British Journal of Multidisciplinary and Advanced Studies:

Agriculture, 4(2),81-100, 2023

Print ISSN: 2517-276X

Online ISSN: 2517-2778

Website: https://bjmas.org/index.php/bjmas/index

Published by European Centre for Research Training and Development-UK

Toxicity and the Effects of Ethanolic Leaf Extract of *Annona senegalensis* on Haematological Parameters in Albino Rats

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doi: https://doi.org/10.37745/bjmas.2022.0175 Published: May 2, 2023

Citation: Mbaya, Y.P., Bobbo, A.G., Barkindo, A.A. and Hayatu, A.I. (2023) Toxicity and the Effects of Ethanolic Leaf Extract of Annona senegalensis on Haematological Parameters in Albino Rats, *British Journal of Multidisciplinary and Advanced Studies*: Agriculture, 4(2),81-100

ABSTRACT: *Ethanolic leaf extract of Annona senegalensis was studied for acute toxicity and some haematological parameters in albino rats. The method described by Karbar as modified by Aliu and Nwude was used for acute toxicity study in 30 albino rats. One hundred rats were randomly selected into five groups of 20 rats each, that is, groups 1-5, group 1 was the control and groups 2-5 were administered the ethanolic leaf extract for 28 days of the doses of 100, 200, 300 and 400 mg/kg respectively and blood samples were collected for the determination of the haematological parameters using standard methods described by Coles and Schalm. The extract was screened for phytochemical and elemental components using standard protocols. The phytochemical screening revealed the presence of carbohydrates, cardiac glycosides, terpenoids and flavonoids. The elemental screening revealed the presence of some elements in milligram per kilogram concentration such as 7.202 for iron, 0.893 for copper, 6.637 for magnesium, 0.014 for lead, 0.070 for chromium, 0.057 for cadmium, 0.604 for cobalt, 1.732 for zinc and 2.362 for manganese. The acute toxicity study was 2400 mg/kg which is classified as non label according to the report of Organization for Economic Cooperation and Development (OECD) which means it is tolerable at that level. The prolonged administration of the ethanolic extract showed significant increase ($P<0.05$) in White Blood Cell (WBC), Red Blood Cell RBC), Packed Cell Volume (PCV), Haemoglobin concentration (HB), Platelet Count (PLT) and Differential Leucocytes Count (DLC) throughout the duration of the study. In conclusion the ethanolic extract is found to be tolerable and an increase in the haematological parameters in the treated albino rats, thus, has a good immune boosting and treatment of anaemia potentials.*

KEY WORDS: Annona senegalensis, Ethanolic Extract, haematological parameters, Acute Toxicity, Rats

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INTRODUCTION

Many indigenous plants are widely used in traditional medicine for the treatment of various diseases. Plant derived herbal medicines are seen to be safe alternatives of synthetic drugs. Most drugs are synthesized from medicinal plants. Plants contain bioactive substances that constitute the plants' therapeutic activity and or pharmacological activity. Plants extracts or their active principles have enormous therapeutic potentials and the continued investigation of their secondary metabolites has led to important breakthrough in pharmacology and has helped tremendously in the development of modern pharmacotherapeutics in Africa and other parts of the world (Adawaren *et al*., 2015). Plants have been used for thousands of years to flavor and conserve food, to treat health disorders and prevent diseases. All over the globe, the use of medicinal plants has significantly supported primary health care.

The annonas are shrubs or small trees whose height varies from 5-11m depending on several factors such as species, climate, soil and crop management. They are erect or somewhat spreading in habit, with grey-brown bark, often rough and corrugated (Pinto, 2005). Annona stems are generally, ferruginous to grayish and tomentose when young, but later becoming glabrous. With few exceptions, annonas are deciduous. The system has thin lateral roots and a taproot that is not as strong as in other tropical fruit trees such as mango (*Mangifera indica* L). The lighter the soil texture, the longer the taproot grows. The taproot of a soursop tree can reach approximately 1.5-1.8m in depth (Pinto, 2005).

The natives in Africa use this plant primarily for food, fruit rich in nutrient, boost food security, foster rural development and support sustainable land care (Anon, 2014). It tends to grow in semiarid to sub-humid regions adjacent to the coast, often, but not exclusively, on coral-based rocks with mostly sandy and loamy soils (Anon, 2014).

Generally, the annonas grow at a range of altitudes, and those with the widest adaptation to altitude are also those with the widest adaptation to latitude. No photoperiod responses have been reported (Cordeiro and Pinto, 2005). Most annonas do not adapt to low temperature, however highland species such as Cherimoya, wild soursop and to some extent custard apple are better adapted to cold weather than the lowland soursop and sugar apple (Cordeiro and Pinto, 2005). Wild soursop is adapted to various altitudes being cultivated from 0-1,800m in Kenya and from 0-2,400m in other parts of East Africa (FAO, 1983; Anon, 2014). It appears to have adaptation within the range of very low to moderately high rainfall regimes occurring in areas with 600-2,500mm, while across Africa requirements are far more than 600mm annual rainfall. It can withstand a relative humidity as low as 44% at midday. The best mean temperatures for wild soursop growth are between 16^{o}C and 30^{o}C (FAO, 1983; Anon, 2014). It is often a solitary plant within woodland savannah

understory, swamp forests, river banks and former crop land left fallow for an extended period (Anon, 2014).

The primary use of this plant is for food, though it has applications in numerous aspects of human endeavour. The flowers, leaves and fruit are edible and culinary, white fruit pulp has a mild, pineapple-like flavour. Wild Soursop fruits are sold in local markets in Africa. The fruit has a pineapple-like odour and sweet taste (FAO, 1983). It keeps for only a few days. It is used in sherbets, ice creams and for making drinks (FAO, 1988). The flower is added to spice or garnish meals; leaves are eaten by humans as vegetables, or browsed by livestock (Anon, 2014). The leaves are also used to create a general health tonic in the treatment of pneumonia, diseases of the eye, stomach and intestines (Cordeiro *et al*., 2005). The leaves have essential oils with parasiticide, antidiarrhoea, rheumatological and antineuralgic properties (Cordeiro *et al*., 2005). Boiled water infusions of the leaves have anti-spasmodic, astringent and gastric properties (Calzavara and Miiller, 1987; Khan *et al*., 1997), help in treatment diabetes and gastric upsets (Calzavara and Miiller, 1987) and are used in kidney ailments (Cordeiro *et al*., 2005). The cooked flowers and petals are used for healing eye inflammations; the treatment requires 2-3 washes a day (Calzavara and Miiller, 1987). The study became necessary in order to investigate this plant in ethanolic base to observe its effect on some blood parameters for its haematological and immunity potentials.

MATERIALS AND METHODS

Phytochemical Study

The aqueous leaf extract of *Annona senegalensis* was subjected to qualitative phytochemical analysis for the presence of carbohydrates, flavonoids, alkaloids, saponins, glycosides, tannins, terpenes, resin, aloes steroids and anthraquinones using standard methods under room temperature as described by Evans (2009b).

Elemental Analysis of the *Annona senegalensis* Leaf

The dried sample of *Annona senegalensis* leaf powder was put in a well labelled crucible and heated in a furnace at 550^{o}C for 3 hours. The hot sample was removed and kept in a desiccator to cool naturally. The sample in the form of ash was digested in a 250 ml beaker with 20 ml of 2 M nitric acid and 10 ml of 35% hydrogen peroxide and heated on a hot plate (>100^{o}C) in a fume cupboard until a clear digest was obtained. After cooling, it was filtered and deionized water was added until it reached 100 ml in a volumetric flask for elemental analysis using atomic absorption spectrophotometer. The elemental concentrations were determined using a Standard Calibration Curve (Sunderman Jr, 1973; Kolthoff and Elving, 1976; Ojo*et al*., 2013).

Acute Toxicity Study

Ethanolic extract of *A. senegalensis* leaf was used for this study. Thirty (30) rats consisting of males and females were randomly divided into 6 groups of five rats each in separate cages. The

rats were marked and housed individually and kept for 7 days in the laboratory to allow them acclimatize. The rats were fed with standard feed (Vital feed, Nigeria) and water *ad libitum*. Group 1 rats served as the control and distilled water was given orally. Groups 2 - 6 rats were treated orally with varying doses of 100, 200, 400, 800, 1600 and 3200 mg/kg respectively of the aqueous extract once. The rats were observed within 24 hours for signs of toxicity and mortality. Dead rats were posted and histopathology was carried out to determine any pathological changes. The median lethal dose (LD_{50}) was calculated using the arithmetic method of Karbar (1931) as modified by Aliu and Nwude (1982).

Effects of Prolonged Administration of Ethanolic Leaf Extract of *Annona senegalensis* in Albino Rats

One hundred (100) rats were randomly divided into five groups of 20 rats each (Groups 1, 2, 3, 4 and 5). Groups 2, 3, 4 and 5 were treated orally with graded doses of 100, 200, 300 and 400 mg/kg of the extract daily for 28 days. Feed and water were given *ad libitum*. Group 1 received normal feed and water only, for the duration of 28 days. The initial body weights of the rats were taken before the extract was administered and thereafter on weekly basis during the treatment period. Blood samples were collected by humanely sacrificing the rats every week before the extract was given. The samples were used for the determination of white blood cell (WBC), red blood cell (RBC), packed cell volume (PCV), haemoglobin (Hb), Platelet count and differential leucocytes count (DLC) (Schalm *et al*., 1975).

Total White Blood Cell (WBC) Count Determination:

The method described by Coles (1986) was used for the test which is similar to that of the RBC except that blood from the tail vein of the albino rat was drawn to 0.5 mark of the WBC pipette and then filled to the 11 mark of the pipette with Turk's solution (WBC diluting fluid) giving a dilution of 1:20. The count was obtained by counting the cells in the four (4) corner squares of the filled counting chamber and the result obtained was multiplied by 1000 to give the total number of cells counted in thousand per cubic millimeter (x $10^3/mm^3$).

Red Blood Cells (RBC) Count Determination:

Improved Neubauer's haemocytometer was used in the determination of the red blood cells count as described by Coles (1986). The tail of the rat was cleaned with cotton wool soaked in methylated spirit, and the tail was lanced and blood from the tail vein was drawn to a 0.5 mark into the RBC diluting pipette. Cotton wool was used to wipe the tip of the pipette so as to remove the blood from the tip, and then Hyem's diluting fluid was drawn into the same pipette containing blood upto the 0.5 mark until the mixture reached the 101 mark where a dilution of 1:200 was obtained. This mixture was rolled gently and thoroughly in between the index finger and the thumb for about two (2) minutes. The counting chamber of the improved Neubauer's slide was cleaned and a clean cover slip was placed thereof. Some few drops were discarded from the tip of the pipette before it was brought into contact with the haemocytometer counting chamber and 1-2 drops of the fluid

were dropped under the cover slip to fill it. This slide (charged slide) was allowed to stand for about two (2) minutes and then the cells in the five (5) of the twenty five (25) tertiary squares were counted using x 40 objective of the light microscope. The counted red blood cells was multiplied by ten thousand (10, 000) to give the number of red blood cells in million per cubic millimeter (x $10^6/mm^3$).

Packed Cell Volume (PCV) Determination:
The microhaematocrit method was used as described by Coles (1986) for the determination of the PCV. The tail of the rat was lanced and blood from the tail vein was drawn into the heparinised capillary tube via capillary action to about three quarter filled. The end of the capillary tube which had contact with the blood was sealed using plasticine and then centrifuged at 5000 revolution per minute (rpm) for 5 minutes. Microhaematocrit reader (Hawslley, England) was used to read the PCV and it was expressed in percentage (%).

Haemoglobin Concentration (Hb) Determination
The measurement of haemoglobin concentration was done colorimetrically using cyanomethaemoglobin method (Coles, 1986). Drabskin's solution was used. Five milliliter (5ml) of Drabskin's solution was dispensed into a test tube and to it was added 0.2ml of blood via pipette and shaken vigorously to form oxyhaemoglobin and then allowed to stand for 3 minutes for colour development. The colorimeter machine was put on and allowed for 10 minutes for it to warm up for the task ahead. The mixture in the test tube was dispensed into a colorimeter cuvette and then placed in the colorimeter to determine its optical density using a filter of 520 nm wavelength. The control test was performed using the same procedure without blood. The concentration of haemoglobin was calculated using the formula below:

$$\text{Hb (g/dl)} = \frac{\text{O.D test}}{\text{O.D standard}} \times \text{concentration of standard} \times \frac{250}{1000}$$

Where, O.D = optical density

Platelet Count
The platelet count was determined using improved Neubauer's haemocytometer as described by Coles (1986). Blood from the tail vein of the rats was drawn into the RBC diluting pipette to exactly 1.0 mark. The tip of the pipette was wiped free of blood. Ammonium oxalate solution was thereafter drawn into the same pipette until the mixture reached the 101 mark to obtain a dilution of 1:100. The solutions were then mixed thoroughly by rolling the pipette between the index finger and the thumb for about 2 minutes. The counting chamber of the improved Neubauer's haemocytometer was cleaned and a clean cover slip placed over it. The first few drops of the mixture from the pipette were discarded. Then, the tip of the pipette was brought in contact with the exposed edge of the haemocytometer counting chamber and then 1-2 drops of the fluid allowed

to flow under the cover slip to fill it. The charged slide was allowed to settle for about 2 minutes and the cells in the five (5) of the twenty-five (25) tertiary squares were counted using x 40 objective of the light microscope. The number of platelets counted was then multiplied by ten thousand to give the number of platelets in billions per liter (x 10^9/L).

Differential Leucocyte Count (DLC) Determination:
The method described by Schalm *et al*. (1975) was used for the differential leucocyte count (DLC). A slide was prepared by placing a drop of blood at one end of grease-free clean slide and a thin blood smear was made using a cover slide and allowed to dry by shaking it vigorously. The slide was then irrigated with one milliliter of Giemsa stain and allowed to stand for 2 minutes to fix it, and to it was gently added 2 milliliters of buffered distilled water (pH 6.8). This slide was allowed to stand for 10 minutes after which it was washed with distilled water and air-dried. The slide was examined under x 100 objective using immersion oil. The white blood cells were counted symmetrically by starting in the body of the smear; moving from left to right proceeding downward until 100 cells were counted and classified. The percentage of the white blood cells (lymphocytes, monocytes, eosinophils, basophils and neutrophils) identified together with the total white blood cell count above, was used to get the number of each kind of cell per litre of blood (X 10^9/L).

RESULTS

Phytochemical Analysis of Ethanolic Extract of *Annona senegalensis* Leaves
The phytochemical analysis of the aqueous extract of *Annona senegalensis* leaf revealed the presence of chemical constituents such as carbohydrates, cardiac glycosides, terpenoids and flavonoids as presented in Table 4.1.

Table 1: Qualitative phytochemical components of the ethanolic leaf extract of *Annona senegalensis.*

Components	Inference
Carbohydrates	+
Soluble starch	-
Anthraquinone s	-
Cardiac glycoside	+
Terpenoids	+
Flavonoids	+
Tannins	-
Phlobatanins	-
Saponins	-
Alkaloids	-

Key:
- = absent
+ = present

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Published by European Centre for Research Training and Development-UK

Elemental Screening of Ethanolic Extract of *Annona senegalensis* Leaves

The result of the elemental screening of the aqueous extract of *Annona senegalensis* leaves is shown in Table 4.2. The extract contains the following elements iron (Fe), copper (Cu), magnesium (Mg), lead (Pb), chromium (Cr), cadmium (Cd), cobalt (Co), zinc (Zn) and manganese (Mn) in parts per million concentrations and their values are 7.202, 0.893, 6.637, 0.014, 0.070, 0.057, 0.604, 1.732 and 2.362 respectively.

Table 2: Elemental concentration of ethanolic leaf extract of the *Annona senegalensis*.

Elements	Concentration (mg/kg)	WHO (1996) Standard (mg/kg)
Iron (Fe)	7.202	0.5 – 50
Copper (Cu)	0.893	1 – 3
Magnessium (Mg)	6.637	10 – 20
Lead (Pb)	0.014	-
Cromium (Cr)	0.070	-
Cadmium (Cd)	0.057	10 – 35
Cobalt (Co)	0.604	-
Zinc (Zn)	1.732	15 – 20
Manganese (Mn)	2.362	-

Keys:
mg/kg =Milligram per Kilogram
- = Not present

Acute Toxicity Study (LD_{50})

Mortality was not observed in any of the doses except at 3200mg/kg which produced 100% death of the animals. The calculated LD_{50} is 2400 mg/kg. There was slight sedation, dyspnea, reduced appetite, coma and eventually death in group six (3200 mg/kg).

Effects of ethanolic leaf extract of *Annona senegalensis* on some haematological parameters in albino rats

The effect of ethanolic leaf extract on WBC in albino rats. There were significant decreases of WBC in all the groups compared to the control in week 1. The mean values in week 1 are in the doses of 100, 200, 300 and 400 mg/kg which have **14.1**±12.20^{c}, 9.5±13.10^{c} , 10.2±15.40^{c} and 9.3±11.20^{c} respectively. There were significant increases in week 2 in all the treatment groups as compared to the control. The mean values in the doses of 100, 200, 300 and 400 mgkg are 15.4±10.90^{b}, 19.8±17.20^{b}, 12.2±11.70^{b} and 14.5±17.40^{b} respectively in week 2. In week 3 there were significant decreases in all the treatment groups as compared to the control. The mean values in the doses of 100, 200, 300 and 400 mg/kg are 7.5±13.50, 12.0±15.70^{c}, 10.9±13.90^{c} and 13.8±9.90^{c} respectively in week 3. In week 4, there were significant decreases in all the groups except in group 2 no significance. The mean values in week 4 in the doses of 100, 200, 300 and 400 mg/kg are 13.93±3.36, 10.81±2.52^{c}, 11.88±1.96^{c} and 12.45±2.83^{c} respectively.

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Table 3: Effects of ethanolic leaf extract of *Annona senegalensis* on mean* white blood cell (WBC) count in albino rats.

Extract dose (mg/kg)	Weeks of Aqueous Extract administration			
	1	2	3	4
WBC (X 10^3/mm^3)				
Control(0)	**19.6**±13.60[a]	10.1±6.40[a]	14.6±10.60[a]	13.15±2.39[a]
100	**14.1**±12.20[c]	15.4±10.90[b]	7.5±13.50[c]	13.93±3.36
200	9.5±13.10[c]	19.8±17.20[b]	12.0±15.70[c]	10.81±2.52[c]
300	10.2±15.40[c]	12.2±11.70[b]	10.9±13.90[c]	11.88±1.96[c]
400	9.3±11.20[c]	14.5±17.40[b]	13.8±9.90[c]	12.45±2.83[c]

Different superscripts in the same column are significantly different at P<0.05.

Key: a = for control groups
b = significantly increased
c = significantly decreased

* = **Mean** ± S.D. based on six observations.

The effect of ethanolic leaf extract on RBC in albino rats. In week 1, there were significant increases in all the groups as compared to the control. The mean values in week 1 in the doses of 100, 200, 300 and 400 mg/kg are 8.55±0.86[b], 7.77±0.23[b], 7.88±0.29[b] and 7.73±0.31[b] respectively. There were significant increases in all the groups except in group 2 that has no change in week 2. The mean values in week 2 in the doses 100, 200, 300 and 400 mg/kg are 7.21±0.25[c], 7.85±0.83, 7.26±0.75 and 7.03±0.98 respectively. In week 3, there were significant decreases in groups 2 and 3 while there were significant increases in groups 4 and 5 as compared to the control. The mean values in week 3 in the doses of 100, 200, 300 and 400 mg/kg are 5.92±0.38[b], 5.77±0.81[b], 7.60±0.75[b] and 7.61±0.42[b] respectively. In week 4, there were decreases in all the groups as compared to the control. The mean values in week 4 in the doses of 100, 200, 300 and 400 mg/kg are 7.12±0.49[b], 6.87±0.37[b], 6.65±0.58[b] and 7.11±0.65[b] respectively.

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Table 4: Effects of ethanolic leaf extract of *Annona senegalensis* on mean* red blood cells count (RBC) in albino rats treated orally for 28 days with the extract.

Extract dose (Mg/Kg)	Weeks of Aqueous Extract administration			
1	2	3	4	
RBC (X $10^6 mm^3$)				
Control(0)	6.97±0.63[a]	6.80±0.34[a]	6.70±0.06[a]	7.79±0.52[a]
100	8.55±0.86[b]	7.21±0.25[b]	5.92±0.38[c]	7.12±0.49[c]
200	7.77±0.23[b]	7.85±0.83[b]	5.77±0.81[c]	6.87±0.37[c]
300	7.88±0.29[b]	7.26±0.75[b]	7.60±0.75[b]	6.65±0.58[c]
400	7.73±0.31[b]	7.03±0.98[b]	7.61±0.42[b]	7.11±0.65[c]

Different superscripts in the same column are significantly different at P<0.05

Key: a = for control groups
b = significantly increased
c = significantly decreased
* = **Mean** ± S.D. based on six observations.

The effect of ethanolic leaf extract on PCV in albino rats. In week 1, there were significant increases in all the treatment groups as compared to the control. The mean values in the doses of 100, 200, 300 and 400 mg/kg are 44.40±1.35, 45.00±2.01[b], 46.30±1.56[b] and 46.50±2.04[b] respectively. In week 2, there were slight decreases in groups 4 and increases in groups 3 and 5 as compared to the control. The mean values in the doses of 100, 200, 300 and 400 mg/kg are 43.80±1.33[b], 44.56±2.46[b], 42.34±1.26[b] and 46.30±3.14[b] respectively. In week 3, there were significant increases in all the treatment groups as compared to the control. The mean values in the doses of 100, 200, 300 and 400 mg/kg are 45.23±2.00, 45.40±1.65, 46.43±2.04 and 46.56±3.24 respectively. In week 4, there were decreases in all the groups as compared to the control. The mean values in the doses of 100, 200, 300 and 400 mg/kg are 41.01±2.83, 39.70±1.65, 39.00±3.23 and 40.76±3.20 respectively.

Table 5: Effects of ethanolic leaf extract of *Annona senegalensis* on mean* pack cell volume (PCV) in albino rats

Extract dose (Mg/Kg)	Weeks of Aqueous Extract administration			
1	2	3	4	
PCV(%)				
Control(0)	42.30±2.14[a]	43.24±1.69[a]	44.24±2.02[a]	43.32±3.17[a]
100	44.40±1.35[b]	43.80±1.33	45.23±2.00[b]	41.01±2.83[c]
200	45.00±2.01[b]	44.56±2.46[b]	45.40±1.65[b]	39.70±1.65[c]
300	46.30±1.56[b]	42.34±1.26[c]	46.43±2.04[b]	39.00±3.23[c]
400	46.50±2.04[b]	46.30±3.14[b]	46.56±3.24[b]	40.76±3.20[c]

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Key: a = for control groups
b = significantly increased
c = significantly decreased
* = **Mean** ± S.D. based on six observations.

The effect of ethanolic leaf extract on haemoglobin in albino rats. In week 1, there were increases in groups 2, 4 and 5 and decrease in group 3 as compared to the control. The mean values in the doses of 100, 200, 300 and 400 mg/kg are 14.29±2.14, 12.41±1.82^{b}, 15.50±2.03 and 14.43±2.40 respectively. In week 2, there were decreases in groups 2 and 3 and increases in groups 4 and 5 as compared to the control. The mean values in the doses of 100, 200, 300 and 400 mg/kg are 12.43±2.32, 13.61±1.80, 15.23±1.90 and 15.52±2.10 respectively. In week 3, there were significant increases in groups 4 and 5 as compared to the control. The mean values in the doses of 100, 200, 300 and 400 mg/kg are 15.32±2.14, 15.41±2.50, 16.03±2.53 and 16.42±2.50 respectively. There were increases in groups 2 and 4 as compared to the control in week 4. The mean values in the doses of 19, 200, 300 and 400 mg/kg are 17.29±2.14^{b}, 14.40±1.82, 17.50±2.03^{b} and 16.43±2.40^{b} respectively.

Table 6: Effects of ethanolic leaf extract of *Annona senegalensis* on mean* haemoglobin concentration in albino rats.

Extract dose (Mg/Kg)	Weeks of Aqueous Extract administration			
1 Hb (g/dl)	**2**	**3**	**4**	
Control(0)	**13**.52±2.38^{a}	14.54±1.34^{a}	15.02±1.81^{a}	16.52±2.38^{a}
100	14.29±2.14^{b}	12.43±2.32^{c}	15.32±2.14	17.29±2.14^{b}
200	12.41±1.82^{c}	13.61±1.80^{c}	15.41±2.50	14.40±1.82
300	15.50±2.03^{b}	15.23±1.90^{b}	16.03±2.53^{b}	17.50±2.03^{b}
400	14.43±2.40^{b}	15.52±2.10^{b}	16.42±2.50^{b}	16.43±2.40

Different superscripts in the same column are significantly different at P<0.05.
Key: a = for control groups
b = significantly increased
* = **Mean** ± S.D. based on six observations.

The effect of ethanolic leaf extract on Platelet in albino rats. There were significant increases in groups 2, 4 and 5 and slight decrease in group 3 as compared to the control. The mean values in the doses of 100, 200, 300 and 400 mg/kg are **473.37**±44.50^{b}, 355.47±42.80^{b}, 352.35±51.99^{b} and 390.20±49.74^{b} respectively. In week 2, there were increases in groups 2, 3, 4 and 5 as compared to the control. The mean values in the doses of 100, 200, 300 and 400 mg/kg are 655.45±46.90^{b}, 808.70±67.70^{b}, 575.26±59.52^{b} and 764.22±63.32^{b} respectively. In week 3, there were slight increase in groups 2 and 3 as compared to the control. The mean values in the doses of 100, 200, 300 and 400

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mg/kg are 561.51±58.30, 632.50±51.83[b], 620.40±50.61[b] and 506.54±58.83[b] respectively. In week 4, there were increases in all the treatment groups as compared to the control. The mean values in the doses of 100, 200, 300 and 400 mg/kg are 614.00±101.63[b], 585.05±67.52[b], 577.50±46.20[b] and 548.34±102.27[b] respectively.

Table 7: Effects of ethanolic leaf extract of *Annona senegalensis* on mean* platelet counts in albino rats.

Extract dose (mg/kg)	Weeks of Aqueous Extract administration			
1	2	3	4	
Platelet (X 10^9/L)				
Control(0)	**415.32**±37.30[a]	536.36±51.61[a]	653.53±48.30[a]	418.75±44.32[a]
100	**473.37**±44.50[b]	655.45±46.90[b]	561.51±58.30[c]	614.00±101.63[b]
200	355.47±42.80[c]	808.70±67.70[b]	632.50±51.83[c]	585.05±67.52[b]
300	352.35±51.99[c]	575.26±59.52[b]	620.40±50.61[c]	577.50±46.20[b]
400	390.20±49.74[c]	764.22±63.32[b]	506.54±58.83[c]	548.34±102.27[b]

Different superscripts in the same column are significantly different at P<0.05.
Key: a = for control groups
b = significantly increased
* = **mean** ± S.D. based on six observations.

Effect of prolong administration of eyhanolic leaf extract of *A. senegalensis*on differential leucocyte counts in albino rats

The effect of prolonged administration of aqueous leaf extract of *A. senegalensis*on differential leucocyte counts in albino rats are presented in Table 4.12. There was no significant difference in the mean values of basophils in all the treatment groups throughout the period of the experiment. There was no significant difference in the mean values of eosinophils in week 1 in all the treatment groups as compared to the control. Whereas in weeks 2, 3 and 4, there were significant increases in the mean values in all the treatment groups as compared to the control. The mean values in week 2 in the doses of 100, 200, 300 and 400 mg/kg are 8.20±1.10, 7.40±0.89, 7.60±1.34 and 11.60±1.82 X 10^9/L respectively. The mean values in week 3 in the doses of 100, 200, 300 and 400 mg/kg are 9.20±1.48, 10.20±0.84, 12.60±1.14 and 12.40±1.14 X 10^9/L respectively. The mean values in the doses of 100, 200, 300 and 400 mg/kg in week 4 are 9.40±0.40, 11.20±1.30, 11.60±1.34 and 12.00±1.23 X 10^9/L respectively.

British Journal of Multidisciplinary and Advanced Studies:
Agriculture, 4(2),81-100, 2023
Print ISSN: 2517-276X
Online ISSN: 2517-2778
Website: https://bjmas.org/index.php/bjmas/index
Published by European Centre for Research Training and Development-UK

The mean values of lymphocytes in week 1 were not statistically significant in all the treatment groups as compared to the control. Whereas in week 2, the mean values significantly decreased in the extract doses of 100, 200, 300 and 400 mg/kg to 51.20±3.11, 49.80±2.17, 50.20±2.59 and 50.80±2.68 X 10^9/L respectively as compared to the control. In week 3, there was significant decrease at 400 mg/kg (48.40±2.70 X 10^9/L) while there were no significant change in the rest of the treatment groups as compared to the control. No significant changes were observed in the mean values of lymphocytes in week 4 at 100 and 200 mg/kg but the values significantly ($P<0.05$) decreased at 300 mg/kg (49.00±3.24 X 10^9/L) and 400 mg/kg (49.60±1.82 X 10^9/L) as compared to the control.

The mean values of monocytes in all the treatment groups significantly ($P<0.05$) increased with all the doses in week 1 and week 2 compared to the control. At weeks 3 and 4 the extract doses of 300 and 400 mg/kg were also significantly increased while the doses of 100 and 200 mg/kg did not produce any significant change as compared to the control.

There was significant ($P<0.05$) decrease in the mean values of neutrophils in all the treated groups in weeks 1, 3 and 4, while in week 2 there was significant decrease at 400 mg/kg dose only and in the other treated groups no significant difference was observed when compared to the control. The mean values in week 1 in the doses of 100, 200, 300 and 400 mg/kg are 31.00±1.23, 31.80±1.48, 32.20±1.48 and 32.00±1.58 X 10^9/L respectively. The mean values of week 3 in the doses of 100, 200, 300 and 400 mg/kg are 31.80±1.64, 28.80±1.79, 26.40±1.34 and 29.20±1.48 X 10^9/L respectively. The mean values in the doses of 100, 200, 300 and 400 mg/kg at week 4 are 29.80±2.17, 27.40±1.82, 27.80±1.92 and 25.80±1.92 X 10^9/L respectively.

British Journal of Multidisciplinary and Advanced Studies:
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Published by European Centre for Research Training and Development-UK

Table 8: Effects of Ethanolic leaf extract of *Annona senegalensis* on differential leucocyte counts (DLC) of albino rats (X 10^9/L)

Parameters (mg/kg)	Dose	Period of Administration (weeks) 1	2	3	4
Basophils	control(0)	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	100	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	200	0.40±0.55	0.40±0.55	0.20±0.45	0.00±0.00
	300	0.20±0.45	0.00±0.00	0.40±0.55	0.00±0.00
	400	0.20±0.45	0.20±0.45	0.60±0.89	0.40±0.55
Eosinophils	control(0)	5.00±0.71	5.40±0.55[a]	6.20±1.30[a]	5.20±0.84[a]
	100	6.00±1.00	8.20±1.10[b]	9.20±1.48[b]	9.40±0.40[b]
	200	6.60±0.89	7.40±0.89[b]	10.20±0.84[b]	11.20±1.30[b]
	300	5.60±0.89	7.60±1.34[b]	12.60±1.14[b]	11.60±1.34[b]
	400	6.40±1.14	11.60±1.82[b]	12.40±1.14[b]	12.00±1.23[b]
Lymphocytes	control(0)	54.60±2.19	55.00±1.87[a]	52.40±2.70[a]	53.80±1.79[a]
	100	54.60±2.07	51.20±3.11[c]	53.20±2.78	53.80±2.49
	200	52.00±1.73	49.80±2.17[c]	55.60±1.14	54.20±1.92
	300	53.60±2.30	50.20±2.59[c]	53.00±2.55	49.00±3.24[c]
	400	54.40±1.50	50.80±2.68[c]	48.40±2.70[c]	49.60±1.82[c]
Monocytes	control(0)	5.40±0.55[a]	5.40±1.14[a]	5.60±0.89[a]	6.00±0.71[a]
	100	8.80±0.84[b]	7.00±1.23[b]	5.80±1.10	7.00±0.32
	200	8.40±1.14[b]	7.80±0.84[b]	5.40±1.14	7.20±1.30
	300	8.40±0.89[b]	8.20±1.30[b]	7.60±1.34[b]	11.80±1.64[b]
	400	7.20±0.84[b]	7.80±1.30[b]	9.40±0.55[b]	12.20±1.92[b]
Neutrophil	control(0)	35.80±1.64[a]	34.20±1.30[a]	35.80±1.30[a]	35.00±1.00[a]
	100	31.00±1.23[c]	33.20±1.48	31.80±1.64[c]	29.80±2.17[c]
	200	31.80±1.48[c]	34.60±1.14	28.80±1.79[c]	27.40±1.82[c]
	300	32.20±1.48[c]	34.00±2.45	26.40±1.34[c]	27.80±1.92[c]
	400	32.00±1.58[c]	29.60±1.67[c]	29.20±1.48[c]	25.80±1.92[c]

Different superscripts in the same column are significantly different at P<0.05.

Key: a = for control groups
b = significantly increased
c = significantly decreased

DISCUSSION AND CONCLUSION

The aqueous leaf extract of *Annona senegalensis* phytochemical screening revealed the presence of carbohydrates, cardiac glycosides, terpenoids and flavonoids whereas soluble starch, anthraquinone, tannins, phlobotannins, saponins and alkaloids were not present. The presence of carbohydrates could be the reason for the significant increase in the mean values of glucose in treated rats. Carbohydrates metabolism yield glucose. The digestion is completed in the small

intestine to the end product, glucose, which is then absorbed into the portal circulation from where it passes to the liver (Nduka, 1999).

Cardiac glycosides have been utilized in the treatment of congestive heart failure, constipation, oedema and microbial infections (Frantisek, 1991). Terpenoids have antimicrobial, antitumour and antifungal activities (Singh and Singh, 2003). They might have given support in enhanced WBC count which is important in fighting infection.

Flavonoids are naturally occurring phenolic compounds in plants. Flavonoids have been shown to possess many pharmacological properties such as: anti-oxidant activities, anti-inflammatory activities, anti- cancer activities and anti- microbial effects hence, flavonoids may have a contributory effect to its fertility properties and other pharmacological effects the plant possesses (Joy and Kuttan, 1998; Kassuya *et al*., 2003; Adeneye*et al*., 2006). Favonoids as an anti-oxidant, has a rejuvenating effects on cells or tissues, it is anti-aging hence can contribute substantially to the fertility effect of this plant (Nita bishop, 2003).

The disease fighting potential of flavonoids stem from their ability to reduce inflammation by preventing the release of histamine (which causes allergic symptoms such as congestion). Flavonoids antagonise free radicals, boost immunity, strengthen blood vessels, and increase blood flow, among other actions. Flavonoids are involved in, gene expression, capillary and cerebral blood flow, platelet aggregation, liver function, enzyme activity and collagen, phospholipid and histamine metabolism (Nita bishop, 2003).They decrease capillary fragility and are therefore employed in case of hypertension and radiation injuries (Nita Bishop, 2003).Hesperidin is regarded as the most important flavone in oranges and has been reported to lower high blood pressure as well as cholesterol in animal studies and also possess strong anti – inflammatory properties (CSIRO, 2004).

The presence of these phytochemicals in the plant as oberved in this study might be the reason for its therapeutic uses by the natives.

Some of the elements found in the extract in this study include: Iron (Fe), copper (Cu), magnesium (Mg), lead (Pb), chromium (Cr), cadmium (Cd), cobalt (Co), zinc (Zn) and manganese (Mn). Their presence in this plant may be responsible for the many therapeutic activities of this plant as claimed by the natives (Shirin *et al*., 2010).

Some of the elements found in the extract are all within the normal range of the WHO standard safety limits (WHO, 1996). Elements such as Pb and Cd are so small in quantity which makes their absorption negligible or impossible for them to produce toxicity (Lalman and McMurphy, 2002). Iron (Fe) is an essential component in the structure of proteins involved in transportation and utilization of oxygen namely haemoglobin, myoglobin and cytochromes. It is also involved in

electron transport chain. Its deficiency includes anaemia, anorexia, reduced growth and increased weight loss (Lalman and McMurphy, 2002). The increased mean values of haematological parameters in this study could be due to the presence of Fe in the extract.

Copper (Cu) is an important cofactor in many enzymes especially those involved in haemoglobin formation, Fe absorption and mobilization, connective tissue metabolism and immune function(Lalman and McMurphy, 2002). The significant increase in the mean values of Hb and WBC could also be attributed to the presence of Cu in the extract.
Magnessium (Mg) is known to activate many different enzymes which is essential in energy metabolism, transmission of genetic code, membrane transport and nerve impulse transmission (Lalman and McMurphy, 2002). This might have contributed in the significant increase in the mean values of glucose.

Cromium (Cr) is implicated in glucose metabolism behaving like insulin. Its presence may be beneficial to diabetic patients(Lalman and McMurphy, 2002).
Cobalt's (Co) primary role is as a building block for vitamin B12 which is manufactured in the rumen. Its deficiency includes reduced appetite and reduced disease resistance (Lalman and McMurphy, 2002). Its presence might have helped in enhancing the immune potential of the extract.

Zinc (Zn) has been reported to have beneficial effects on atherosclerotic patients. Subnormal plasma Zn level has been reported in patients with atherosclerosis (Shirin *et al*., 2010). The presence of Zn and Cu in the plant may be correlated with its anticancer property, as both elements are required in growth and proliferation of normal cells. Zinc concentration decreases in cancer patients whereas Cu concentration increases (Shirin *et al*., 2010). It is believed that low concentration of plasma Zn in cancer patients is due to the increased requirement of Zn by cancer tissues (Shirin *et al*., 2010). This seems to be reasonable because of the fact that tumor cells have high rate of DNA synthesis and most of the enzymes involved in the nucleic acid synthesis are Zn dependent (Shirin *et al*., 2010). Deficiencies of these elements may cause different diseases.

Manganese (Mn) is important in bone growth and formation in young animals and in maintaining optimum fertility in female cattle. Its role in metabolism include its serving as a component of the enzymes pyruvate carboxylase, arginase and superoxide dismutase. Its deficiency includes skeletal abnormalities, low reproductive performance, abortions, still births and low birth weight (Lalman and McMurphy, 2002). Its presence in this extract might have not been sufficient enough to maintain the pregnant rats or its absorption was interfered with in the process of administration of the extract leading to its deficiency, thus causing the abortion observed in the female study.

In this study, 3200 mg/kg was the dose that produced100% mortality. The calculated LD_{50} of the aqueous leaf extract of *A. senegalensis* administered orally was 2400 mg/kg (Aliu and Nwude,

1982).The rats treated with 100, 200, 400, 800 and 1600 mg/kg doses produced no mortality, suggesting that the plant could be safe at those levels. It may therefore be considered nontoxic; although this does not predict the lethal dose in humans or other animals, it however provides a guide for choosing the dose for use in sub-chronic studies.Generally, the smaller the LD_{50} value, the more toxic the substance is and vice versa. These doses 100, 200, 400, 800 and 1600 mg/kg considered to be non harmful because the higher the LD_{50} the less toxic and the smaller the LD_{50} the more toxic the substance. This agrees with the report of Organization for Economic Cooperation and Development (OECD, 1998) which classifies: very toxic as<5 mg/kg, toxic as>5<50 mg/kg, harmful as>50 < 500 mg/kg and no label as>500 < 2000 mg/kg. On the other hand, the report of the Environmental Protection Agency (EPA) [OECD, 1998], United States classifies toxicity as follows: very toxic as ≤ 50 mg/kg, toxic as>50 ≤500 mg/kg, harmful as> 500 - 5000 mg/kg and no label as> 5000 mg/kg. From the above, every substance has to be handled with care. This extract is nontoxic based on OECD but for EPA (USA) it is harmful (OECD, 1998). Assessment of haematological parameters can be used to determine the extent of deleterious effect of foreign compounds, including plant extracts on the blood. The significant ($P < 0.05$) increase in the mean values of red blood cell count (RBC), packed cell volume (PCV) and haemoglobin concentration (Hb)at different dosages of *A. senegalensis* ethanolic leaf extract are used in this study implies that the plant extract has potential haematological effects. Its effects on RBC, PCV and Hb imply that there were increases in the oxygen carrying capacity of the blood and the amount of oxygen delivered to the tissue, since RBC is very important in transferring respiratory gases (De Gruchy, 1976). This suggests that the extracts have the potential to stimulate erythropoietin release in the kidney which is the humoral regulator of RBC production (Polenakovic and Sikole, 1996; Sanchez-elsner *et al*., 2004).Thus it can also be deduced that *A. senegalensis* has a stimulatory effect on the bone marrow, which is responsible for production of red blood cells and white blood cells (Omodamiro and Nwankwo, 2013). The increases in RBC, PCV and Hb showed that *A. senegalensis* can be useful in the treatment of anaemia and other blood disorders.

The mean values of the total WBC count exhibited a dose dependent increase throughout the 28 days (4 weeks) of the treatment period. This might suggest possible immune modulatory effects of the extract through their dynamic regulation of information molecules such as cytokines. This offers an explanation for the effect of the extract on the immune system which might be due to the presence of flavonoids in the extract. Flavonoids boost immunity. Since *A. senegalensis* increased the amount of white blood cells, it may be useful in the treatment of infections (Spellman *et al*., 2006; Anjana *et al*., 2010; Omodamiro and Nwankwo, 2013).

Platelets are an essential part of blood coagulating mechanism. They act as plugs around the opening of a wound and release certain factors that are necessary for the formation of a blood clot to prevent blood loss. The platelets also maintain the integrity of the blood vessels by plugging the gaps in the endothelial lining (Ochei and Kolhatkar, 2007). In this study, the increase in the platelet counts suggest that the extract has the properties of blood clotting and maintaining the integrity of

blood vessels. Also the increased platelete counts could be due to the presence of flavonoids in the extract since flavonoidsare involved in platelete aggregation (Nita-Bishop, 2003).

The extract elicited a boost in the white blood cells. White blood cells (leukocytes) are involved in fighting infection and clearing off damaged or dead cells and tissues in the body (Jeremy *et al*., 2001). However, excessive count of white blood cells (WBC) are implicated in uraemia, inflammation and tissue necrosis (Vasudevan and Sreekumari, 2000).The significant ($P<0.05$) increase in WBC and significant decrease in lymphocyte counts (lymphocytopaenia) in weeks 2 and 4 and week 3 at 400 mg/kg BW dose requires careful examination, as the increase may be due to uraemia, inflammation and tissue necrosis. Reduction in the blood level of neutrophils (neutropaenia) at all the doses and the weeks of treatment may indicate tissue damage, anaphylaxis and splenomegaly and in treatment with certain drugs (Topley, 1998; Ochei and Kolhatkar, 2007). Eosinophils play an important role in the defense mechanism of the body against parasites. During parasite infection and allergic condition, it is known to increase. Eosinophils are responsible for detoxification, disintegration and removal of foreign bodies by secretion of lethal substances like cytokines. The significant increase in eosinophil level at all the doses in weeks 2, 3 and 4 may mean that the animals were probably predisposed to tissue damage and anaphylaxis due to the presence of flavonoids in the extract (Topley, 1998; Ochei and Kolhatkar, 2007; Sembulingam and Sembulingam, 2012).

The blood basophil levels observed in this study, did not produce any statistically significant change. The increase observed in the blood monoctye level in all the groups treated suggest support and immune boosting capabilities of the ethanolic extract of *A. senegalensis* due to the presence of flavonoids.This is because, monocytes play important role in the body defense mechanism. Along with neutrophils, they provide the first line of defense. Monocytes are phagocytic; they also secrete interleukin-1 that make them effective macrophages in tissues (Tracey and Cerami, 1994; Sembulingam and Sembulingam, 2012).

In conclusion, the ethanolic leaf extract has some bioactive compounds such as carbohydrates, cardiac glycosides, terpenoids and flavonoids. The extract also has some elements which include iron (Fe), copper (Cu), magnesium (Mg), lead (Pb), chromium (Cr), cadmium (Cd), cobalt (Co), zinc (Zn) and manganese (Mn). The ethanolic leaf extract is found to be tolerable and an increase in the haematological parameters in the treated albino rats, thus, has a good immune boosting and treatment of anaemia potentials.

Acknowledgments
I wish to sincerely thank our prestigious university, Modibbo Adama University Yola through which the TETFund Institutional Base Research (IBR) grant was achieved. A big thank you also to my team in the persons of Dr. A.G. Bobbo, Dr. A.A. Barkindo and Mal. Ahmed Ibrahim Hayatu for the technical assistance.

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