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# Haematological and Biochemical Changes Observed Following Sub-chronic Administration of Crude Methanol Extracts of *Stellaria media* and *Cajanus cajan* to Wistar Rats

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***Authors' contributions***

*This work was carried out in collaboration between all authors. Author BOO designed the study and carried out the experiments. Authors OAO and GOO performed the statistical analysis, wrote the protocol, wrote the first draft of the manuscript and managed the literature searches. Author ABS designed and supervised the study and carried out the final editing of the submitted manuscript. All authors read and approved the final manuscript.*

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## ABSTRACT

**Aims:** *Stellaria media* and *Cajanus cajan* are nutritive plants used as food and as components of several herbal remedies either singly or in combination with other medicinal plants in Southwestern Nigeria. This study aimed to evaluate its effect on haematology and serum biochemistry after a sub-chronic administration to Wistar rats.
**Study Design:** Wistar rats were randomly grouped into four groups and administered methanol extracts of *S. media* at doses of 100 mg/kg, 500 mg/kg and 1000 mg/kg respectively per os. Same grouping and treatment was carried out for methanol extract of *Cajanus cajan*.
**Place and Duration of Study:** Department of Animal Science, Obafemi Awolowo University, Ile-Ife, Nigeria between June and October, 2010.

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**Methodology:** The study evaluated the effect of the extract on haematology and serum biochemistry of rats administered with the extract by assessing changes in PCV, MCV, WBC and differential counts while ALT, AST, total protein, albumin, serum bilirubin and creatinine levels were used to assess effects on the liver and kidneys.
**Results:** The study shows no toxic effect in the haematology rather an immunostimulatory effect is seen with both plants. Lymphocyte counts were between 4.6–8.6 $X10^3/mm^3$ for *S. media* and 8.3-11.0 $X10^3/mm^3$ for *C. cajan* compared to the control rats (4.3 $X10^3/mm^3$). A possible hepatotoxicity was observed for *C. cajan* after sub-chronic usage. A non-significant ($P>0.05$) increase in AST levels were observed with significantly ($P<0.05$) decreased protein levels of 3.3-3.8 g/dl compared to control rats (5.9 g/dl).
**Conclusion:** Lower doses of ≤100 mg/kg are recommended and caution should be taken when using *Stellaria media* or *Cajanus cajan* at high doses and or for prolonged administration.

*Keywords: Stellaria media; Cajanus cajan; haematology; serum biochemistry.*

## 1. INTRODUCTION

Since ancient times, crude plant extracts and herbal decoctions have been used as remedy for several disease conditions that afflict man and animals. The wide range of pharmacological actions of medicinal plants such as anti-inflammatory, antibacterial, antianaemic amongst others, has been attributed to the phytocompounds contained in the plants [1]. These phytocompounds mediate the aforementioned effects in the human or animal body by similar, but in most cases identical ways as those understood for chemical components of conventional drugs. Thus, herbs possess the ability of being effective as conventional drugs but this also confers on them the potential to bring about harmful adverse reactions in the body [2]. The aim of this study was to assess the potential toxicity of two medicinal plants; *Stellaria media* and *Cajanus cajan*. These edible and nutritious medicinal plants have been used in folkloric medicine and they have been proven to possess anti-inflammatory and analgesic activities [3,4], amongst other medicinal properties [5,6,7,8].

*S. media* commonly known as chickweed belongs to the family Caryophyllaceae (Pink family). It is called 'Awede' in Yoruba, Southwestern Nigeria. It is a cosmopolitan vegetable that has been used in traditional medicine as a tonic, diuretic, demulscent, expectorant and laxative. It has been utilized externally to relieve itching and inflammation due to it soothing and moisturizing effects. The methanol extract of *S. media* has been shown to possess antileishmanial activities [9]. It has been reported to ease rheumatic pain and used to treat abscesses [10]. Brown [11] reported its use to soothe inflamed skin and to provide relief to swollen and painful haemorrhoids. *S. media* has been reported to contain ascorbic acid, beta carotene, B vitamins, flavonoids, triterpenoids and saponin glycosides [12], while Oyebanji et al. [3] reported its anti-inflammatory and analgesic effects.

*C. cajan* is an important legume in semi-arid tropics and is cultivated in more than 25 tropical and sub tropical countries [13]. It is commonly known as Pigeon pea and is locally called 'Otili/Feregede' in Yoruba. C. cajan is a member of the Fabaceae family. It is often grown as a cover crop or occasionally as a windbreak hedge. Pigeon pea is widely used alone or in combination with other medicinal plants to mitigate various conditions and diseases. Decoction of its leaves is used for treatment of cough, diarrhea, abdominal pain and aphthous stomatitis. It has been used in treating sores, skin irritations, measles and jaundice [14]. Some herbal practitioners maintain that it diminishes swelling of internal organs. In Brazil, it is used as a remedy for fever and ulcers as well as inflammations and blood disorders [15]. Duker-Eshun et al. [16] reported hypoglycaemic, antisickling and anti-plasmodial properties of *C. cajan*, and the anti-nociceptive and anti-inflammatory effect was reported by Oyebanji et al. [4].

The exploration of therapeutic plant extracts is necessary due to incidences of drug resistance and toxic effects of some chemically synthesized drugs. For efficient and safe usage of these medicinal plants, it is necessary to establish their toxicity profile. Haematological and biochemical indices serve as baseline information required for evaluation of the risk or toxicity of a plant extract.

Therefore, this study investigated the toxic potential of *S. media* and *C. cajan* using changes in the haematology and serum biochemistry of Wistar rats administered with the methanol extracts for 30 consecutive days. The phytochemical profile of the plants was also carried out.

## 2. MATERIALS AND METHODS

### 2.1 Plant Collection and Extraction

The leaves and stem of *S. media* and *C. cajan* were procured at the local Bode market, Ibadan in Southwestern Nigeria. Authentication of the plants was carried out at the herbarium of the Department of Botany, Obafemi Awolowo University, Ile-Ife. The plants were air dried and ground to powder using industrial blending machine. The powders were macerated in methanol for 72 hours and the resulting extracts were filtered. The filtrates were concentrated in rotary evaporator at 35°C followed by freeze drying of the extracts. The resultant crude methanol extracts were stored in a dessicator and fresh batches were reconstituted for each experiment.

#### 2.1.1 Phytochemical screening

Phytochemical screening of the crude methanol extracts of *S. media* and *C. cajan* was carried out to determine the presence of phytocompounds according to the method of Trease and Evans [17].

### 2.2 Experimental Animals

Wistar rats weighing 150-170g were utilized for the experiment. They were kept in standard cages in the Animal Health Laboratory of the Department of Animal Sciences, Obafemi Awolowo University, Ile-Ife. The animals were fed with commercial rat diet and water *ad libitum* and acclimatized for two weeks prior to the experiment. Strict animal use and care protocols were observed and the animals were handled humanely.

#### 2.2.1 Acute toxicity assay

Methanol extract of *S. media* and *C. cajan* were reconstituted in distilled water and orally administered to Wistar rats at the dose of 100, 500, 1000, 2000 or 5000 mg/kg. The rats were observed within 24 hours for signs of toxicity and or death.

#### 2.2.2 Sub-chronic toxicity assay

The rats were divided into four groups per extract consisting of one control and three test groups. Group 1 was the control rats for the experiment and administered with distilled water *per os*. The test groups were administered with crude methanol extract of *S. media* at doses of 100 mg/kg, 500 mg/kg and 1000 mg/kg respectively. This grouping pattern was repeated for *C. cajan* extract. All treatments were administered *per os* for 30days. After the experimental period, blood was collected from the retro-orbital sinus into non-heparinized bottles to obtain serum for biochemical assays and also collected in EDTA bottles for haematology.

### 2.3 Statistical Analysis

All data were reported as Mean ± SEM and comparison between groups were done with one way ANOVA (analysis of variance) and values with $p < 0.05$ were considered statistically significant.

## 3. RESULTS AND DISCUSSION

### 3.1 Phytochemical Screening

The phytochemical screening of the methanol extract of *S. media* showed the presence of phlobatannins and saponins while the methanol extract of *C. cajan* possessed tannins, phlobatannins and saponins (Table 1). Determination of phytochemicals present in plants may suggest likely toxicity(ies) which may be caused by the plant and also explain other toxicities observed.

### 3.2 Acute Toxicity Test

No signs of toxicity or mortality were observed in all the rats administered with the extracts at doses of 100, 500, 1000, 2000 or 5000mg/kg in the acute toxicity test for both extracts and no death was recorded.

### 3.3 Sub-chronic Toxicity

#### 3.3.1 Haematology

*3.3.1.1 Stellaria media*

In the sub-chronic toxicity test, red cell indices of rats administered methanol extract of *S. media* showed no anaemia although slight decreases in packed cell volume (PCV) of rats treated with

**Table 1. Phytochemical screening of crude methanol extracts of *Stellaria media* and *Cajanus cajan***

| Phytochemical | *Stellaria media* | *Cajanus cajan* |
|---|---|---|
| Tannins | - | + |
| Flavonoids | - | - |
| Phlobatannins | + | + |
| Cardiac glycosides | - | - |
| Free anthraquinones | - | - |
| Combined anthraquinones | - | - |
| Alkaloids | - | - |
| Saponins | + | + |

100 mg/kg and 1000 mg/kg respectively. A significant increase in MCV of rats given 1000 mg/kg of the extract was also observed. The white cell counts of experimental rats showed leucocytosis in the group dosed with 1000 mg/ kg of the extract (Table 2).

The slight decrease in PCV of rats administered methanol extract of *S. media* for 30 days was comparable to control group. These values, however, were not indicative of clinical anaemia. There was a corresponding increase in MCV but no significant changes in MCH and MCHC values recorded for the group of rats given 1000 mg/kg of the extract when compared with control group. This finding is in agreement with the work of Esenowo et al. [18], who reported increased MCV values in mice treated with methanol extracts of *S. media*. Increases in MCH and MCV are indicative of macrocytic anaemia in which there is reticulocytosis; an initial response when erythropoiesis is stimulated [19]. An increase in WBC, neutrophils and lymphocyte counts indicative of an immunostimulatory property was recorded in rats treated with methanol extract of *S. media* at a dose of 1000 mg/kg. No significant change in eosinophils was observed which is indicative of the maintenance of homeostatic functions. These findings substantiate the use of *S. media* in ameliorating anaemia and its use in management of burns. The possession of tannins and saponins in the plant could be responsible for its antibacterial and antimicrobial effects as saponins possess cholesterol binding properties [20], formation of foams in aqueous solutions and bitterness. Tannins are astringents and quicken the healing of wounds and burns [21].

*3.3.1.2 Cajanus cajan*

Decreased PCV values were recorded for the experimental rats compared to the control group with anaemia evident in the group of rats given 500 mg/kg of the methanol extract of *C. cajan*. A significant ($p<0.05$) decrease in Hb and increased MCV were also observed in rats administered 500 mg/kg of the extract. White blood counts of treated rats showed significant increases at all doses compared to the control group (Table 3).

This finding is in consonance with that of Igene et al. [22] who investigated haematological responses of broiler chickens fed boiled pigeon pea. No significant change in MCHC but increase in MCV was obtained in 500 mg/kg methanol extract of *C. cajan* treated groups. This is suggestive of a responsive anaemia as indicated by macrocytosis of the red blood cells. A marked increase in WBC and differential count (neutrophils and lymphocytes) obtained in treated groups compared to control group corroborates its use in antibacterial infections and disease conditions as it possesses immunostimulating properties with increased antibody production as indicated by proliferation of lymphocytes.

**3.3.2 Biochemistry**

*3.3.2.1 Stellaria media*

Significantly higher values of ALT were recorded in groups administered methanol extract of *S. media* compared to the control group. Rats given extract at 500 mg/kg however had significantly lowered AST values compared to control. Increase in globulin content of extract treated rats compared to control was observed. No significant difference in bilirubin clearance was recorded but a higher amount of serum creatinine was observed in rats given extract at 100 mg/kg (Table 4).

**Table 2. The effect of sub-chronic administration of crude methanol extract of *Stellaria media* on haematology of Wistar rats**

| Group | PCV (%) | RBC ($10^6$/mm$^3$) | Hb (g/dl) | MCHC (g/dl) | MCV (*f*l) | MCH (ρg) | WBC ($10^3$/mm$^3$) | Neut ($10^3$/mm$^3$) | Lymph ($10^3$/mm$^3$) | Eosin ($10^3$/mm$^3$) |
|---|---|---|---|---|---|---|---|---|---|---|
| Control | 42.8±1.51[a] | 13.8±0.4[a] | 15.0 ± 0.4[a] | 35.0±0.4 | 32.1±1.2[a] | 11.4±0.5 | 7.8±2.3[a] | 2.8±1.2[a] | 4.3±0.8[a] | 0.2±0.1 |
| 100 mg/kg | 35.0±2.1[a] | 12.4±9.7[a] | 12.2 ± 0.8[a] | 34.9±0.1 | 29.3±3.4 | 10.2±0.3 | 7.3±1.5[a] | 2.4±5.8[a] | 5.1±5.8[a] | 0.2±1.8 |
| 500 mg/kg | 41.4±1.24 | 13.4±7.6 | 14.5 ± 0.4[a] | 35.0±0.0 | 32.5±3.0 | 11.3±1.0 | 8.4±1.2 | 3.3±1.1 | 4.6±0.7 | 0.3±0.6 |
| 1000 mg/kg | 37.3±2.2[a] | 10.2±9.1 | 12.3 ± 0.7[a] | 33.0±0.1 | 36.0±2.2[a] | 11.7±1.1 | 12.4±1.8[a] | 4.9±1.1[a] | 8.6±4.6[a] | 0.4±0.2 |

*Values with superscript a are statistically (p< 0.05) significantly different compared to control values*

**Table 3. Effect of sub-chronic administration of methanol extract of *Cajanus cajan* on haematology of Wistar rats**

| Group | PCV (%) | RBC ($10^6$/mm$^3$) | Hb (g/dl) | MCHC (g/dl) | MCV (*f*l) | MCH (ρg) | WBC ($10^3$/mm$^3$) | Neut ($10^3$/mm$^3$) | Lymph ($10^3$/mm$^3$) | Eosin ($10^3$/mm$^3$) |
|---|---|---|---|---|---|---|---|---|---|---|
| Control | 42.8±1.5[a] | 13.7±0.4 | 15.0±0.4[a] | 35.0±0.4[a] | 32.0±1.2[a] | 11.4±0.5 | 7.8±2.3[a] | 3.1±1.1[a] | 4.2±0.8[a] | 0.2±0.1[a] |
| 100 mg/kg | 37.2 ±2.2[a] | 10.6±8.0 | 15.6±1.2 | 34.3±0.2 | 35.0±8.0[a] | 11.3±3.4 | 13.4±0.8[a] | 4.6±3.2[a] | 8.5±4.0[a] | 0.3±0.3[a] |
| 500 mg/kg | 25.4±2.9[a] | 9.2 ± 7.0 | 8.8±1.1[a] | 34.4±0.2 | 35.8±4.0[a] | 12.4±1.4 | 15.8±1.8[a] | 4.5±4.3[a] | 11.0±4.9[a] | 0.3±0.8[a] |
| 1000 mg/kg | 29.8±2.2[a] | 9.6±7.2 | 8.9±1.2[a] | 33.1±0.2[a] | 32.3±7.0 | 10.3±2.8 | 11.9±0.9a | 3.3±3.2[a] | 8.3±6.2[a] | 0.1±0.3 |

*Values with superscript a are statistically (p< 0.05) significantly different compared to control values*

**Table 4. Changes in serum biochemistry of Wistar rats administered with methanol extract of *Stellaria media* for 30 days**

| Group | AST (U/L) | ALT (U/L) | T Prot (g/dl) | ALB (g/dl) | Glob (g/dl) | T BIL (mg/dl) | Dir BIL (mg/dl) | Creat (mg/dl) |
|---|---|---|---|---|---|---|---|---|
| Control | 38.8±6.0[a] | 22.5±1.6[a] | 5.1±0.3[a] | 3.7±0.2[a] | 1.4±0.1[a] | 0.5±0.1 | 0.3±0.6 | 0.09±0.1[a] |
| 100 mg/kg | 33.0±9.1 | 27.7±7.0[a] | 4.1±1.0[a] | 2.3±1.0[a] | 1.8±0.1[a] | 0.3±0.1 | 0.3±0.1 | 0.23±0.2[a] |
| 500 mg/kg | 25.8±1.5[a] | 28.5±22.0[a] | 5.9±0.3[a] | 3.9±0.1 | 2.0±0.2[a] | 0.6±0.1 | 0.3±0.6 | 0.12±0.0 |
| 1000 mg/kg | 31.0±8.2 | 25.5±6.0[a] | 5.0±1.4 | 2.9±1.0 | 2.1±0.4[a] | 0.6±0.2 | 0.3±0.5 | 0.11±0.1 |

*Values with superscript a are statistically (p< 0.05) significantly different compared to control values*

**Table 5. Changes in serum biochemistry of Wistar rats administered with methanol extract of *Cajanus cajan* for 30 days**

| Group | AST (U/L) | ALT (U/L) | T Prot (g/dl) | Alb (g/dl) | Glob (g/dl) | T Bil (mg/dl) | Creat (mg/dl) |
|---|---|---|---|---|---|---|---|
| Control | 25.75±1.5[a] | 28.5±22.0[a] | 5.9 ± 0.3[a] | 3.9 ± 0.1[b] | 2.0±0.2 | 0.6 ± 0.1[a] | 0.11 ± 0.04[a] |
| 100 mg/kg | 30.0±7.1[a] | 23.4±5.0[a] | 3.8 ± 0.3[a] | 2.3 ± 1.0[a] | 1.5±0.7 | 1.9 ± 0.2[a] | 0.20 ± 0.9[a] |
| 500 mg/kg | 33.0±0.8[a] | 29.5±7.5 | 3.3 ± 0.3[a] | 1.3 ± 0.8[a] | 2.0±0.5 | 1.8 ± 0.1[a] | 0.18 ± 1.1[a] |
| 1000 mg/kg | 26.0±1.2 | 25.2±5.4[a] | 3.8 ± 0.4[a] | 2.2 ± 0.5[a] | 1.6±0.1 | 1.7 ± 0.3[a] | 0.22 ± 0.1[a] |

*Values with superscript a are statistically ($p< 0.05$) significantly different compared to control values*

The significant increase in ALT with decreased AST values for rats given methanol extract of *S. media* is noteworthy. High levels of ALT have been attributed to the damaged structural integrity of the liver. Contrary to the findings of this study, Gorina et al. [23] reported a decrease in serum activities of transaminases and bilirubin concentration in rats treated with water soluble polysaccharide fraction of *S. media* against $CCl_4$ induced hepatitis. Higher levels of globulin were observed in treated rats compared with the control group. Increased globulins could be attributed to an immunostimulatory effect of the methanol extract of *S. media*. By extrapolation, the methanol extract of *S. media* could be hepatotoxic for sub-chronic administration although seemingly safe at doses of 500mg/kg, as indicated by decreased AST levels it should be used with caution for prolonged administration. This study recorded a higher value of serum creatinine for rats treated at a dose of 100 mg/kg although there was no significant difference in bilirubin concentration when treated groups were compared with the control. This could be indicative of the absence of nephrotoxicity with the sub-chronic administration of the methanol extract of *S. media*.

*3.3.2.2 Cajanus cajan*

Increased AST values were recorded for experimental rats treated with methanol extract of *C. cajan* compared to the control. Significantly lower values of total protein and albumin levels were observed for rats administered extracts at all doses as compared against the control group. Increased serum bilirubin and creatinine levels were also recorded in treated rats (Table 5).

This study revealed an increase in AST, ALT and bilirubin values in *C. cajan* treated rats. Ighodaro and Amole [24] reported no significant changes in both serum enzymes when aqueous leaf extract of *C. cajan* was administered to rats for 14 days at 500mg/kg. However, at 1000 mg/kg, they recorded increased activities of serum enzymes. This point to a probable hepatotoxicity when *C. cajan* is consumed at high doses and or over a long duration of time. Lower protein and albumin concentrations were obtained from rats in the control group as compared to treated groups which further corroborates hepatotoxicity as protein production is a function of an intact healthy liver. Increased creatinine levels suggest a possible activity in kidneys of treated rats. Aly and colleagues [25] reported an increase in serum total urea and creatinine and showed the ameliorative effect of *C. cajan* and *Caesalpinia gilliesii* against acetaminophen overdose-induced renal damage.

## 4. CONCLUSION

Prolonged consumption of methanol extracts of *S. media* and *C. cajan* although not haematoxic, evoke haematological instability but possess immunostimulatory properties. Caution with prolonged administration is necessary especially with *C. cajan* with respect to hepatotoxicity concerns.

## CONSENT

It is not applicable.

## ETHICAL APPROVAL

As per international standard or university standard, written approval of Ethics committee has been collected and preserved by the authors.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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