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Blood Pressure Lowering Effect of *Adenanthera pavonina* Seed Extract on Normotensive Rats

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Abstract: The effect of *Adenanthera pavonina* (AP) seed extract on the blood pressure of normotensive rats was evaluated. Twelve adult male Wistar rats divided into 3 groups of 4 animals each were used and were treated orally with normal saline (control group), propanolol (positive control, and was given at 1mg/kg) and 200mg/kg of AP seed extract over a 4- week period. Condon manometer was used to measure the mean arterial blood pressure. The mean arterial blood pressure of the normal saline treated animal was 60mmHg, those of propanolol treated animals was 23mmHg while the 200mg/kg extract treated group was 30mmHg. Phytochemical screening showed that the extract contained cardiac glycosides, tannins, saponins, alkaloids and flavonoids. Cyanogenetic glycosides and anthraquinones were absent. The sodium level for the 200mg/kg group was significantly lower than that of control group. The total bilirubin, total protein and the globulin fraction were significantly higher in the extract treated groups compared to the control group. Histopathological examination showed that the extract did not cause any significant lesion changes in the liver, kidney and even the testes. The study showed that *Adenanthera pavonina* seed extract have the potential to cause a blood pressure lowering effect. The serum biochemistry changes may suggest that the extract has a tonic effect on the kidneys and the liver and these organs play central role in drug metabolism. Absence of significant lesion in the kidney, liver and testes may indicate that the plant is safe for medicinal use.

Keywords: *Adenanthera pavonina,* Propanolol, blood pressure, rats, histopathology, biochemistry.

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1. Introduction

Hypertension or high blood pressure is a common disorder which if not effectively treated, results in a greatly increased probability of coronary thrombosis, strokes, and renal failure [1]. Treatment of hypertension reduces cardiovascular risk, and this has been a major focus of campaigns aimed at reducing cardiovascular mortality and morbidity [2]. A number of international guidelines suggest that blood pressure should be reduced at least to below 160/90mmHg to normalize cardiovascular risk in patients with hypertension. In patients at higher baseline risk of cardiovascular disease, for example those with diabetes, the recommendations are that the target blood pressure should be substantially lower than 130/85mmHg. This recommendation is based on the view that the absolute risk of a cardiovascular event in these patients is much greater, and therefore, the absolute benefit of treatment is larger [2].

Adenanthera pavonina is endemic to Southeast China and India, with first reports being recorded in India. The tree has been introduced throughout the humid tropics. It has become naturalized in Malaysia, Western and Eastern Africa and most island nations of both the Pacific and the Caribbean. It belongs to the family Leguminosae and subfamily Momosoideae [3]. In terms of medicinal uses, the seeds have been found to be effective in treating cardiovascular diseases in pregnancy. The ground seeds are used to treat boils and inflammatory reactions. Decoction of leaves is used to treat gout and rheumatism [4].

A methanol extract of seeds of *Adenanthera pavonina* was evaluated for pharmacological effects in animal models. The study demonstrated the anti-inflammatory and analgesic effect of *Adenanthera pavonina* extract [5]. Acute toxicity studies revealed that the extract produced reduced motor activity. The LD_{50} value of the extract was found to be 1.36g/kg [5].

Studies have reported the presence of cardiac glycosides in the methanol extract of *Adenanthera pavonina* seeds [5], although there is no known study on the cardiovascular effect of the extract. This study is therefore designed to determine the presence of cardiac glycosides in *Adenanthera pavonina*, hence assess the phytochemical constituents of the seeds of this plant, evaluate the effect of the methanol extract obtained from the seeds on the blood pressure, and then assess the safety or otherwise of this extract in experimental animals.

2. Materials and Methods

2.1 Collection and Authentication of Plant Materials

The seeds of *Adenanthera pavonina* were collected from the Staff School compound of the University of Ibadan, Nigeria. They were authenticated at the Forestry Research Institute of Nigeria (FRIN), Ibadan, where the voucher specimen was deposited at the herbarium. The Forestry Herbarium Identification (FHI) number is 107848.

2.1.1 Sample Preparation and Extraction

Seeds were air dried and powdered with an electronic blender. Sample was put in a conical flask, and then one litre of methanol was added. It was stirred continuously and was kept for about 72 hours. It was then filtered and the filtrate was dried with a rotatory evaporator to yield 3.94% of the extract.

2.2 Phytochemical Screening

Standard phytochemical methods were used to test for the presence of saponins, alkaloids,

tannins, anthraquinones, cardiac glycosides, cyanogenetic glycosides and flavonoids [6, 7, 8, 9, 10] and these tests are described below:

2.2.1 Saponins

About one gram of the powdered sample was boiled with 10 mL's of distilled water for ten minutes. The samples were filtered while hot, cooled and the following tests were carried out:

a. Frothing: 2.5 mL of the filtrate was diluted to 10mLs with water and was shaken vigorously for 2 minutes. Frothing observed indicates a positive test.
b. Emulsification: 2.5mL of the filtrate was shaken vigorously for 2 minutes with a few drops of olive oil. An emulsified layer indicates a positive test.

2.2.2 Alkaloids

About one gram of the powdered sample was extracted with 10 mL of 10% hydrochloric acid by boiling for five minutes on a water bath. The extract was filtered and the pH of the filtrate was adjusted to about 6 by adding a few drops of dilute ammonia solution and tested with litmus paper after which few drops of Dragendorff's, Mayer's and Wagner's reagent were added separately to aliquots of the filtrate in the test tubes. A reddish brown, cream and reddish brown precipitate respectively indicates a positive test.

2.2.3 Tannins

About 1 gram of the powdered sample was boiled with 10 mL of the distilled water for five minutes, filtered while hot and a few drops of ferric chloride reagent was added to the filtrate. A red colouration indicates a positive test.

2.2.4 Anthraquinones

One gram of the powdered sample was extracted with 10 mL of 10% sulphuric acid, containing traces of ferric chloride solution for 15 minutes. It was filtered while hot and the cooled filtrate was extracted while hot with 2 equal volumes of chloroform. The presence of a rose pink colour in the aqueous layer indicates a positive test.

2.2.5 Cardiac Glycosides

One gram of the powdered sample was extracted with 10 mL of 80% alcohol for 5 minutes on a water bath; the extract was filtered and diluted with an equal volume of distilled water. A few drops of lead acetate solution was added, shaken, and filtered after standing for a few minutes. The filtrate was extracted with aliquots of chloroform. The combined chloroform extracts were divided into two portions and Keller Killiani and Kedde tests were carried out on them.

2.2.6.1 Keller Killiani test

The extract was evaporated to dryness and 3 mL of ferric chloride reagent was added to the cooled residue in a clean test tube. 2 mL of concentrated sulphuric acid was gently poured down the side of the test tube. A purple or reddish brown ring at the interface and green colour in acetic acid layer indicates a positive test for 2-de-oxy sugar.

2.2.6.2 Kedde test

The dry residue was mixed with 1 mL of 2% 3, 5 –dinitrobenzoic acid in ethanol. The solution was made alkaline with 5% sodium hydroxide. A brown purple colour indicates a positive test for the presence of unsaturated lactone ring.

2.2.7 Cyanogenetic glycosides

0.5 gramme of the powdered sample was placed in three different test tubes A, B, C. Test tubes A and B were mixed with water with a suspended moist sodium picrate paper in the neck of the tube, trapped by means of cork. Test tubes B and C were kept at room temperature while test tube A was placed on a boiling water bath. At the end of about half an hour, a change in colour of the sodium picrate paper indicates a positive test.

2.2.8 Flavonoids

A small quantity of the extract was dissolved in dilute sodium hydroxide and hydrochloric acid was added to the mixture. A yellow solution that turns colourless on addition of hydrochloric acid indicates the presence of flavonoids.

2.3 Antihypertensive effects of Adenanthera pavonina in rats

The animals used in this study were 12 male Wistar rats weighing between 240-350gms. They were maintained in the central animal house of the College of Medicine University of Ibadan, Nigeria. They were kept in rat cages and fed commercial rat cubes (Ladokun and Sons Livestock Feeds, Nigeria Ltd.) and allowed free access to clean fresh water in bottles *ad libitum*.

The twelve animals were divided into 3 groups of 4 animals per group. While group A rats served as control, groups B and C animals were administered with 1mg/kg and 200mg/kg doses of propanolol and the extract respectively. The extract, propanolol and distilled water were administered orally to the animals once daily for 28 days using stomach canula. On the 29^{th} day, the mean arterial blood pressure of the animals was measured. All experimental protocols were in compliance with University of Ibadan Ethics Committee on Research in Animals as well as internationally accepted principles for laboratory animal use and care.

2.3.1 Measurement of arterial blood pressure

The direct method, which is used to measure arterial blood pressure in animals, was adopted [11]. The animals were given suitable anaesthesia i.e. urethane and this was administered through the intraperitoneal route. The tracheal incision chosen for the study was made on the anterior tracheal wall, and a cannula inserted into the trachea. The tracheal cannula allowed for free flow of air to avoid any disturbance during the experimental procedure. The carotid artery was cannulated and connected to a mercury manometer. The antihypertensive effect of the extract was measured and the results were then compared with those obtained for rats treated with propanolol and control.

Table 1. Phytochemical screening of the powdered samples of the seeds of *Adenanthera pavonina*

SN	Test	Observation	Inference
1.	Test for saponins a. Frothing b.Emulsification	 Frothing was observed. The formation of emulsified layer was observed.	 Presence of saponins suspected (+). Presence of saponins confirmed (+).
2.	Test for alkaloids a. Mayers' reagents b. Dragendorff's reagent c. Wagner's reagent	 A cream precipitate was observed. A reddish-brown precipitate was observed. A reddish-brown precipitate was observed.	 Alkaloids present (+) Alkaloids present (+++). Alkaloids present (+)
3.	Test for tannins	A red colour was observed.	Tannins present (+)
4.	Test for anthraquinone	Absence of a rose colour in the aqueous layer was observed.	Anthraquinone absence (-)
5.	Test for cardiac glycosides a. Keller-Killiani. b. Kedde test.	 a. A reddish-brown ring was observed at the interphase and a green colour was observed in the acetic layer. b. A brown purple colour was observed.	 a. Cardiac glycosides present (+). b.Cardiac glycosides present (+).
6.	Test for cyanogenetic glycosides	There was no change in colour of the sodium picrate paper	Cyanogenetic glycosides absent (-).
7.	Tests for flavonoids	A yellow solution that turned colourless on addition of hydrochloric acid was observed.	Flavonoids present (+).

+ = present, +++ = high concentration, - = absent.

2.4 Safety evaluation of the seeds of Adenanthera pavonina rats

The animals used in this study were 20 male Wistar rats weighing between 240-280gms. They were maintained in the central animal house of the College of Medicine University of Ibadan, Nigeria. They were kept in rat cages and fed commercial rat cubes (Ladokun and Sons Livestock Feeds, Nigeria Ltd.) and allowed free access to clean fresh water in bottles *ad libitum*.

The twenty animals were divided into 4 groups of 5 animals per group. While group A rats served as control and received distillated water, groups B, C and D animals were administered with 50mg/kg, 100mg/kg and 200mg/kg doses of the aqueous extract respectively. The extract was administered to the rats per os once daily using stomach canula for 21 days. All experimental protocols were in compliance with University of Ibadan Ethics Committee on Research in Animals as well as internationally accepted principles for laboratory animal use and care.

2.4.1 Technique for Obtaining Serum Samples

Blood samples were collected by cervical decapitation from diethyl ether anaesthetized rats into clean non-heparinised bottles and allowed to clot. The serum was separated from the clot and centrifuged according to groups into clean bottles for biochemical analysis.

2.4.2 Determination of serum biochemical parameters

Total protein was measured using biuret reaction while albumin was measured by colorimetric estimation using the sigma Diagnostics albumin reagent (Sigma® Diagnostic, U.K.), which contained bromocresol green (BCG). Globulin was obtained from the difference between total protein and albumin. Aspartate aminotransferase (AST), alkaline phosphatase (ALP) and alanine aminotransferase (ALT) were determined using a photoelectric colorimeter (Gallenkamp® and Sons Ltd.; England) as described by Toro and Ackermann [12] and Duncan *et al* [13]. Serum urea and creatinine levels were determined using photoelectric colorimeter (Gallenkamp® and Sons Ltd. England) as described by Toro and Ackermann [12] and Coles [14].

2.5 Histopathology

The liver, kidney and testes of all the animals were fixed in 10% buffered formalin in labeled bottles. Tissues were processed routinely and embedded in paraffin wax. Sections 5-µm thick were cut, stained with haematoxylin and eosin and examined under the light microscope.

2.6 Statistical analysis

Results were subjected to the student's t-test and were considered significant at $P<0.05$ [15].

Table 2. The antihypertensive effects of the methanol extract of the seeds of *Adenanthera pavonina* (n=4).

Group	Mean arterial blood pressure (mmHg)
A. (Control)	60±3.4
B. (Propanolol) (1mg/kg)	23±4.2
C. Extract (200mg/kg dose)	30±3.8

3. Results and Discussion

The phytochemical screening of the methanol extract of the seeds of *Adenanthera pavonina* showed the presence of the following: tannins, alkaloids, saponins, flavonoids, and cardiac glycosides. The results however showed that anthraquinone and cyanogenetic glycosides were absent (Table I). The results thus showed that the methanol extract of the seeds of *Adenanthera pavonina* is rich in cardiac glycosides, alkaloids, saponins, tannins, and flavonoids. Alkaloids usually have marked physiological action on human or animals. Saponins on the other hand are of great pharmaceutical importance because of their relationship to compounds such as the sex hormones, cortisones, diuretic steroids, vitamin D and cardiac glycosides. Tannins like alkaloids are substances which show protein precipitation and are related to the physiological effects of herbal medicines. Flavonoid containing plants have influence on arachidonic acid metabolism, thus could have anti-inflammatory, antiallergic, antithrombotic or vasoprotective effects. Cardiac glycosides are steroidal glycosides which exert a slowing and strengthen effect on the failing heart [16]. The implication of all these is that this plant is of great medicinal importance. The presence of cardiac glycosides in this plant has further confirmed its medicinal use as antihypertensive agent. The presence of flavonoids in this plant is therefore evidenced of its anti-inflammatory properties. This therefore supports the earlier work of Olajide et al. [5] that the plant has anti-inflammatory property.

Table 3. Effects of methanol extract of *Adenanthera pavonina* on the serum chemistry of rats. (n=5)

Paramaters	Control	50 mg/kg	100 mg/kg	200 mg/kg
Total Protein (g/dL)	5.8±0.2	7.4±1.4[d]	8.4±0.3[d]	8.0±0.8[d]
Albumin (g/dL)	3.4±0.2	3.6±1.1	4.6±0.2[d]	4.2±0.9
Globulin (g/dL)	2.4±0.1	3.8±0.5[d]	3.9±0.3[d]	3.5±±0.7[d]
ALT (U/L)	60.5±1.3	18.0±2.2[d]	25.0±3.6[d]	34.3±9.1[d]
AST (U/L)	102.5±1.3	180.8±28.6[d]	20.5±4.4[d]	33.8±7.2[d]
ALP (U/L)	104.8±2.2	257.5±38.4[d]	92.5±3.9[d]	111.0±4.5[d]
Total bilirubin (mmol)	0.3±0.1	0.6±0.1[d]	0.5±0.1[d]	0.6±0.1[d]
Urea (mg/dL)	39.5±1.9	21.5±4.2[d]	23.8±8.9[d]	54.0±19.4
Na^+	141.5±1.3	146.0±7.0	144.5±2.6[d]	137.0±0.8[d]
K^+	4.6±0.2	6.1±0.2 [d]	37.4±2.2 [d]	39.2±3.2 [d]
HCO_3^-	18.0±1.6	22.3±1.7 [d]	20.5±1.3 [d]	20.5±2.1

Superscripted items indicate significant values (P<0.05) from control.
Note: Mean±S.D.

The results of the effect of the methanol extract of the seeds of *Adenanthera pavonina* on the blood pressure of Wistar rats showed that both the extract and propanolol caused significant reduction of the mean arterial blood pressure when compared with those of the control (Table II). The study showed that the antihypertensive effect of this extract on the mean arterial blood pressure is comparable to that of propanolol, an adrenergic antagonist, though at a very high dose of the extract. Propanolol efficacy in treating hypertension as well as most of its toxic effects results from non-selective beta blockade. It decreases blood pressure as a result of decrease in cardiac output [17]. The presence of cardiac glycoside in this plant known to slow and strengthen a failing heart may have accounted for its antihypertensive effect. Besides, it is known that tannins have diuretic effect hence its presence in this plant may have contributed to the antihypertensive effect. Since flavonoids are known to possess vasoprotective effect, its presence in this plant may have ensured that they relax smooth muscles of arterioles thereby decreasing systemic vascular resistance [17].

The results of the safety evaluation of the aqueous extract of the seeds of *Adenanthera pavonina* on rats showed that all the doses caused significant increase in the levels of total protein and globulin. In the case of albumin, only the 100mg/kg dose caused significant increase but the other 2 doses caused no significant change (Table III). It is inferred that alteration in the plasma total protein is most often due to decrease in the quantity of albumin, which may be accompanied by a relative hyperglobulinaemia [13, 14]. In this study, all protein experienced an increase, suggesting that there is increased protein synthesis or mobilization. The increase in globulin level may indicate that the plant could have the potential to stimulate immune response by increasing antibody production (immunoglobins) [18], and this also could be responsible for its use as a medicinal plant. For the liver enzymes, all the doses caused significant decrease in the level of ALT relative to the control. In the case of AST, 50mg/kg dose caused significant increase while the other 2 doses caused significant decrease. For ALP, 50mg/kg and 200mg/kg doses caused significant increase while the 100mg/kg dose caused a significant decrease. ALT level is known to increase in liver disease and it has been used as a tool for measuring hepatic necrosis, especially in small animals [17]. Increase in serum ALP may be considered as an indicator of cholestasis in early stages or mild circumstances preceding other indicators e.g. hyperbilirubinemia [19]. The observations of significant decrease in the levels of the liver enzymes may indicate that the extract of *Adenanthera pavonina* has hepatoprotective effects. The results for total bilirubin showed that all the doses caused significant increase while for urea, 50mg/kg and 100mg/kg doses caused significant increase but the 200m/kg dose caused no significant difference. The results of this study with respect to the serum electrolytes showed that all the doses caused significant increase in the level of potassium ions. In the case of sodium ions, 50mg/kg dose caused no significant difference, but the 100mg/kg dose caused a significant increase while the 200 mg/kg dose caused a significant decrease. For bicarbonate ions, while 50 and 100 mg/kg doses

caused significant increase in the level of this electrolyte, the 200mg/kg dose caused no significant difference (Table III). The extract caused significant increase in the level of all the electrolytes assayed in this study. It is particularly interesting to note that potassium sparing effect is the hall mark of some diuretics [17]. This may be significant in explaining the antihypertensive potential of this plant.

Histopathological changes due to the methanol extract of the seeds of *Adenanthera pavonina* was also embarked upon. Liver, kidneys, and testes from the experimental animals were harvested and processed for histology. The results showed that in all the organs examined none showed any visible lesion indicating that the doses used were not high enough to cause any toxic change. This may then indicate that this plant is relatively safe for use as a medicinal herb.

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