.* (2021), Baptisa *et al.* (2020) and Nweke *et al* (2019) documented significant reductions in liver enzymes with *Anacardium occidentale* extracts, suggesting enhanced liver protection. Moreover this study, contradict Anaziah (2023) who stated that ethanolic leaves of *Anacardium occidentale* at low dose 200mg and high dose of 400mg has toxic effect on the liver. As well contradicts Famurewa *et al. (*2015) who reported a significant increase in ALP, AST, ALT, and total bilirubin at 400mg aqueous extract of the plant. The discrepancy between this study and previous research could be due to differences in experimental conditions, including dosages, duration of treatment, and variations in the extraction process.

Blood indices such as Erythrocyte count (RBC), packed cell volume (PCV), hemoglobin concentration (Hb), and leukocyte count (WBC) are vital indicators of optimal blood function. An elevation in the plasma levels of these parameters suggests a significant hematopoietic effect, implying that the extract may possess the ability to stimulate blood cell production and development. From the study, *Anacardium occidentale* caused a significant increase in RBC, PCV, Hb, and WBC for the group that received the highest dose of the extract at 800mg/kg body weight when compared to the control group, while, blood platelets had a non-significant reduction across the groups when compared to the control. The assessment of erythrocyte-related parameters, including red blood cell count, is a crucial component of evaluating circulatory health and diagnosing anaemia. These indices also serve as valuable markers of bone marrow function, providing essential insights into its capacity to produce erythrocytes and overall efficiency of the hematopoietic system in mammals (Zubairu *et al*., 2021). The significant increase in red blood cell (RBC) and packed cell volume (PCV), following the administration of *Anacardium occidentale* suggests that the leaf extract may have stimulated erythropoiesis (the production of red blood cells). This could have been achieved by enhancing or maintaining the release of erythropoietin, a hormone produced by the kidneys that regulates RBC production. The packed cell volume (PCV) represents the percentage of blood volume comprised of red blood cells, with normal values ranging from 48% in males to 38% in females. As a vital component of a complete blood count, PCV is evaluated alongside hemoglobin levels, white blood cell count, and platelet count. The assessment of PCV serves as a straight-forward and dependable method for identifying anaemia or polycythemia. Platelet count can serve as a valuable diagnostic tool for identifying various diseases and conditions that affect blood clotting, such as, thrombocytopenia or thrombosis (Zubairu *et al*., 2021). Platelet count can be used in the evaluation of various hematological disorders, including bleeding disorders, bone marrow diseases, and thrombotic conditions. An elevated platelet count may indicate that the bone marrow is producing an excessive number of platelets, which can be a response to underlying conditions such as inflammation, infection, or malignancy. Conversely, a low platelet count can indicate bone marrow dysfunction, immune-mediated platelet destruction, or consumption due to excessive bleeding or clotting. In the absence of an identifiable cause, an elevated platelet count is classified as primary or essential thrombocytosis. Primary thrombocytosis is a distinct entity, separate from secondary thrombocytosis, which occurs in response to an underlying condition or stimulus. A low platelet count, also known as thrombocytopenia, can lead to impaired blood clotting, increasing the risk of bleeding complications. The cause of thrombocytopenia may be genetic, resulting from inherited traits that affect platelet production. In other cases, the cause may be unknown or unrelated to a medical condition, such as autoimmune disorders, bone marrow dysfunction, or certain medications that affect platelet production (Zubairu *et al*., 2021). In context to the study, platelet count had a non-significant reduction, indicating that the extract does not have a significant impact on platelet count and may not have any effect on coagulation factors or processes.

The administration of cashew leaf extract to rats resulted to a significant elevation of hemoglobin levels (Akpotu *et al*., 2022). This finding suggests that the extract does not compromise the blood’s oxygen-carrying capacity or its delivery to peripheral tissues. Moreover, the increase in hemoglobin indicates that the extract does not induce anaemia, a condition characterized by a deficiency in red blood cell or hemoglobin (Akpotu *et al*., 2022). The preservation of oxygen delivery to tissues is a critical aspect of maintaining physiological homeostasis. The white blood cell (WBC) play a crucial role in the body’s immune system, by fighting infections and diseases,

inflammation and phagocytosis. From the research made by Adeleke *et al*., (2022), an increase in WBC is associated with a range of conditions, including inflammation, allergic reactions, leukemia, tissue damage resulting from physical trauma, and systemic illnesses. While a decrease in white blood cell count may indicate an infectious disease (Osman, 2013). From the study, this suggests that the ethanolic leaf extract of *Anacardium occidentale* may be having an effect on the immune system, potentially leading to increased white blood cell production. However, it is important to note that an elevated WBC count does not necessarily mean that the condition is present.

**5.0 CONCLUSION AND RECOMENDATIONS**

**5.1 Conclusion**

The study investigated the phytochemical, nutritional, and toxicological properties of the ethanolic leaf extract of *Anacardium occidentale* (cashew) on liver and haematological biomarkers in male albino rats. The results of the study reveal that the ethanolic leave extract contains several beneficial phytochemicals such as flavonoids, tannins, phenolic compounds, saponins, phytosterols, terpenoids, and triterpenoids, with no presence of alkaloids and cardiac glycosides. These compounds are known for their antioxidant, anti-inflammatory, and antimicrobial properties, contributing to the extract's overall health benefits.

The nutritional analysis revealed moderate levels of proteins and amino acids, and low levels of carbohydrates and reducing sugars, indicating the extract's potential in supporting dietary protein intake without significantly affecting blood sugar levels. The acute toxicity test demonstrated that the ethanolic leaf extract of *Anacardium occidentale* is safe at doses ≤ 5000 mg/kg body weight, as there was no mortality observed at this highest dose in the test animals.

The liver function tests indicated that ethanolic leave extract of *Anacardium occidentale* had a significant effect on certain liver biomarkers. Total protein and albumin levels increased significantly in the treatment groups compared to the control group. However, total bilirubin levels decreased significantly in the high-dose group, suggesting improved liver function and reduced haemolysis. There were no significant changes in AST, ALT, and ALP levels, this suggests that the ethanolic leaf extract of *Anacardium occidentale* does not induce hepatotoxicity and may even have a hepatoprotective effect at certain doses.

Overall, these result provides preliminary evidence for the extract’s potential to modulate hematological parameters, and further research is justified to fully elucidate it’s effects on blood cell production and development and highlights *Anacardium occidentale* as a valuable resource for pharmacological research and traditional medicine, warranting further exploration of it’s therapeutic potential.

**5.2 Recommendations**

There should be more extensive studies to confirm the long term safety and efficacy of the ethanolic leaf extract of *Anacardium occidentale* in different animal models and potentially in human trials to fully understand its therapeutic potential and safety profile. In terms of dosage regulation; while the extract has shown potential health benefits, it is crucial to regulate the dosage to avoid potential risk effects, especially at higher concentrations.

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