

Full Length Research Paper

Phytochemical screening and toxicological effects of *Amblygonocarpus andongensis* aqueous stem bark extract in wistar albino rat

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***Amblygonocarpus andongensis* is being used as antipsychotic. Hence, it may portend high risk of toxicosis. In view of this, phytochemical screening and toxicological effects of aqueous stem bark extract of the plant was studied in wistar albino rats. Qualitative screening of the stem bark extract revealed the presence of alkaloid, hydrolysable tannin, saponin, glycoside, cardiac glycoside, saponin glycoside and anthraquinone glycoside. Quantitative screening revealed 4.04% alkaloid, 4.94% saponin and 14.38% true tannins. Subchronic dose (900 mg/kg) caused increased and decreased significant levels of basophils and eosinophils ($P < 0.05$) respectively. Serum biochemistry revealed increased alkaline phosphatase level ($P < 0.05$). A mild periportal and perivascular lymphocytic infiltrations of the liver and moderate focal lymphocytic infiltration of the meninges (focal nonsuppurative meningitis) which are suggestive of mild toxicity of *A. andongensis* were observed. Hence, the plant is toxic at dose level of 900 mg/kg body weight when administered for a period of 28 days.**

Key words: Toxicity; *Amblygonocarpus andongensis*, phytochemicals, stem bark, brain, liver, rat.

INTRODUCTION

Amblygonocarpus andongensis (Fabaceae) known in Hausa as Kolon itche, is a tall tree of 6 to 25 m in height

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having wide crown with glabrous leaves (2-5) and 5-9 pairs of leaflets distantly placed. The flower is white or yellow, fragrant, paired with dense axillary Eduardo et al., 2000, recimose. It has small calyx and pod is tetragonal and glossy (Shahina, 1989). It is widely spread in tropical Africa and usually found in moist and sandy habitats (Exell and Torre, 1989). Roger (2000) also reported the plant to be an economic tree that can be found in the west central Africa. Paul et al. (2000) had reported the presence of this tree in Nigeria. Osemeobo and Ujor (1999) also reported the presence of this tree in Nigeria and particularly in the Northern part of the country.

The tree was described to have clear potential therapeutic and economic values (Paul et al., 2000; Roger, 2000). It has activities against venomous stings, bites, diarrhea, dysentery, constipation, pulmonary problems and fish poisons (Burkill, 1985). The plant has long-standing history of antipsychotic effect reported in wistar albino rats. The plant was reported to be mildly toxic in rats, caused weight loss and reduced mortality caused by amphetamine in wistar rats (Ebbo et al., 2008; 2009). The plant showed dose dependent potential antipsychotic effect in Wistar rats (Ebbo et al., 2010). In spite of all these claims, there is no documentation on phytochemical analysis, haematological and biochemical effects of *A. andongensis* in wistar albino rat. This is because traditional medicine in Africa is not codified but verbally passed unto apprehentice as folk medicine (Ohaeri, 1989).

MATERIALS AND METHODS

Collection of the plant materials

The plant samples were obtained from Anka, the headquarter of Anka Local Government Area of Zamfara State, Nigeria and identified by Mallam Umar Denge a botanist in the Herbarium of the Department of Biological Science, Usmanu Danfodiyo University, Sokoto where a voucher specimen has been deposited.

Preparation of aqueous extract

Stem barks of the plant were obtained, dried under shade to a constant weight and pounded into fine powder using pestle and mortar. One hundred gram of the pulverized bark of *A. andongensis* powder was placed in a conical flask and 500 ml of distilled water added and shaken manually for 6 h, but allowed to stand for 18 h and filtered into beaker using Whatman (No. 1) filter paper. The filtrate was concentrated at 50°C and kept in the refrigerator until ready for use (Eduardo et al., 2000).

Phytochemical analyses

Phytochemical analyses of the plant were carried out using aqueous bark extract of *A. andongensis* for the presence of alkaloids, cardiac glycosides, saponins, tannins, steroids, flavonoids, anthraquinones, total glycosides, and reducing sugars

using the methods of Trease and Evans (1989), Haborne (1973), and Edeoga et al. (2005).

Experimental animals

Twelve adult Wistar albino rats were obtained from Zoological Garden of Usmanu Danfodiyo University Sokoto Nigeria and acclimatized for two weeks. Laboratory animal care was provided to the animals according to the NIH (1985) guidelines and procedures were followed in this study as directed by the Department of Pharmacology, College of Health Sciences, Usman Danfodiyo University Sokoto and approved by research ethics committee.

Sub chronic administration of aqueous extract

The rats of either sex weighing 156.75±10.26 g and divided into two groups of 6 each were used for the study. Six out of the twelve rats received 30% of the limit dose (3000 mg/kg) of oral *A. andongensis* aqueous stem bark extract for a period of 4 weeks. The control rats were administered water only. Grower's marsh® and water were provided *ad libitum*.

Haematology

Blood sample (0.5 ml) was collected from the tail vein at the end of 4th week from all the rats into ethylene diammine tetraacetic acid (EDTA) bottles for haematological and biochemical analyses. Full blood cells count was done using the method of Baker (1985). Brain, kidney, liver, heart and skeletal muscle harvested from each rat under the effect of anaesthetic ether for histopathology (Rivera et al., 2001).

Biochemistry

Another blood sample (2.0 ml) was collected from the tail vein for serum biochemistry. Total protein was determined using biuret method (Tietz, 1995). Albumin was determined using bromocresol green method (Doumas 1971). But conjugated bilirubin and total bilirubin were determined using the method of Jendrassik and Grof (1938). Whereas serum glutamic aspartate amino transferase and alanine aminotransferase were determined using the method of Reitman and Frankel (1957).

Statistical analysis

The data on haematological and biochemical parameters were expressed as mean±S.D. Test for significance was performed at 5% level between mean parameters of the control and experimental rats using student 't' test unpaired (Petrie and Watson, 2002).

RESULTS

Results of phytochemical screening carried out on aqueous stem bark extract of *A. andongensis* revealed the presence of alkaloid, hydrolysable tannins, saponin, flavonoid, cardiac glycoside, saponnin glycoside and anthraquinone glycoside. Whereas flavonoid and flavonols were absent. The results of quantitative screening

Table 1. Qualitative and quantitative screening of *A. andongensis* aqueous stems bark extract.

Secondary metabolites	Result
Alkaloid	4.012%
Tannins (hydrolysable)	14.38%
Saponin	4.94%
Flavonoid	+
Flavonols	-
Glycoside	-
Cardiac glycoside	+
Saponin glycoside	+
Anthraquinone glycoside	+

+ = presence, - = absence.



Figure 1. Brain showing moderate lymphocytic infiltration of meninges (focal non-suppurative meningitis) (x 400).

of *A. andongensis* aqueous stem bark extract revealed 4.01, 4.94 and 14.38% of alkaloid residue, saponin and true tannins (Table 1).

Haematology showed significant increased and decreased ($P < 0.05$) levels of eosinophils and basophils in the control and experimental animals respectively. But packed cell volume (PCV), white blood cells (WBC), red blood cells (RBC), lymphocytes, neutrophils and monocytes were not significantly affected ($P > 0.05$).

Biochemistry revealed significant increased level of alkaline phosphatase ($P < 0.05$). But all other investigated parameters such as total bilirubin, conjugated bilirubin, total protein, albumin amino transferase and aspartate amino transferase were not increased significantly ($P > 0.05$).

Histopathology revealed mild periportal and perivascular lymphocytic infiltration of liver (Figure 2) and moderate lymphocytic infiltration of the meninges (focal nonsuppurative meningitis) of the brain (Figure 1). But kidney had no visible lesion (Figure 3).

DISCUSSION

The presence of alkaloid in the extract corroborates the report of Ajao et al. (2018) indicating that alkaloid has been responsible for antipsychotic effect of the plant. However, the presence of hydrolysable tannins, saponin, cardiac glycoside, saponin glycoside, flavonoid and anthraquinone glycoside in the plant, agrees with the report of Aliyu and Yusuf (2013) indicating that the plant contains phytotoxic principles. This agrees with the report of Akinloye et al. (2003) indicating that tannin and saponin are antinutritional substances. These substances can cause haemolysis and nutrient malabsorption (Conning, 1993). The presence of alkaloid residue (4.01%), saponin (4.94%) and true tannins (14.38%) in the stem bark of *A. andongensis* (Table 2) may suggest the ability of the plant to cause both beneficial and toxic effects in animals.

Saponin cause haemolysis, nutrient malabsorption and abnormal haemopoiesis (Moody et al., 2003), the plant may be useful in treatment of polycythemia vera in human.

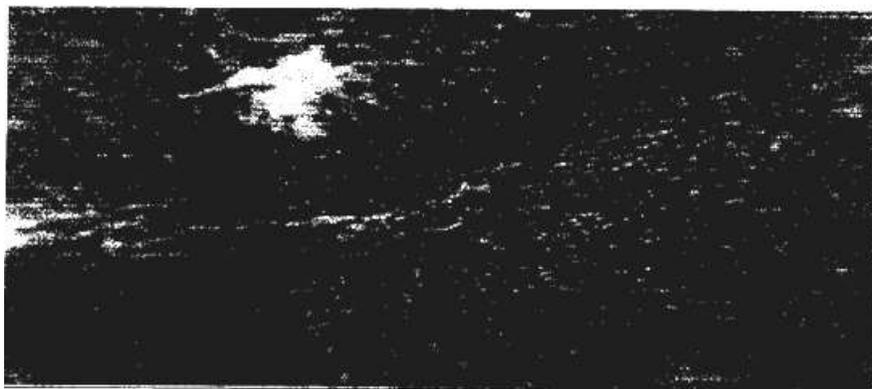


Figure 2. Liver shows mild peripotal and perivascular lymphocytic infiltrations (x 400).

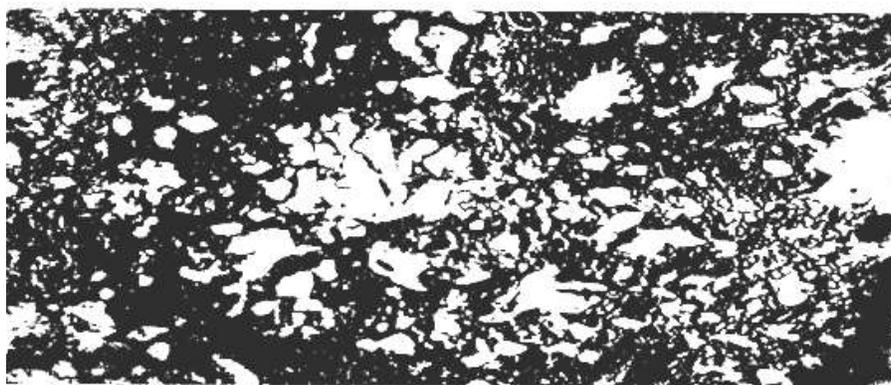


Figure 3. Kidney shows no visible lesion (x 400).

Table 2. Effects of *A. andongensis* aqueous stem bark extract on haematological and biochemical parameters of wistar albino rats.

Indices	Control	Experimental
Packed cells volume (%)	50±0.98	48.33±1.72
Red blood cells (%)	4.96±0.33	6.11±0.23
White blood cells (%)	7.55±0.35	6.33±0.59
Lymphocytes (%)	70.5±1.38	73.5±2.17
Neutrophils (%)	21.5±1.0	19.7±1.86
Monocytes (%)	5±0.59	5.7±0.49
Eosinophils (%)	3±0.26	1.0±0.37 ^a
Basophils (%)	0.0±0.0	0.16±0.17 ^a
Total bilirubin (µg/dl)	0.27±0.01	0.25±0.02
Conjugated bilirubin (µg/dl)	0.14±0.0	0.18±0.02
Total protein (g/dl)	6.75±0.2	7.4±0.28
Albumin (g/dl)	3.4±0.05	3.71±0.23
Aspartate amino transferase (µg/L)	39.5±2.67	41±0.22
Alanine amino transferase (µg/L)	21±2.63	22±0.53
Alkaline phosphatase (µg/L)	31.25±1.69	42.2±3.04 ^a

Values are expressed as mean ± S.D., n = 6, a = statistically significant (P<0.05).

The presence of tannin may be responsible for its antiulcer (Oluranti et al., 2012) and anti-diarrhoeic activities (Ugwah et al., 2014). Saganuwan (2009) had earlier attributed the pharmacological property of plants to its chemical principles. The presence or absence of such principles may depend on the methods of extraction. However, the higher content of true tannins may be responsible for its astringent activity. Tannins are capable of turning animal hides into leather by binding proteins to form water-insoluble substance that are resistant to proteolytic enzymes (WHO, 1998). Since tannin contains phenol, it may have antimicrobial activity. Quattrouichi (2016) had also reported that bark, roots and seeds are poisonous, whereas fruits are used as tonic, stimulant, anticonvulsant, and anti-inflammatory agent. But powdered pods are used to treat skin diseases, and leaf extract is stomachache. Decoctions of root are emetic, vermifuge, antipyretic, antimalarial, anticolic, anticough and anti-dote for food poisoning. Powder from the pod is used as fish poison and insecticide (Quattrouichi, 2016). Significant increased level of eosinophils in the control animals cannot be explained however, the basophilia observed in the experimental rats may be attributable to stress or the presence of glucocorticoid principle in the test plant. The eosinopenia observed in the present study agrees with the report of Tvedten (1989) indicating that eosinopenia is usually due to stress or exogenous glucocorticoid treatment. Hence, the plant may have glucocorticoid principle. However, the significant increased level of basophilia observed in the experimental extract treated group as compared to the control group may also suggest antilipaemic property of the plant. This agrees with report of O'keefe et al. (1987) indicating that basophilia in the absence of an eosinophilia has been associated with altered lipid metabolism. Basophil is one of the sources of heparin which enhances the activity of lipoprotein lipase along blood vessels in clearing fat from blood (Tvedten, 1989). Invariably *A. andongensis* may have ability to cause decreased blood cholesterol level. However, the observed basophilia in the experimental animals further agrees with the report of Tvedten (1989) indicating that basophilia is caused by the same process causing an eosinophilia or is associated with lipaemia. But the significant ($P < 0.05$) difference between control value and the experimental value of alkaline phosphatase may be associated with age or the injected aqueous extract of *A. andongensis*. The extract may have mild deleterious effect on the liver as shown by mild periportal and perivascular lymphocytic infiltrations of liver (Figure 2). However, since there is no significant increased level of alanine aminotransferase and aspartate aminotransferase, the increased level of alkaline phosphatase may also be of bone origin. Serum alkaline phosphatase is commonly increased in animals less than 6 to 8 months (Willard et al., 1989) perhaps because of bone formation. Mild

periportal and perivascular lymphocytic infiltrations of the liver (Figure 2) and moderate focal lymphocytic infiltration of the meninges (Figure 1) are suggestive of the ability of the plant to cause liver and brain damage. Hence there is need to investigate the intensity of long-time effects of *A. andongensis* aqueous stem bark extract on vital organs of wistar albino rats. Information obtained from such investigation may give a lead to understanding of mechanism of its toxicological action. However, Nwinyi et al. (2006) reported that the plant is relatively safe when large dose is administered for short period of time.

Conclusion

Qualitative analysis of the aqueous stem bark extract revealed the presence of alkaloid, hydrolysable tannin, saponin, glycoside, cardiac glycoside, saponin glycoside and anthraquinone glycoside. But quantitative analysis revealed 4.04% alkaloid residue, 4.94% saponin and 14.38% true tannins. The plant caused eosinopenia, basophilia, increased alkaline phosphatase, mild periportal and perivascular lymphocytic infiltration of the liver and non-suppurative meningitis which are suggestive of mild toxicity. But the extract has no evident toxicity effect on kidney.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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