Original Article

Life Science

Effects of Aqueous Extract of *Solanum melongena* on Blood System of Wistar Rats

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ABSTRACT [ENGLISH/ANGLAIS]

Eggplant (*Solanum melongena*) fruit, an important vegetable, has high content of free reducing sugars, anthocyanin, phenols, glycoalkaloids, amide proteins and medicinal properties. But the fruit was at one time believed to be extremely dangerous mainly because of its relationship with Solanaceae (nightshade) family. In this study, we investigated the effects of eggplant on the blood system (with special attention to haematological and serum electrolytes parameters). After toxicity test to ascertain the reasonable dosage, 20 rats were randomly divided into 4 groups: 1 control and 3 treatment groups; n = 5. The rats in group A (Control) were administered 0.9% physiological saline, while the treatment groups B, C, and D were administered graded doses of the *Solanum melongena* fruit extract of 200, 400 and 800 mg/kg body weight, respectively. The animals were dosed orally (using rat oral cannula), once daily for 8 weeks, after which they were sacrificed and their blood were analysed. The results showed that administration of extract of *S. melonena* elicits electrolyte changes. Though, *S. melonena* does not stimulate erythropoiesis, and does not have haematoxic effect, it was found to have possibility of enhancing immunity (especially the cell-mediated immunity). *S. melonena* may also cause some weight reduction (which may, in some cases, be desirable). However, extensive understanding of the molecular basis of the noted effects is still a puzzle.

Keywords: Eggplant, blood, electrolyte, Wistar rat, haematoxic effect

RÉSUMÉ [FRANÇAIS/FRENCH]

Aubergine (Solanum melongena) fruits, un légume important, a un contenu élevé desucres réducteurs libres, des anthocyanes, des phénols, glycoalcaloïdes, des protéineset amides propriétés médicinales. Mais le fruit était à un moment considéré commeextrêmement dangereux en raison principalement de sa relation avec Solanacées(morelle) de la famille. Dans cette étude, nous avons étudié les effets de l'aubergine sur le système sanguin (avec une attention particulière aux paramètres hématologiques et le sérum d'électrolytes). Après essai de toxicité pour déterminer la dose raisonnable,20 rats ont été divisés au hasard en 4 groupes: 1 contrôle et 3 groupes de traitement, n = 5. Les rats du groupe A (contrôle) ont été administrées à 0,9% du sérum physiologique, tandis que les groupes de traitement B, C et D ont été administrées des doses graduées de l'extrait de fruits Solanum melongena de 200, 400 et 800 de poids corporel mg / kg, respectivement. Les animaux ont reçu par voie orale (en utilisant une canule orale chez le rat), une fois par jour pendant 8 semaines, après quoi ils ont été sacrifiés et leur sang ont été analysés. Les résultats ont montré que l'administration de l'extrait de S. melonena suscite des changements électrolytiques. Bien que, S.melonena ne stimule pas l'érythropoïèse, et n'a pas d'effet haematoxic, il a été constatéà avoir la possibilité de l'immunité d'amélioration (en particulier l'immunité à médiation cellulaire). S. melonena peut également causer une certaine perte de poids (qui peut,dans certains cas, être souhaitable). Cependant, la compréhension approfondie de labase moléculaire des effets notés est toujours un casse-tête.

Keywords: Aubergines, du sang, des électrolytes, le rat Wistar, effet haematoxic

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Accepted/Accepté: May, 2012

Full Citation: Wahab AA, Abdel Aziz MM, Alo OE, Oluwatobi SE, Omotoso OD. Effects of aqueous extract of *solanum melongena* on blood system of wistar rats. World Journal of Life Sciences and Medical Research 2012;2(5):159-65.

INTRODUCTION

Solanum melongena L. (eggplant, aubergine (UK), melanzana (India) [1]) fruit is one of the most important vegetable crops grown on over 1.7 million hectares worldwide [2, 3]. It contains a higher content of free reducing sugars, anthocyanin, phenols, glycoalkaloids (solasodine) and amide proteins. Its bitterness is accounted for by the presence of glycoalkaloids.

Eggplant has some medicinal properties, and patients with liver problems and/or diabetes find it helpful [4]. But because of the plant's relationship with Solanaceae (nightshade) family, the fruit was at one time believed to be extremely dangerous [5].

In this study, we investigated the effects of eggplant on the blood system (with special attention to haematological and serum electrolytes parameters).

MATERIALS AND METHODS

Site of experiment

The experiment was carried out at the laboratory of Human Physiolology, Faculty of Medicine, Ahmadu Bello University Zaria. Nigeria.

Animals

Thirty three adult albino Wistar rats bred and maintained at the Experimental Animal Unit of the Vector/Parasitology Unit of Nigerian Institute for Trypanosomiasis and Onchocerciasis Research (NITOR), Federal Ministry of science and Technical, Kaduna State Nigeria, were used for this study. The rats used for this study were in the range of 180- 210 grams in weight. Water and feed were provided *ad libitum*.

The animals were kept in adequately ventilated laboratory with similar conditions of temperature, relative humidity and light/dark cycle of 12-12 hours. The home of the animals was made of aluminum with open tops covered with iron wires gauze on which food was placed. The animals were allowed to acclimatize to the laboratory conditions for 2 weeks, prior to the treatment phase of the experiment. Hygienic was maintained.

Plant Material (Eggplant)

Fresh fruits of *Solanum melongena* were bought from "Kasuwar Sabo" market in Zaria, Kaduna State of Nigeria. Botanical identification was performed at the Herbarium section of Biological Science Department of Ahmadu Bello University, Zaria, Kaduna State, Nigeria, and given a voucher number 1939.

Extraction of Plant Material

The fruits were shade-dried for 10 days and ground into powder. An aqueous extract was made from 1 kg of *Solanum melongena*, which was soaked in distilled water (5L) and the mixture boiled for 15 minutes. The heated decoction was taken and allowed to cool at room temperature; it was then filtered and evaporated to dryness in water bath of 60 ^{o}C. A brownish residue weighing 60.2 g (yield of extraction, 6.02%) was obtained and kept in air tight bottles in a refrigerator until use. The working solution was prepared at a final concentration of 800 mg/ml as described by Pierrie *et al*. [6]. the extraction was carried out in department of Pharmacognosy Laboratory, Ahmadu Bello University, Zaria. The working solution was prepared at a final concentration of 150 mg/ml in distilled water.

Acute Toxicity Studies

The lethal dose (LD_{50}) of the plant extract was determined using the method of Lorke [7]. The study of *S. molengena* on Wistar rat toxicity was divided into two phases. Nine Wistar rats were used in the first phase in three divided groups of three each, named group A, B and C. Groups A, B, C were respectively administered (orally) 10, 100, and 1000 mg of the extract per kg body weight. The treated animals were observed for 24 hours, after which, no death was recorded. In the second phase, four Wistar rats were divided into one rat each into groups D, E, F, and G. Group D received extract at a dose of 1000 mg/kg body weight orally, group E, F and G treated with the extract at a dose of 1000, 1600, 2900 and 5000 mg/kg body weight respectively, and they were then closely observed for 24 hours. Also, at the end of the second phase no death was recorded. This means that $LD_{50} > 5000$ mg/Kg, and that the extract is safe and non-toxic at these doses.

Experimental Design

The remaining 20 rats were randomly divided into 4 groups: 1 control and 3 treatment groups; n = 5. The rats in group A (Control) were administered 0.9% physiological saline, while the treatment groups B, C, and D were administered graded doses of the *Solanum melongena* fruit extract of 200, 400 and 800 mg/kg body weight, respectively. The animals were dosed orally (using rat oral cannula), once daily for 8 weeks.

Animal Sacrifice and Sample Collection

Twenty-four hours after the last treatments, the rats were sacrificed by cervical dislocation, and blood samples was collected through cardiac puncture using one syringe for each of the rats. For each rat, some of the blood sample (about 2.0 ml) was immediately put into ethylenediaminetetraacetic acid (K_3 EDTA type) bottle (for analysis of haematological indices), and the remaining blood sample (about 3.0 ml) was put into lithium heparinised bottles (for analysis of serum electrolytes and proteins).

Determination of Red Blood Cell Count (RBC)

To determine RBC, red cell pipette was used to suck the blood up to 0.5 mark by placing the pipette into the blood in a more or less horizontal position. After sucking the

blood into the pipette, the tip of the pipette was wiped off. It was then placed in the watch glass containing diluting fluid. On doing so, the pipette was rotated until the fluid reaches the 101 mark [8].

The counting chamber and the cover slip were cleansed with cotton wool. To the counting chamber, the cover slip was placed over the central platform, 1 cm of the fluid in the stem was expelled from the pipette and the remaining 100 cm content was mixed thoroughly. A drop of the blood solution was then introduced into the counting chamber by approximating the tip of the pipette to one end of the counting chamber. The counting chamber was mounted on the stage of the microscope and a magnification of x 40 was used to view the corpuscles. Counting was done after allowing the corpuscle to settle for few minutes. The counting was done in an L-shape pattern so as to avoid counting those cells on the boundary twice. Five regions of the counting chamber were counted [8].

Determination of White Blood Cell (WBC) Count and Differential WBC Count

WBC and differential WBC were similar to WBC count, except that the magnification (power) of the light microscope was set at ×10 and ×100 respectively, instead ×40 in RBC count. Also, the WBC diluting fluid and Leishman stain were used, and the counting was in four regions of the counting with the counting chamber of the RBC in the middle [9].

Determination of Packed Cell Volume (PCV)

The blood sample collected was introduced into the capillary tubes using the syringe. The tubes were then sealed by blue flames from spirit lamp. The sealed capillary tubes were then spun in microhaematocrit centrifuged for 30 minutes at 5 g. The respective PCV's were then read from microhaematocrit reader [9].

Calculations of Red Blood Cell Indices

Red cell indices (Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), and Mean Corpuscular Haemoglobin Concentration (MCHC)) were calculated as described by Jain [10].

MCV = PCV x 10 / RBC (fl)
MCH = HbC x 10 / RBC (pg)
MCHC = HbC x 100 / PCV (g/dl)

Statistical Analysis

All data were expressed as the Mean ± Standard Error of Mean (SEM). Analysis of Variance, and then Scheffe's post hoc test [11] were used to analyse the data. Any test with a $p < 0.05$ was considered significant.

RESULTS

The results of this study show that body weights were not significantly ($p > 0.05$) affected by the treatments (Figure 1), but there was at least a small reduction in the body weight in groups B and D (Figure 2).

There was reduction in the PCV of all the treatment groups relative to that of the rats in the control group but the reduction was only significant ($p < 0.05$) for group C (Figure 3). The mean total red blood cell count of rats in each of the Experimental groups B and C were lower compared with those in the control group with a significant decrease ($p<0.05$) only in group C (Figure 3). There was a non-significant ($p > 0.05$) decrease in the mean Hb concentration recorded for rats in the Experimental groups B and D, but significant ($p < 0.05$) decrease in group C (Figure 3).

Each of MCV, MCH, and MCHC was not significant different throughout the groups (Figure 4). On the other hand, WBC was increased in all the experimental groups relative to the control, but the increase were significant for only group B ($p < 0.01$) and group D ($p < 0.05$) (Figure 5). However, the differential count did not show any significant difference between there treatment groups and the control (Figure 5).

As for the serum electrolytes, Na^+ levels for the rats in all the experimental groups B, C and D decreased significantly ($p < 0.001$) when compared to the control group (Figure 6). But there was no significant difference in the serum K^+ level between the control and the treatment groups. On the other hand, serum Ca^+ level was significantly ($p < 0.01$) higher for group C but not for groups B and D (Figure 6). The mean serum Cl^- levels in the experimental rats increased relative to the control, but only group C was statistically significant ($p < 0.05$), while HCO^{3-} and HPO^{4-} decreased significantly in all the treatment groups (Figure 7).

There was significant ($p < 0.001$) decrease in serum creatinine level, and blood urea nitrogen for all the treatment groups relative to the control group (Figure 8). Significant increase in the Total serum protein and Serum Albumin levels were noted in both groups B and C, but not in group D. But there was no significant difference in the Serum Globulin (Glb) levels for all the groups.

DISCUSSION

Relative to the rats in the control group, the PCV values of rats in the experimental groups were observed to have

decreased dose-dependently (except for group D) (Figure 3). The observed (but dosage-inconsistent) slight decrease in the PCV goes in line with the noted pattern for both red blood cells count and haemoglobin concentration (Figure 3). The non-significant change in MCV, MCH and MCHC values suggests that eggplant (*Solanum melongena*) does not really have any haemopoeitic, and may not even have any serious haematotoxicity or general toxic effect as previously believed [5]. Our findings up to this point is (somewhat) in line with those of Adamson and Longo [12]. Slight decrease in haemoglobin concentration and circulating RBC with no change in values of MCV, MCH and MCHC may produce normocytic hypochromic red blood cells. This could only be possible in group C rats (administered 400 mg of extract/Kg body weight) where the decrease in haemoglobin concentration and circulating RBC was noted, and thus may not signify any particular significant threat/toxicity from eggplant.

The differential cell analysis showed that there was an increase in the mean lymphocyte values. Increase in circulating lymphocyte values is associated with enhanced immunological status of the body, especially the cell-mediated immune response. Our findings here agree with those of Saba *et al*, [13] and those of Lowenthal *et al*. [14] who suggested that eggplant may favour the cells involved in immunity. The findings in this study (figure 5), therefore, confirm that eggplant enhances cell-mediated immunity and thus boost body's immune system. Knowing that increased circulating neutrophils serve as an index of bacterial infection in the body [13], the non-significant change in the value of circulating neutrophils even in rats treated with 800 mg/kg does not seem to have any special implication with respect to the administered extract, except that it may suggest that the animals were safe from sepsis and that other observed results can, in fact, be considerably trusted (as the influences of most non-random factors were kept out). In addition, one may still argue/believe that the reduction of mean value of circulating neutrophils in this study is somewhat (indirectly) due to the antimicrobial effect of the extract, which is in accordance with the work of Jorge *et al*. [15].

Serum sodium ion level was lower in the experimental rats studied. This may be due to presence of some sodium depleting base compounds in the extract or excessive excretion of sodium by the kidneys, but the later reason could not be affirmed since no specific test was done to very that. As (more) sodium is excreted, it takes with it (more) water, which could in turn result in weight loss [16, 17]. This may, thus, explain part of the moderate weight loss noted in the extract-treated rats (figure 1).

The noted reduction is calcium level could be linked to the fact that oxalate reduces calcium absorption from the gut. Thus the presence of oxalates in the extract, which could lead to reduced absorption of calcium from the intestine, is implicated. This agrees with the findings by Whitaker and Stommel [18]. In some other cases (not studied, in this research), another possible explanation for similar reduction in calcium level is related to the fact that hypocalcaemia results from chronic generalized renal failure [19]. But since accumulation of oxalates over time can cause derangement of renal functions, one could see a possibility (although, not experimentally confirmed fact) that the noted reduced calcium level could have very well resulted from any slight compromise in the renal tubules ions transport system [20] of the extract-treated rats. However, the significantly decreased ($p < 0.001$) serum creatinine level, and bilirubin level are much more supportive of enhanced renal (and liver) functions [21, 22].

Figure 1: This figure shows comparison of initial and weight before sacrifice of control and experimental rats

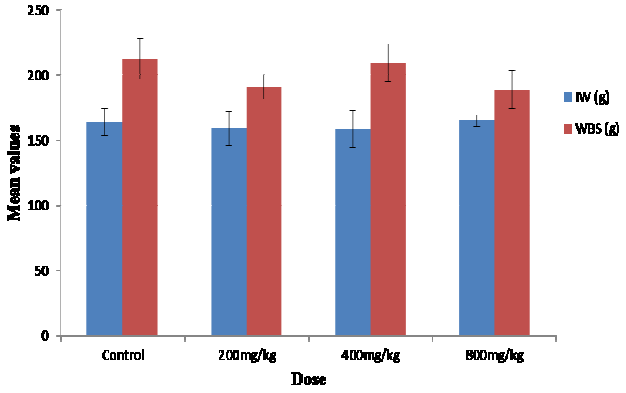


Figure 2: This figure shows comparison of weight change (wc) across the four groups

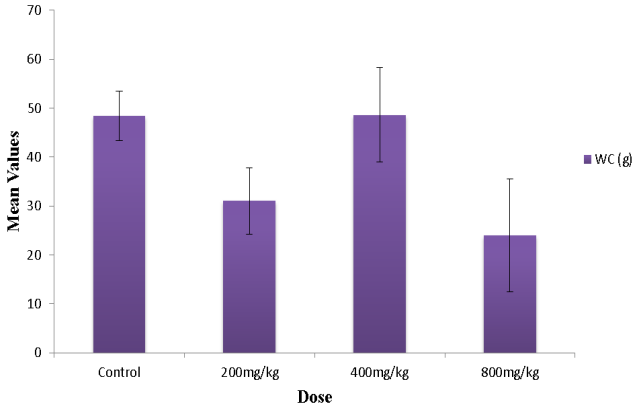


Figure 3: This figure shows comparison of hb concentration, pcv and rbc in control and experimental rats

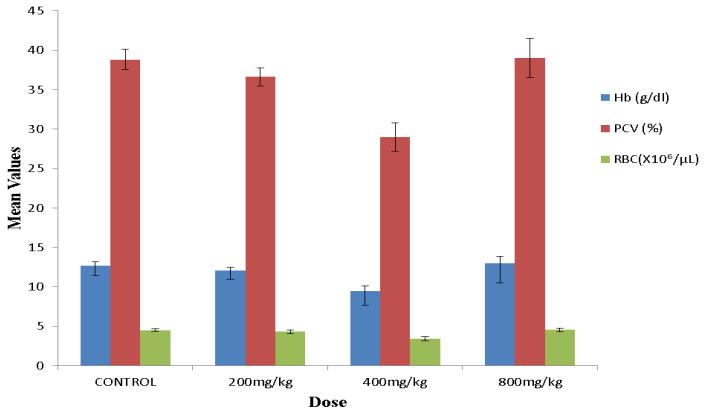


Figure 4: This figure shows comparison of mcv, mch and mchc in control and experimental rats

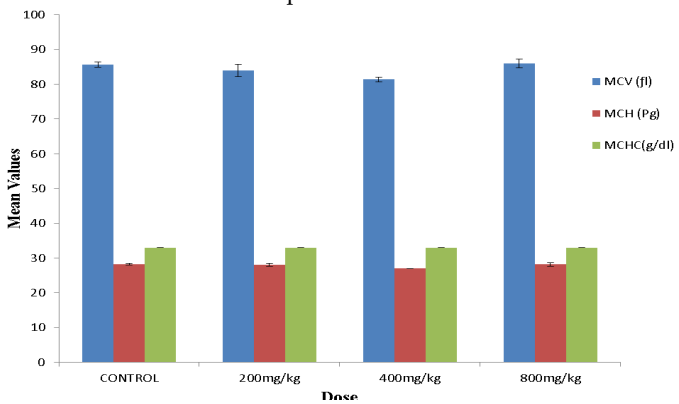
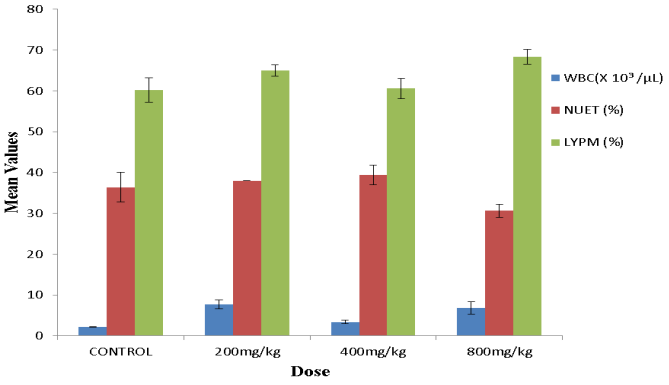


Figure 5: This figure shows comparison of wbcs, lymp and nuet in control and experimental rats



CONCLUSION

It is concluded that prolonged administration of extract of *S. melonena* may elicit electrolyte changes. Though, *S. melonena* does not stimulate erythropoiesis, and does not have haematoxic effect, the results from this study suggests that it may enhance immunity (especially the cell-mediated immunity). *S. melonena* may also cause some weight reduction (which may, in many cases, be highly desirable). However, further studies would be required to ascertain the molecular basis of the noted effects.

Figure 6: This figure shows comparison of some serum cations in control and experimental rats

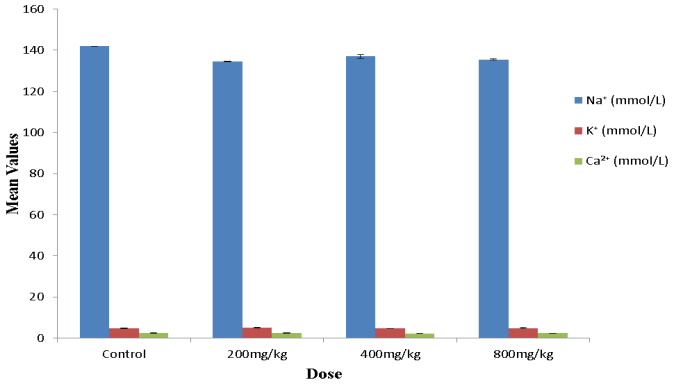


Figure 7: This figure shows comparison of some serum anions in control and experimental rats

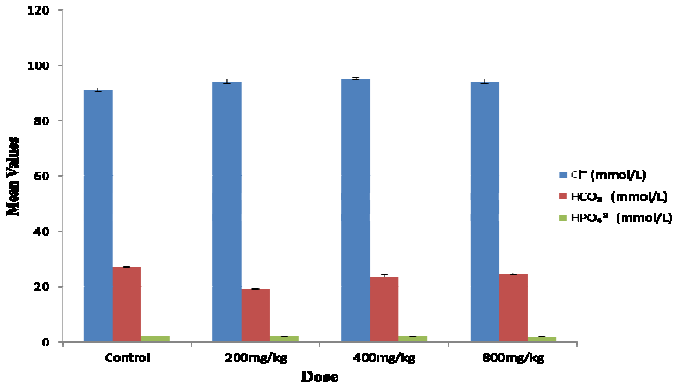


Figure 8: This figure shows comparison of some serum metabolites in control and experimental rats

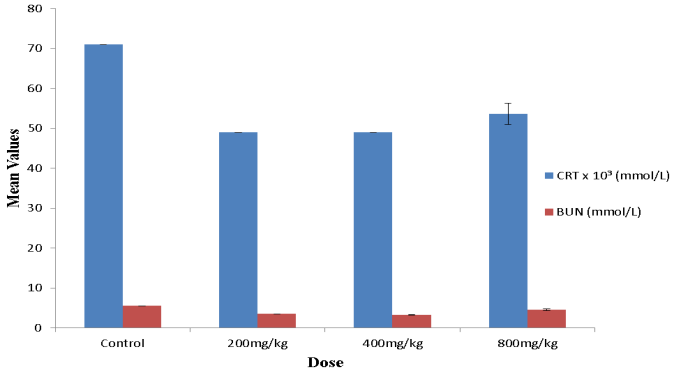
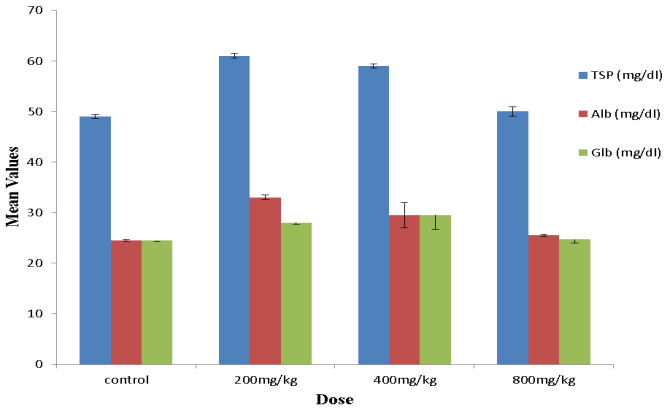


Figure 9: This figure shows comparison of tsp, alb and glb in control and experimental rats



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ACKNOWLEDGEMENT / SOURCE(S) OF SUPPORT

Nil

CONFLICT OF INTEREST

No conflict of interests was declared by authors