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Hepatoprotective Effects of Ethanol Leaf Extracts of *Andrographis paniculata* and *Tapinanthus bangwensis* on Male Albino Wistar Rats

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Abstract
Assessment of hepatoprotective properties of ethanol leaf extracts of *Andrographis paniculata* and *Tapinanthus baugwensis* on male albino Wistar rats weighing 74 – 186 g was conducted for one month. Twenty-five animals were randomly assigned to five groups of five rats each. Groups 1 and 2 were treated orally with 200 mg/kg and 300 mg/kg combined leaf extracts of *A. paniculata* and *T. bangwensis*, respectively, at a 50:50 dosage ratio. Groups 3 and 4 were treated with 200 mg/kg each of *A. paniculata* and *T. bangwensis*, respectively. Group 5 was not treated with either of the plant extracts and served as a control. The animals were allowed free access to commercial rat mash and water throughout treatment. At the end of treatment, serum AST, ALT, and ALP showed no significant difference ($p>0.05$) compared to the control. No significant difference ($p>0.05$) was also observed among the treatment groups. Serum total protein revealed no significant difference ($p>0.05$) in the treatment groups when compared to the control, and there was no significant difference ($p>0.05$) among the treatment groups. Serum albumin showed a significant decrease ($p<0.05$) in group 3 when compared to the control. Total bilirubin and direct total bilirubin levels showed a significant decrease ($p<0.05$) in the control group when compared to the treatment groups, but no significant difference ($p>0.05$) was recorded among the treatment groups. No significant difference ($p>0.05$) was also observed in serum indirect bilirubin levels in the treatment groups when compared to the control and when compared among the treatment groups. Therefore, these results implied that single and combined leaf extracts of *A. paniculata* and *T. bangwensis* exhibited hepatoprotective properties as they did not cause any adverse effect on the liver function parameter.

Keywords: Hepatoprotective, *Andrographis Paniculata*, *Tapinanthus Bangwensis*, Albino Wistar Rats, Liver Enzymes

Introduction

The use of medicinal plants with therapeutic properties has been in the system since the dawn of civilization. Plants possess the ability to synthesise a wide range of chemical compounds that are employed to perform important biological functions. Long before the discovery and development of modern scientific medicines, traditional healing methods have been in use and are still in practice today in every culture (Aithal et al., 2002). These plants are known to contain substances that could be used to produce drugs. That is why the use of medicinal plants is a core component at the primary healthcare level due to availability, acceptability, compatibility, and affordability. However, a few such medicinal plants are *Andrgraphis paniculata* and *Tapinanthus bangwensis*, *Andrographis paniculata* is an important medicinal plant and is widely used around the world (Akbar, 2011; Karbir et al., 2014). The plant has been ethnobotanically used for the treatment of snake bites, bug bites, diabetes, dysentery, fever, and malaria (Hajiaghee & Akhondzedeh, 2012). *A. paniculata* has been reported to possess diterpenoids, diterpenes, glycosides, lactone, and flavonoids as its bioactive compounds. The leaves and other parts of *A. paniculata* have been discovered to have a broad spectrum of pharmacological effects including anticancer (Rajagopal et al., 2003), antidiarrheal (Gupta et al., 1993), antihepatitis (Sharma et al., 1991), anti-HIV (Nanduri et al., 2004), anti-hyperglycemic (Subramanian & Amawi, 2006), anti-inflammatory (Sheeja et al., 2006) antimicrobial and antimalarial (Wiart et al., 2005), antioxidant, cardiovascular, cytotoxic, hepatoprotective and immunostimulatory properties (Tan & Zhang 2004; Nanduri et al., 2004; Akowuah et al., 2008). On the other hand, *Tanpinanthus bangwensis*, generally referred to as mistletoe, is a well-

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known evergreen parasitic plant, which grows on deciduous trees in a ball-like bush. Traditionally, the extract of mistletoe has been used against a variety of diseases such as disorders in the female reproductive system, cancer, arthritis, rheumatism, epithelial tumors, hypertension, asthma, nervousness, and epilepsy (Evans, 2005; Mishra et al., 2007). Preliminary phytochemical screening of the leaf of *T. bangwensis* by Nwze et al. (2004) revealed the presence of alkaloids, tannins saponins, steroids, and flavonoids. The liver is a central organ in living organisms that is responsible for the metabolism of carbohydrates, protein, lipids, etc. It is a key organ involved in the detoxification of xenobiotics. However, the liver is a direct victim of toxicity that could result in liver disease. Elevation or depression in serum liver enzymes such as alanine transaminase (ALT), aspartate transaminase (AST), and alkaline phosphatase (ALP) and levels of total protein, albumin, and bilirubin results in liver dysfunction (Ghout et al., 2010). Therefore, that was why the hepatoprotective effect of ethanol leave extracts of *A. paniculata* and *T. bangwensis* was assessed.

Materials and Methods

Collection and Preparation of Plant Sample

Fresh leaves of *Andrographis paniculata* and *Tapinanthus bangwensis* were collected at different locations in the botanical garden at Akwa Ibom State Polytechnic, Ikot Osurua, Ikot Ekpene. The two leaves were authenticated by a Taxonomist in the Department of Botany and Ecological Studies, Faculty of Sciences, University of Uyo, Akwa Ibom State, Nigeria. The leaves were plucked from their stem, washed with distilled water to remove dirt, sliced separately with a knife into tiny pieces, and dried separately at room temperature for 3 days. The dried leaves were later ground separately using a clean, dry mortar and pestle, after which 500 g of each of the samples were separately soaked in 150 ml of 70 percent ethanol for 72 hours (Essien et al., 2023). The macerated leaf extracts were differently filtered using Whatman No.1 filter paper using a funnel. The filtrates were then separately concentrated for 3 consecutive days in a water bath at a temperature of 40- 50 ^{0}C (Essien et al., 2022), after which the slurry form of the extracts obtained was preserved in the racks obtained were preserved in a refrigerator at 4 ^{0}C (Essien et al., 2022), after which slurry form of the extracts obtained were preserved in a refrigerator at 4 ^{0}C for further use (Essien et al., 2023).

Experimental Design, Grouping, and treatment of the Animal

A total of twenty- five (25). Healthy adult male albino Wistar rats weighing 74-186 g were obtained from a disease-free stock of the animal house, Biochemistry Unit, Department of Chemical Sciences, Akwa Ibom State Polytechnic, Ikot Osurua, Ikot Ekpene, Akwa Ibom State, Nigeria. The animals were housed in cages with five sizeable compartments of wooden bottom and mesh top, randomly assigned five animals per five groups. The rats were maintained under standard conditions of temperature and natural light-dark cycle for 7 days of acclimatization in the Animals House, Akwa Ibom State Polytechnic, Ikot Osurua. Groups 1 and 2 were treated orally with 200 mg/kg and 300 mg/kg combined leave extracts of *A. paniculata* and *T. bangwensis* respectively, at a 50:50 dosage ratio. Groups 3 and 4 were treated with 200 mg/kg each of *A. paniculata* and *T. bangwensis* respectively. Group 5 was not treated with either of the plant extracts and served as a control. The treatment lasted for one month, however, all animals were allowed free access to commercial rat mash and water throughout the treatment period.

Collection of Blood Samples and Preservation of Serum.

Immediately after the expiration of treatment, the rats were fasted overnight (12 hours) after which they were anesthetized under chloroform vapor and were sacrificed by dissecting medioventrically and blood was obtained via cardiac puncture using a syringe and needle into sterile EDTA sample bottles and then centrifuged at 3,000 rpm for 15 minutes to separated serum from the plasma (Essien et al., 2022). The serum was used to determine the liver function parameters; AST, ALT, ALP, total protein, albumin, total bilirubin, and direct and indirect bilirubin.

Methods

Determination of Aspartate Transaminase (AST).

To two clean sample tubes labeled blank and sample, 0.1 ml of distilled water and serum sample were poured into respectively. Thereafter, 0.5ml of the substrate prepared (R1) was added into each tube and incubated at 37 ^{0}C for 30 minutes. Then, 0.5 ml of 2,4-dinitrophenyl-hydrazine solution (R2) was added, mixed and allowed to stand for 20 minutes at 20-25 ^{0}C and finally, 5.0 ml of sodium hydroxide (0.4 ml) was added to each tube, mixed and the absorbance of the sample was read against the reagent blank after 5 minutes using spectrophotometer at the wavelength of 456 nm (Reitman & Frankel, 1975).

Determination of Alkaline Phosphatase (ALP)

Exactly 0.1ml of serum sample was pipetted into a test tube, 0.5 ml of the reagent was added to the sample and it was mixed and the initial absorbance was read immediately then, it was mixed further and the absorbance was

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read at 1,2 and 3 minutes intervals all at 405 nm with a spectrophotometer at room temperature. (Englanhondt, 1970).

Determination of Alanine Transaminase (ALT).
To two sample tubes labeled blanks and sample, 0.1ml of distilled water and serum sample was measured into each respectively, then, 0.5ml of the substrate prepared (RL) was also added, mixed, and incubated at 37 ^{0}C for 30 minutes. Thereafter, 0.5 ml of 2,4-dinitrophenyl 1-hydrazine solution (R2) was added to all the tubes, incubated, and allowed to stand at room temperature for 20 minutes. Finally, 5ml of Sodium hydroxide (0.4 M) was added to each tube and the absorbance of the samples was read against a reagent blank with a spectrophotometer at 546 nM (Reitman & Frankel, 1957).

Determination of Total Protein
The total protein in the serum sample was determined based on the method by Tietz (1995). A standard commercial kit supplied by Randox Laboratory Ltd, UK was used. Exactly 20 µl of distilled water, 20 µl of standard (CAL), and 20 µl of serum sample were pipetted into three respective test tubes labeled blank, standard, and sample. Then, 1000 µl Buiret reagent (R_1) was pipetted into each of the tubes, mixed, and incubated at 25 ^{0}C for 30 minutes. The absorbance of the sample and standard were read against the blank at 546 nm with a spectrophotometer.

Determination of Albumin
Albumin concentration the in the serum samples was determined based on the method by Grant et al. (1987). Exactly 10µl each of distilled water, standard (CAL), and serum samples were pipetted into three separate test tubes labeled blank, standard, and sample respectively. Then, 3000 µl of Bromocresol (BCl) reagent (R_1) was measured into each test tube, mixed, and incubated at room temperature for 5 minutes, after which the absorbance of the sample and standard were read against the reagent blank at 578 nM with a spectrophotometer.

Determination of Total Bilirubin
Total Bilirubin was assayed with the method by Jendrassik and Grof (1938). Exactly 200ul of reagent 1 (Sulphanilic acid) was pipetted into two different test tubes labeled blank and samples respectively. Then, 50 µl of reagent 2 (Sodium nitrate) was added to the sample test tube and 100 µl of reagent 3 (Caffeine) was added to each of the test tubes followed by the addition of 200 µl serum sample to each tube. The content of each tube was mixed and incubated for 10 minutes at room temperature after which 1000 µl of reagent 4 (Tartrate) was added to each test tube, mixed, and incubated for 30 minutes at 25 ^{0}C. The absorbance of the sample was read against that of the reagent blank at 578 nM with a spectrophotometer.

Total Bilirubin Concentration (mg/dl) = 10.8 X A_{TB}

Where, A_{TB} = Absorbance of Total Bilirubin

Determination of Direct Bilirubin
Direct bilirubin in the sample was assayed according to the method by Jendrassk and Grof (1938). Here, two test tubes were labeled blank and sample respectively, and 50ul of reagent 2 (Sodium nitrate) was measured into the sample labeled sample. Again, 2000 µl of 0.9 % Sodium Chloride (NaCl) solution was put into the respective test tubes, followed by 200 µl of the sample, mixed and incubated for 10 minutes at room temperature, after which the absorbance of the sample was read against that of the blank at 546 nM. Direct bilirubin concentration was calculated using the formula below;

Direct Bilirubin (mg/dl) = 14.4 X A_{DB}

Where, A_{DB} = Absorbance of direct bilirubin

Determination of Indirect Bilirubin
Indirect bilirubin was determined as the difference between total bilirubin and direct bilirubin as shown below;

Indirect Bilirubin (mg/dl) = Total Bilirubin – Direct Bilirubin

Statistical Analysis

Data obtained from the analysis were subjected to a one-way analysis of variance (ANOVA). Statistical significant differences were obtained at ($P<0.05$) by Bonferoni's multiple range test. The results were expressed as mean ± standard error of the mean (SEM) estimated using Statistical Package for Social Science (SPSS) version 23.

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Results

Table 1: Mean serum liver Enzymes in Albino Wistar rats treated with ethanol leaf extracts of *Andrographis paniculata* and *Tapinanthus bangwensis*.

Groups	ALT (IU/L)	AST (IU/L)	ALT (IU/L)
1	19.18 ± 0.19	17.58 ± 0.99	0.58±0.02
2	21.90 ± 0.88	24.11 ± 1.34	0.040±0.04
3	26.00 ± 4.93	14.68 ± 1.46	0.040±0.01
3	35.15 ± 8.55	15.65 ± 1.10	0.32±0.03
5 (Control)	29.29 ± 3.01	29.87 ± 1.94	0.048±0.01

Result are Expressed as mean ± SEM (N=5)

Table 2: Mean serum Total Protein, Albumin, Total bilirubin, Direct and indirect Bilirubin in Albino Wistar Rats Treated with Ethanol Leaves extract of *Andrographis paniculata* and *Tapinauthus bangwensis*.

Groups	Total Protein (g/dl)	Albumin (g/dl)	Total bilirubin (mg/dl)	Direct bilirubin (mg/dl)	Indirect bilirubin (mg/dl)
1	5.39 ± 0.19	3.35 ± 0.23	2.74 ± 0.29	2.16 ± 0.17	0.58 ± 0.15
2	5.59 ± 0.23	3.65 ± 0.13	2.68 ± 0.38	1.93 ± 0.47	0.61 ± 0.17
3	5.45 ± 0.49	3.10 ± 0.35	2.83 ± 0.29	2.07 ± 0.13	0.76 ± 0.20
4	5.29 ± 0.46	3.39 ± 0.33	2.79 ± 0.52	2.31 ± 0.38	0.48 ± 0.16
5(control)	5.50 ± 0.30	3.51 ± 0.21	1.99 ± 0.28	1.24 ± 0.25	0.64 ± 0.14

Results are Expressed as mean ± SEM (N=5)

Discussion

The liver is a critical organ in the human body responsible for an array of functions that help support metabolism, immunity, digestion, detoxification, and vitamin storage, among other functions. Liver function tests check the levels of certain enzymes and proteins in the human blood. Levels that are higher or lower than normal can mean liver problems. The pattern and degree of elevation of these parameters along with the overall clinical picture can provide hints to the underlying cause of these problems (Coon & Ernest, 2004). Most diseases are generated from the exposure of the overall metabolic or biochemical function of the body cells, tissues, or organs to certain substances such as drugs. Meanwhile, in this work, hepatoprotective properties of ethanol extracts of *Andrographis paniculata* and *Tapinanthus bangwensis* were assessed. Serum levels of alkaline phosphatase (ALP), Alanine Transaminase (ALT), Aspartate transaminase (AST), total protein, albumin, total bilirubin, direct and indirect bilirubin were the liver function indices assayed. The results revealed no significant difference in the level of serum ALP, ALT, and AST in the treatment groups of animals when compared to the control group, and when compared among the treatment groups. Meanwhile, abnormal levels of ALP in the blood most often indicate a problem with the liver, gallbladder, or bone (Niufa, 2010). They may also indicate malnutrition, kidney, cancer tumors, intestinal issues, a pancreas problem, or a serious infection. The normal range of ALP (20-140 IU/L) varies from person to person and this depends on one age, blood type, gender, and whether one is pregnant (Jody & Charnow, 2010). The normal range of ALP runs higher in children and decreases with age because of bone growth and development in children. A decrease in serum ALP may be due to zinc, folic acid, and vitamin C deficiencies, hyperthyroidism, excess vitamin D intake, and low phosphorus levels (Nanduri et al., 2004). However, serum ALP in this study which ranged from 0.032-0.058 IU/L fell within its normal range for humans.

Furthermore, mean Serum ALT levels in all treatment groups were within the normal range of 8 – 48IU/L for humans. Liver diseases such as liver hepatitis or cirrhosis are the most common reason for higher serum ALT levels above the normal range. Very high levels (more than 10 times normal) are usually due to acute hepatitis, sometimes due to a viral infection (Ninfa, 2010). ALT levels are usually not as high in chronic hepatitis, often less than 4 times normal. In this case, ALT often varies between normal and slightly increased. Again, the mean Serum AST level in all treatment groups fell within the normal range of 7-55 IU/L for humans. The results of this work indicated that ethanol leaves extracts of *A. paniculata* and *T. bangwensis* at various dosages; Single and combined treatment exhibited no adverse effects on the liver enzymes. Serum ALT and AST are considered to be two of the most important enzymes that indicate liver injury when elevated, although ALT is more specific to the liver than AST. Sometimes AST is compared directly to ALT and an AST/ALT ratio is calculated. This

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ratio may be used to distinguish between different causes of liver damage and to help recognize heart or muscle injury (Wang et al., 2010). According to Strove and Makarova (1994), ALT/AST ratio greater than one is indicative of liver damage. However, in this study, the ratio of ALT/AST in all treatment groups was less than one, indicating that the liver was not injured by the extracts. Subsequently, the impacts of treatment of the animals with varying (combined and single) doses of the two leaf extracts on Serum ALP, ALT and AST was not feasible. No. Dose-dependent effects of the extracts were observed on the enzymes. More so, Serum total protein did not show any significant difference in the treatment groups of animals when compared to the control and when compared among the treatment groups. This was in line with the report of Egbewande et al. (2011) who reported no significant difference among treatments in total protein and albumin in their work on the utilization of African mistletoe (*T. bangwensis*) leaf meal by broiler chickens. Serum albumin showed a significant decrease in group 3 when compared to the control. This result is in accord with the report by Bukoye and Musban (2011) who recorded a significant decrease in albumin levels at a dose treatment of 250 mg/kg and 500 mg/kg upon treatment of mice with *A. paniculata*. Serum albumin in this study which ranged from 3.10 to 3.65 mg/dl fell within the normal range (3.5-5.0 g/dl) for humans. Serum total bilirubin and direct bilirubin levels in this study showed a significant decrease in the control when compared to the treatment groups, though there was no significant difference among the treatment groups. No significant difference was also observed in indirect bilirubin levels in the treatment groups when compared to the control. These results were in contrast with the work by Ofem et al. (2014) who reported a significant decrease in Serum bilirubin upon administration of *A. paniculata* and *T. bangwensis* on mice. However, Egbewande et al. (2011) recorded no significant difference in bilirubin levels. These variations may be because of the duration of treatment, dose administered or species of animals used.

Conclusion

There were no adverse effects of ethanol leave extracts of *A. Paniculata* and *T.* bangwensis in their combined and single dosages on Serum ALP, ALT, AST, total protein, albumin, and bilirubin in albino Wistar rats. Therefore, the leaf extracts exhibited hepatoprotective properties.

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