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Toxicological Assessment of Chromatographic Fraction obtained From Aqueous extract of Acacia nilotica Seedpod in Experimental Rats

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ABSTRACT: The toxicological effect of the chromatographic fraction B of Acacia nilotica on serum biochemical parameters in rats was investigated. The aqueous extract of Acacia nilotica was subjected to solvent partition using hexane, butanol and ethylacetate. The n-butanol fraction was subsequently subjected to column chromatography. Column fraction B was administered to rats in graded doses of 5mg/kg, 10mg/kg and 15mg/kg bw orally for twenty eight days. The Packed cell volume (PCV), hemoglobin (Hb), red blood cell (RBC) and white blood cell (WBC) levels were not significantly ($p > 0.05$) different at all same dose levels when compared with the control group. Serum total protein, globulin, total and conjugated bilirubin, urea, creatinine and uric acid levels of group administered the highest dose were significantly reduced ($P < 0.05$) when compared to the control group. However, at concentration of 5mg/kg bw and 10mg/kg bw of the administered extract there was no significant ($p>0.05$) changes in total protein, albumin and globulin levels, suggesting no major adverse effects on protein metabolism or liver function. Activity of liver aminotransferases are significantly increased ($P < 0.05$) at the highest dose level (15 mg/kg bw). The activity of kidney alkaline phosphatase was significantly reduced ($P < 0.05$) at the highest dose level. The toxicological assessment suggest that administration of Fraction B of Acacia nilotica pod should be with caution as higher dose may cause mild alteration in kidney and liver function.

Keywords: Acacia Nilotica, N-Butanol Fraction, Column Chromatography, Toxicity.

INTRODUCTION

Traditional medicine is extensively utilized throughout the world to treat an array of maladies. In a study by Abubakar et al., (2022), it was discovered that 92% of the respondents treated a range

of health problems, including COVID-19, polio, yellow fever, and lassa fever, with unconventional medicine. According to Daniyal et al., (2019), Salimon & Yakubu, (2020), and Erezina et al., (2023), the main factors encouraging more people to use folkloric medicines are accessibility, affordability, being more in line with patients' beliefs and ideologies, being assumed to have a less toxic effect, having fewer side effects associated with developing resistance, and fulfilling the desire for individualized healthcare.

The evaluation of toxicity in traditional herbal treatments is often overlooked, even though it should be a priority for legitimate and well-documented herbal medicinal products, just as it is for legitimate and extensively researched conventional drugs (O. et al., 2015). Abou-Arab & Abou Donia, (2000), Thanaboripat et al., (2007) and, Kneifel et al., (2002) all report that even herbal medicines that are thought to be non-toxic may contain pollutants like pathogenic microbes, aflatoxins, and heavy metals due to the formulation process or as a result of the acquisition of metals (like cadmium) from the soil.

Acacia nilotica, a member of the Fabaceae family, also commonly known as the Egyptian thorn or gum arabic tree, is a versatile plant species well known for its wide range of industrial and therapeutic uses. Due to its medicinal qualities, it has been widely used in traditional medical systems throughout different historical periods. The bark, leaves, and seeds of the Acacia nilotica tree have shown a wide range of biological activity, including anti-inflammatory, antioxidant, antibacterial, and anti-diabetic properties.

Several acute toxicity studies have demonstrated the relatively low toxicity of Acacia nilotica extracts or fractions, providing initial evidence of its safety. For instance, in a study by Ganie et al., (2018), acute oral toxicity tests on an Acacia nilotica bark extract in mice showed no mortalities or significant signs of toxicity at the highest tested dose, suggesting a wide margin of safety. Similar findings were reported in acute toxicity studies conducted on Acacia nilotica leaf extracts by Umar et al., (2025) and on gum extracts by Gupta et al., (2017), further reinforcing the overall safety of Acacia nilotica.

Currently, the toxicological properties of chromatographic fraction B of Acacia nilotica seed pod have not been investigated in rats. In this study, we aim to investigate the toxicological properties of chromatographic fraction B of Acacia nilotica seedpod, employing in vivo experimental model. The findings of this study will help expand our understanding of the safety considerations associated with the utilization of Acacia nilotica and aid in the development of evidence-based recommendations for its practical applications.

Experimental Animals

A total of twenty-four healthy Wistar rats weighing 131.25 ± 10.40 g were purchased from the Department of Biochemistry (Animal Holding Unit), University of Ilorin, Nigeria. All the Wistar rats were handled with great care according to the guidelines for caring for laboratory animals (NIH publication number 82-23, revised 1985). They had unfettered availability to feed (Premier

Feeds, Ibadan, Nigeria), as well as unlimited access to tap water, in a well-ventilated house with a 25 to 29°C temperature range, a 12-hour light/dark cycle period, and 45 to 55 percent humidity.

Reagents and Assay Kits

Assay kits produced by Randox Laboratory Ltd. in Co-Antrim, UK, were used to determine albumin, total and conjugated bilirubin, alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), uric acid, and bilirubin. All other reagents and chemicals used were of standard analytical grade, prepared using distilled water, and stored in airtight reagent bottles, except as otherwise stated.

Preparation of Aqueous Extract of Acacia nilotica Pod

The dried pod from the tree was pulverized into fine powder using a blender. A known amount (150 g) of the sample was extracted in 1500 mL of distilled water for 24 hours. The mixture was then filtered using muslin cloth and filter paper. The filtrate was concentrated on a water bath.

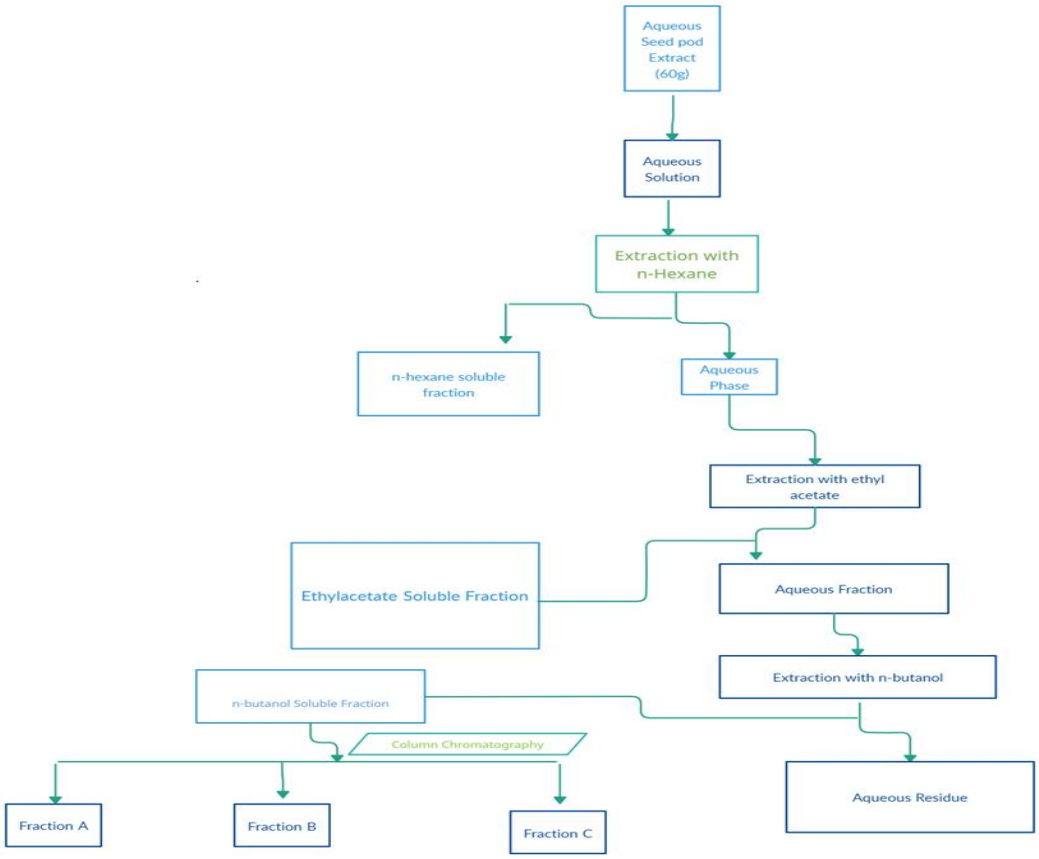
Solvent-partitioning of Aqueous Extract of Acacia nilotica Seedpod

Aqueous extract of Acacia nilotica seedpod was partitioned (Figure 1) by using solvents of increasing polarities. This was done using the protocol designed by Kupchan et al., (1973). In brief, 60 g of crude aqueous extract of Acacia nilotica seedpod was solubilized using 30 mL of distilled water. This was fractionated sequentially with n-hexane, ethyl acetate and n-butanol using a separating funnel. The solvent-partitioned fractions obtained were evaporated till dry using rotary evaporator. The resulting fractions are ethyl acetate, n-butanol and aqueous residue fractions.

Column Chromatography n- Butanol Fraction of Aqueous Extract of Acacia nilotica Pod

The n-butanol partitioned fraction of aqueous extract of Acacia nilotica pod was subjected to column chromatography as described by Jayaprakasha et al., (1998). Silica gel slurry was prepared by suspending 70 g of silica gel (60 - 200 μm) in 100 ml of the mobile phase; Toluene: Ethyl acetate: Formic acid (5: 4: 1). This was packed into a column (60 cm ×18 mm). N-butanol fraction (7 g) was dissolved in a little quantity of n-butanol and then impregnated onto silica gel. This was dried for 40 minutes at 100°C in an oven. Then it was loaded onto the column. The mobile phase was used for elution. The eluates (fractions) were collected into beakers. Twenty three (23) fractions were collected. Few drops of each fraction was spotted on an activated TLC plate and allowed to dry up. The plate was developed in a TLC chamber containing Toluene: Ethyl acetate: Formic acid (5: 4: 1) solvent system. The plate was removed and air dried. Separation was detected using iodine crystals. Fractions with similar Rf values were pooled together. The (three) pooled fractions were obtained: Fraction A, B and C.

Figure 1: Schematic Representation of the Partitioning of Aqueous Extract of Acacia nilotica pod



METHOD

Animal Grouping and Dosing of Plant Extracts

Twenty-four Wistar rats were randomly selected into four groups (A, B, C, and D) of six animals each. Rats in group A received 1.0 mL of distilled water orally, and for 28 days, the other three groups (B, C, and D) were administered orally a graded dose of Fraction B of Acacia nilotica at 5, 10, and 15 mg/kg body weight, respectively.

Preparation of Serum and Tissue Supernatants

Twenty-four hours after the 28-day treatment (on the 29th day), the animals were euthanized under diethyl ether, and blood samples were collected. Uncoagulated blood was used for a hemological assay, while coagulated blood was centrifuged at 3000 rpm for five minutes. The serum was collected and stored at -4°C for biochemical analysis. The abdomen and thorax were opened; the liver and kidneys were removed, cleaned, weighed, and then transferred into a 0.25 M sucrose solution. These were blotted with ash-free filter paper, cut thinly, and homogenized in an ice-cold

0.25 M sucrose solution (Akanji & Ngaha, 1989). The tissue homogenate was centrifuged at 10,000 rpm for 15 minutes, and the supernatant was used for different biochemical estimations.

Hematological and Biochemical Analysis

An automatic hematology analyzer (Sysmex Hematology System, Model KX-21W, Kobe, Japan) was used to carry out the hematological analysis. Using the techniques described by Gornal et al. (1949), Doumas, (1971), Jendrassik & Grof, (1938), Tietz (1995), Veniamin & Varkirtzi, (1970), and Bartels & Bohmer, (1972), in that order, the following parameters were measured: total protein, albumin, total and conjugated bilirubin, globulin, urea, and creatinine. Utilizing methods described by Reitman & Frankel, (1957), Wright et al., (1972), and Aebi, (1984), the activities of ALT, AST, and ALP were assessed respectively.

Data Analysis

The data were expressed as the mean ± SEM of six replicates. Means were analyzed using one-way analysis of variance and complemented with the Duncan multiple range tests. The Statistical Package for Social Sciences, Version 23.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analyses, while differences were considered statistically significant at $p < 0.05$.

RESULT AND DISCUSSION

The result of this showed no significant differences ($p>0.05$) between the treatment group's levels of packed cell volume (PCV), hemoglobin (Hb), red blood cell (RBC) and white blood cell (WBC) and the control groups (Table 1). Serum total protein, globulin, total and conjugated bilirubin, urea, creatinine and uric acid levels of group administered the highest dose were significantly reduced ($P<0.05$) when compared to the control group. However, at concentration of 5 mg/kg body weight and 10 mg/kg body weight of the administered extract there was no significant ($p>0.05$) changes in total protein, albumin and globulin levels, suggesting no major adverse effects on protein metabolism or liver function (Table 2).

Activity of liver aminotransferases are significantly increased ($P<0.05$) at the highest dose level (15 mg/kg body weight). The activity of kidney alkaline phosphatase was significantly reduced ($P<0.05$) at the highest dose level (Table 3).

Table 1: Hematological indices of rats administered chromatographic Fraction B of A. nilotica

Dose (mg/kg)	Red blood cell (10^6/µL)	White blood cell (10^3/µL)	Packed cell volume (%)	Hemoglobin (g/dL)
Control	8.01 ± 0.33[a]	17.97 ± 0.43[a]	52.87 ± 0.63[a]	11.90 ± 0.54[a]
5	8.06 ± 0.23[a]	22.83 ± 0.27[b]	57.23 ± 0.94[b]	12.13 ± 0.31[a]
10	8.12 ± 0.15[a]	23.60 ± 0.42[b]	57.60 ± 0.46[b]	12.03 ± 0.19[a]
15	7.71 ± 0.25[a]	23.35 ± 0.52[b]	54.78 ± 0.87[a]	11.43 ± 0.20[a]

Values are mean ± standard error of mean. n=6, values with different superscript are significantly different from control ($P < 0.05$)

Table 2: Liver and kidney function indices of rats orally administered chromatographic Fraction B obtained from n-butanol partitioned fraction of Acacia nilotica seedpod

Parameter	Control	5 mg/kg bw	10 mg/kg bw	15 mg/kg bw
Total protein (g/dL)	8.91± 0.21[a]	8.96 ± 0.25[a]	8.86 ± 0.19[a]	7.71 ± 0.11[b]
Albumin (g/dL)	4.39 ±0.12[a]	4.45 ± 0.15[a]	4.40 ± 0.03[a]	4.17 ± 0.05[a]
Globulin (g/dL)	4.53 ± 0.20[a]	4.18 ± 0.17[a]	4.30 ± 0.15[a]	3.74 ± 0.11[b]
Total bilirubin (µmol/L)	28.23 ± 0.58[a]	25.42 ± 0.77[a]	23.74 ± 0.86[b]	22.25 ± 0.77[b]
Conjugated bilirubin (µmol/L)	12.16 ± 0.49[a]	12.47± 0.89[a]	13.37 ± 0.46[a]	10.95 ± 0.18[b]
Urea (mMol/L)	8.22 ± 0.52[a]	9.13 ± 0.46[a]	9.85 ± 0.32[a]	6.16 ± 0.27[b]
Creatinine (µmol/L)	25.98 ± 0.39[a]	24.52 ± 0.98[a]	16.31 ± 0.75[b]	19.02 ± 0.50[b]
Uric acid (mg/dL)	4.78 ± 0.18[a]	3.24 ± 0.22[b]	5.60 ± 0.23[a]	3.81 ± 0.32[b]

Values are Mean±SEM of six replicates. Values with different superscripts across the columns are significantly different ($p< 0.05$).

Table 3: Effect of treatment with chromatographic Fraction B of Acacia nilotica seedpod on activity of selected enzymes of rat liver, kidney and serum

	Alanine aminotransferase (U/L)			Aspartate aminotransferase (U/L)			Alkaline phosphatase (U/L)		
Dose (mg/kg)	Serum	Liver	Kidney	Serum	Liver	Kidney	Serum	Liver	Kidney
Control	22.84 ± 1.339[a]	41.98 ± 2.448[a]	36.20 ± 3.328[a]	15.92 ± 0.933[a]	99.56 ± 1.174[a]	55.66 ± 2.15[a]	12.79 ±0.601[a]	54.05 ± 1.588[a]	19.29 ± 0.236[a]
5	24.17 ± 1.576[a]	50.84 ± 1.734[b]	29.23 ± 1.837[a]	26.82 ± 1.439[b]	88.28 ± 0.362[b]	54.85 ± 1.28[b]	25.91 ±0.949[b]	61.93 ± 0.309[b]	22.37 ± 0.896[b]
10	22.87 ± 1.520[a]	57.18 ± 0.653[b]	13.25 ± 1.216[b]	22.22 ± 1.985[c]	94.37 ± 0.302[c]	54.85 ± 1.270[a]	29.29± 0.205[c]	65.27 ± 1.105[c]	26.60 ± 0.2045[c]
15	14.00 ± 0.302[b]	50.74 ± 0.365[a]	42.77 ± 2.274[a]	17.86 ± 0.770[a]	33.43 ± 0.383[d]	33.85 ± 0.428[c]	40.42 ±0.653[d]	52.90 ± 1.103[a]	13.13 ± 0.005[d]

Values are mean of six replicates ± SEM. Values with different superscripts are significantly different from each other ($p < 0.05$).

The general population has noticed a remarkable increase in the use of herbal formulations, likely due to the belief that they provide safer and more natural alternatives to conventional drugs (Latha et al., 2010). However, worries about the efficacy, toxicity, and safety of herbal medicines continue to exist. By examining the effects of chromatographic Fraction B of A. nilotica on liver and kidney functions, this study sought to allay these worries.

To get toxicity-related information that is typically not found by direct examination of organs and body weight analysis, hematology parameters are analyzed. In order to diagnose anemia in the majority of animals, red blood indices such as HB, WBC, and PCV are the most helpful markers (Weingand et al., 1996). In comparison to the control, the chromatographic Fraction B of A. nilotica had no discernible effect on RBC, PCV, WBC, or HB. These findings suggest that the chromatographic Fraction B of A. nilotica had no noticeable harmful effects on plasma erythrocytes at any of the doses examined, and that no macrocytic or microcytic anemia was present (Rogers, 2011).

Both the liver and the kidney are essential organs in charge of metabolism, detoxification, and waste product removal from the body (Knight et al., 2006).

To fully assess the potential toxic consequences on the liver and kidney, biochemical indicators are required. Indicators of overall protein status and liver function include total protein and albumin (Johnson et al., 2013; Oettl et al., 2008). It is possible that the extract in the present study had little or no impact on hepatic protein synthesis or protein metabolism because there were no major alterations in total protein or albumin levels at doses of 5 mg/kg body weight. The degradation of red blood cells by the liver is indicated by the levels of direct and total bilirubin (Pratt & Kaplan, 2000). Lower levels of bilirubin might be a sign of improved liver health; therefore, the significant decrease in direct bilirubin and total bilirubin may point to a potential favorable effect of plant extract on bilirubin metabolism or liver function.

The blood levels of the enzymes ALT and AST, which are mainly situated in liver cells, can be a sign of liver inflammation or damage (Pratt et al., 2000). Particularly in the group receiving the highest dose (15 mg/kg), the increase in AST levels and decrease in ALT levels raise the possibility of hepatic injury or stress as a result of the higher plant extract dose.

ALP is an enzyme present in various tissues, including the liver, bones, and intestines. Changes in ALP levels can indicate liver dysfunction or bone-related issues (Kuo & Chen, 2017). The extract at different doses caused significant variations in ALP levels compared with the control group. These changes may reflect alterations in liver or bone metabolism prompted by the extract.

The findings signify that plant extract at the tested doses did not exert major harmful effects on protein metabolism or liver function, as evidenced by the relatively stable levels of total protein and albumin as well as the reduction in the levels of Direct Bilirubin, and Total Bilirubin. However, changes observed in AST, ALT and ALP suggest potential dose-dependent side effects on liver health and cellular integrity.

Uric acid, and creatinine are waste products that are excreted by kidneys and their intensity in blood can indicate renal function (Wani & Pasha, 2021). The significant decreases in uric acid level at dose of 15 mg/kg suggest a potential influence of the plant extract on the reabsorption of metabolic products in renal tubules. Moreover, creatinine levels were significantly decreased across all doses of the plant extract, indicating a potential disruption in kidney filtration or clearance (Brown et al., 2015).

CONCLUSION

The chromatographic Fraction B of A. nilotica may not be entirely safe for use as evidenced by the distortion of hematological index and functional derangements of the liver and kidney at higher dosages. Thus, the prolonged usage of the plant at higher doses is vehemently discouraged.

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