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# Acute Toxicity and i*n-vivo* Effects of Leaf Extracts of *Byrsocarpus Coccineus* Shum &Thonn in Pregnant Rat Uterus

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**ABSTRACT**

Medicinal plants are the primary source of medicines and main ingredients used by traditional medicine practitioners. *Byrsocarpus coccineus* Schum and Thonn is one of such plants that have been used in Africa to treat different ailments including augmentation of labour. The aim of this study is to determine the acute toxicity and to screen the *in vivo* uterotonic effects of the ethylacetate leaf extract of *Byrsocarpus coccineus* in pregnant rat uterus. Leaves of the *Byrsocarpus coccineus* were collected, air dried, pounded and extracted using ethanol, ethylacetate, N-butanol and water. The extracts obtained were then used for the acute toxicity study, while the ethylacetate extract was used to assess the *in vivo* activity in pregnant rat uterus. Ethylacetate and aqueous leaf extracts *Byrsocarpus coccineus* was found to be relatively non toxic, whereas N-butanol was found to be toxic in rats and mice. Ethanol leaf extract was found to be only relatively toxic in mice. Ethylacetate leaf extract of *Byrsocarpus coccineus* potentiated the delivery of pregnant rats on days 21 of pregnancy. The results of the abortificient effect of the ethyl acetate extract on the pregnant rats showed no significant difference between the treatment groups compared with the control ($p>0.05$). There was a significant increase in haemoglobin, white blood cell, platelets and aspartate aminotransferase ($p<0.05$). Ethylacetate leaf extract of *Byrsocarpus coccineus* is relatively safe and was found to potentiate the delivery of pregnant rats with no significant change in hepatic and renal functions and this supports the traditional use of this plant to induce labour at terms.

## INTRODUCTION

Traditional medicine practice in Nigeria is regarded as the source of livelihood and major source of income to about 200,000 traditional medicine practitioners in Nigeria. Medicinal plants are the primary source of medicines and main ingredients used by traditional medicine practitioners (Federal Ministry of Health, 2000). Today in many countries modern medicine has replaced plant medicine with many synthetic products but it must be emphasized that almost 30% of pharmaceutical preparations are still obtained directly from plants (Marini-Bettolo, 1980). A large percentage of studies have been carried out on herbal traditional medicines and significant amount of laboratory data have been generated and published on their efficiency (Gamaniel et *al*., 1997). *Byrsocarpus coccineus* schum and thonn (family connaraceae) is one of such plant that has been known and used in traditional medicine in several parts of West Africa (Dalziel, 1937). *Byrsocarpus coccineus* is popularly known in Ghana by the Twi and Gar people as "awenda" or "awende." In Northern Nigeria, it is referred to by the Hausas as "Tsamiyar kasa or kimbar maharba." The Fulani people call it "wangarabubi or yangara-bubihi", while the Bassange people call it "Kogi." In the southern part of Nigeria the Yoruba people call it "Oke abolo" or "Mybo-apepea" (Dalziel, 1937). Kilba people in Adamawa State call it ''mblakiki''. *Byrsocarpus coccineus* have been shown to be useful in oro-pharngeal, dermatological, urogenital tract and haematological problems (Irvine, 1961; Akindele and Adeyemi, 2006; Amos *et al*., 2002; Okunji *et al*., 1988; Amadu *et al*., 2006). In central Nigeria the leaf decoction of *Byrsocarpus coccineus* have been used traditionally to augment labour. Despite the diverse growing use of

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this plant in our community, the adverse effects of this plant have not been reported. This and several other reasons prompted us to evaluate the toxicity and *in vivo* uterotonic effects of this plant.

## MATERIALS AND METHOD

### Identification, collection and authentication of plant materials

Samples of the plant material *Byrsocarpus coccineus* were collected from Idu, Abuja during the month of April 2009 under the guide of a professional plant collector Mr.Yakubu Habi of the Department of Medicinal Plant Research and Traditional Medicine of the National Institute of Pharmaceutical Research and Development Abuja where a voucher specimen number (3452) was assigned and deposited at the herbarium for future reference.

### Processing and extraction of the powdered plant material

The leaves of *Byrsocarpus coccineus* were carefully separated from the other morphological parts of the plant and washed clean with water, air dried under shade for seven days pounded with pestle and mortar into fine particles. Six kilograms of the powdered leaves of *Byrsocarpus coccineus* was extracted by marceration with 6 L of N-hexane to cover the powder. The set up was closed tightly for 72 hours with occasional agitation and stirring. Afterwards the mark was filtered, squeezed for remaining N-hexane and were air dried under shade until it was completely free from n-hexane. The mark was then macerated with absolute ethanol. The same procedure as indicated for n-hexane was repeated. Hexane was recovered by rotary evaporator. Ethanol extract was then subjected to evaporation using rotary evaporator. Two hundred grams of dried and crude ethanol extract of *Byrscocarpus coccineus* leaf was suspended in 150 ml of distilled water and successively extracted with 500 ml x 3 ethylacetate and 500 ml x 3 N-butanol. At every stage of the partitioning and before switching over to the next organic phase, it was ensured that the organic phase exhaust the aqueous phase of the needed ingredients and this was indicated by colour change in the organic phase. In addition it was also ensured that the next partitioning organic solvent does not interfere with the previous one; the two organic phases are used though successively but strictly exclusively. The ethyl acetate and N-butanol pooled fractions were filtered and evaporated to dryness separately at reduced pressure under rotary evaporator and the dried fractions stored in the desiccator until constant weight was obtained. The percentage yields for ethanol, ethylacetate, N-butanol and aqueous leaf extracts of *Byrsocarpus coccineus* were 16.7%, 3.9%, 1.2% and 1.0% respectively.

### Acute toxicity studies

The acute toxicity ($LD_{50}$) of leaf extracts of *Byrscocarpus coccineus* was determined using modified Lorke's method (1983) in both rat and mouse using oral and parenteral route. In oral route the dose levels of 2 g/kg, 3g/kg, 4g/kg and 5 g/kg was used in phase I. The mortality observed at 4g/kg due to ethylacetate and aqueous leaf extracts prompted the phase II in which a dose levels of 3100 mg/kg, 3200 mg/kg, 3400 mg/kg and 3800 mg/kg in rats was adopted. Based on the result of phase II the $LD_{50}$ was calculated: Square root of dose that kill multiply by the square root of dose that did not kill (Lorke, 1983). Similarily, the dose levels of 1 mg/kg, 10 mg/kg, 100mg/kg and 1000 mg/kg was used for all the extracts in phase I intraperitonially. Appropriate dose levels for phase II was determined for the ethanol and N-butanol leaf extracts in both rats and mice.

### In vivo experiment (Oderinde *et al.*, 2002)

*Effect of ethylacetate extract on pregnant rats: pilot study to determine suitable dose for the experiment (phase 1)*

Matured adult female rats were selected from the animal house of Faculty of Pharmacy University of Maiduguri for the research. Vaginal smear for each rat was done to monitor ovulation and estrous cycle. Only rats with normal estrous cycle were selected for the experiment. Twenty five animals were selected and sub grouped into five groups (N = 5) A, B, C, D and E. In each group each female was matched with one male. On the 25/11/2010 the females were examined to determine evidence of mating. The presence of spermatozoa was determined by microscopic examination of the vaginal smear the following morning. The presence of spermatozoa indicated conception and represented day one of pregnancy (Oderinde *et al*., 2002). Group A, B, C and D were given 125 mg/kg, 250 mg/kg, 500 mg/kg and 1000 mg/kg of the ethyl acetate extract respectively on days 17, 18 and 19 of pregnancy. Group E served as control and was given only the diluent dimethyl/sulphoxide

*Administration of ethylacetate extract, oxytocin and dimethyl sulphoxide (phase ii)*

Using the same method as in phase I (three groups); A, B and C of ten adult female rats each in oestrous phase were paired with equal number of males overnight. Mating was determined the following day after vaginal swabbing as in phase I on 09/01/201. Group A was given 2 iu/kg subcutaneous oxytocin made by Rotemedica Germany 10iu/ml, equivalent of 16.66mg, NAFDAC Registration number 04-6981 Manufacturing date 06/2010,Expiration date 06/2013 on days 17,18,and 19 of pregnancy. Group B was given 250 mg/kg of the ethyl acetate extract on day 17, 18 and 19 of pregnancy. Group C was given only dimethylsulphoxide on days 17, 18 and 19 of pregnancy.

### Effect of ethylacetate extract on blood of rats

Female adult rats were grouped into two; A and B each group having ten rats. Group A was given 250 mg/kg of the ethylacetate extract everyday for seven days. Group B served as control. On day seven, blood samples were taken from the two groups and analyzed for haematological and biochemical parameters. Twenty (20) rats of both sexes weighing between 160 to 235 g were obtained from Faculty of Pharmacy University of Maiduguri animal house. They were maintained in standard conditions of temperature and light/night cycles. They were allowed free access to commercial poultry feed and water *ad libitum.* The twenty (20) rats were grouped into two groups, of ten

rats each. The bloods of the rats were collected from the tail vein for the analysis of haematological parameter. Thereafter, the rats were sacrificed by decapitation and the blood drained into plain bottles for some serum biochemistry. Blood samples were collected from the tail vein of each rat for the analysis of red blood cells count (RBC), white blood cell counts (WBC), packed cell volume (PCV), haemoglobin concentration (Hb), and haematological indices, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) were determined by calculation.

*Red blood cell count*

The red blood cell pipette has a graduation mark o.5 and 1 on the capillary stem below and 101 above the bulb and it has a red bead. Blood was sucked up to 0.5 mark and immediately the Hamey's diluting fluid was drawn up to 101 mark. The blood was mixed thoroughly with the diluting fluid for five minutes. The cover slip was pressed on the counting chamber. The first few drops of the fluid from the pipette were discarded and the tip of the pipette was brought in contact with the exposed part of the chamber and the fluid flowed under the cover slip. The cells were allowed to settle down for 2 minutes and the cells in five central squares of the chamber were counted under x 40 objective of light microscope. The number of red cells counted was multiplied by ten thousand (10, 000) to give number of red cells in millions per cubic millilitres (or $x10^6mm^3$) (Jain, 1986).

*White blood cells*

The red blood cell pipette has a graduation mark 0.5 and 1 on the capillary stem below and 11 above the bulb and it has a white bead. Blood was sucked up to 0.5 mark and immediately the diluting fluid was drawn up to 11 mark. The blood was mixed thoroughly with the diluting fluid for five minutes. The cover slip was pressed on the counting chamber. The first few drops of the fluid from the pipette were discarded and the tip of the pipette was brought in contact with the exposed part of the chamber and the fluid flowed under the cover slip. The cells were allowed to settle down for 2 minutes and the cells in five central squares of the chamber were counted under x 40 objective of light microscope. The number of red cells counted was multiplied by fifty (50) to give number of red cells in millions per cubic millilitres (or $x10^3mm^3$) (Jain, 1986).

*Packed cell volume and Haemoglobin concentration*

The blood collected was allowed to run into the heparinized capillary tube by capillary action until the tube is about three quarter filled. The end of the tube in contact with the blood was sealed with plasticine and centrifuged at the rate of 15, 000 revolutions per minute (rpm) for five minutes (5min.) and read with micro-Haematocrits reader and expressed as percentage (%) (Jain, 1986). Colorimetric method for the determination of haemoglobin concentration was used. $0.2cm^3$ of blood was pipetted and diluted in a test tube containing 5ml of Drabskin solution and the mixture was allowed to stand for five minutes (5minutes). The absorbance of the solution was read using colorimeter at a wavelength of 540nm (Jain, 1986). The haematological indices were calculated using the formula by Jain (1986).

*Aspartate aminotransferase (ASAT)*

Activities of serum Aspartase, was assayed by the methods of Reitman and Frankel (1957). Zero point two ml of serum with 1.0 ml of substrate (aspartate and α-keto-glutarate in phosphate buffer $p^H$ 7.4) was incubated for an hour. One ml of DNHP solution was added to arrest the reaction and kept for 20 minutes in room temperature. After incubation, 1ml of 0.4N NaOH was added and the absorbance was read at 540 nm. Activities expressed as IU/L.

*Alanine aminotransferase (ALAT)*

Activities of serum Alanine aminotransferase, was assayed by the methods of Reitman and Frankel (1957). Zero point two ml of serum with 1.0 ml of substrate (alanine and α-keto-glutarate in phosphate buffer $p^H$ 7.4) was incubated for 30 minutes. 1.0 ml of DNHP solution was added to arrest the reaction and kept for 20 minutes in room temperature. After incubation, 1.0 ml of 0.4 N NaOH was added and the absorbance was read at 540 nm. Activities expressed as IU/L.

*Alkaline phosphatase (Alk. Phos.)*

Based on the method of King and Armstrong (1937) alkaline phosphatase activity was assayed using disodium phenyl phosphate as substrate. The colour developed read at 680 nm after 10 minutes. Activities of alkaline phosphatase expressed as IU/L.

*Total protein*

Zero point two milliliters of serum, standard solution and distilled water were mixed in separate test tubes with 1.0 ml of Biuret reagent. The mixtures were incubated for 30 minutes at 20 to 25°C. The absorbance of each ($A_{sample}$) and ($A_{standard}$) were measured against the blank, at a wavelength of 546nm (Reitman and Frankel, 1957). Sodium, chloride, hydrogen bicarbonate and potassium concentration of plasma were determined by standard flame photometry. Urea and creatinine were determined by the method described by Harrison (1947).

## RESULTS

**Acute toxicity study of Ethanol, Ethylacetate, N-butanol and aqueous leaf extracts of *Byrsocarpus coccineus***

The $LD_{50}$ for ethanol extract was found to be greater than 1 g/kg for rats and 282.84 mg/kg for mice by intrapritoneal (IP) route, while for the ethylacetate extract in both rats and mice was found to be greater than 1 g/kg by IP route. However, the $LD_{50}$ by oral route for the ethylacetate extract was calculated as 3.30 g/kg in rats. The lethal dose for N-butanol extract was found to be 141.42 mg/kg by IP in rats and 89.44 mg/kg by IP in mice, whereas for water extract it was found to be greater than 1 g/kg by

IP for both rats and mice. $LD_{50}$ in rats was found to be 3.46 g/kg for rats when the water extract was administered orally (Table 1).

**Table. 1:** Acute toxicity study of leaf extracts of *Byrsocarpus coccineus.*

| *Extracts* | *Route* | *$LD_{50}$* | |
|---|---|---|---|
| | | *Rats* | *Mice* |
| A | | >1g/kg (IP) | 282.84mg/kg (IP) |
| B | IP, Oral | >1g/kg (IP); 3.3g/kg (Oral) | >1g/kg (IP) |
| C | IP | 141.42mg/kg(IP) | 89.44mg/kg (IP) |
| D | IP, Oral | >1g/kg (IP); 3.46/kg (Oral) | >1g/kg (IP) |

A = Ethanol extract, B = ethylacetate extract, C = N-butanol extract,
D = Water extract, LD50 = Lethal dose, IP = Intraperitoneal

**Invivo effect of Ethyacetate extract of *Byrsocarpus coccineus* in pregnant rat (phase 1)**

All the five rats that took 125 mg/kg of Ethylacetate leaf extract delivered on day 23 of pregnancy, while those that received 250 mg/kg, 500 mg/kg and 1000 mg/kg delivered on day 21 of preganacy. Four out of 5 rats that were given DMSO gave birth on day 24, while the remaining rat gave birth on day 25 (Table 2).

**Table. 2: I**n vivo effects of Ethylacetate extract of *Byrsocarpus coccineus* in pregnant rat uterus (Phase I).

| *Group* | *Dose of Ethylacetate (mg/kg)* | *Route of administration* | *Date of delivery* | *No. of Rats delivered (N=5)* |
|---|---|---|---|---|
| A | 125 | Oral | Day 23 | 5 |
| B | 250 | Oral | Day 21 | 5 |
| C | 500 | Oral | Day 21 | 5 |
| D | 1000 | Oral | Day 21 | 5 |
| E* | 0.5 ml | Oral | Day 24 | 4 |
| | | | Day 25 | 1 |

* = control group (Dimethylsulfoxide)

**Table 3:** Invivo effect of Ethylacetate extract of *B. coccineus* on pregnant rat .

| Group | Dose | Route of administration | Date of Abortion/ Delivery | No. of Rats Aborted/Delivered (N=10) |
|---|---|---|---|---|
| A | 2 IU/kg | Subcutaneous | Day 19* | 7 |
| | | | Day 20* | 3 |
| B | 250 mg/kg | Oral | Day 21 | 8 |
| | | | Day 22 | 2 |
| C | 0.5 ml | Oral | Day 24 | 6 |
| | | | Day 25 | 4 |

A = Oxytocin (Positive control)., B = Ethylacetate., C = DMSO (Negative control)., * = Abortion., *B. coccineous = Byrsocarpus coccineous*

**Invivo effect of Ethyacetate extract of *Byrsocarpus coccineus* in pregnant rat (phase 2)**

Seven rats that were given 2 IU/kg of oxytocin subcutaneously, aborted their pregnancies on day 19, while the remaining three aborted their pregnancies on day 20. Eight rats that received 250 mg/kg of Ethyl acetate leaf extract of *Byrsocarpus coccineus* delivered successfully on day 21, while the remaining 2 rats delivered on day 22. Six and 4 rats that took DMSO as negative control had their successful delivery on day 24 and day 25 respectively (Table 3).

**Effect of ethyl acetate extract on pregnant rats/ number of litters**

The results of the abortificient effect of the ethyl acetate extract on the pregnant rats are shown in table 4. The results show that there was no significant ($p>0.05$) difference between the treatment groups compared with the control in the phase I (Result of the effect of ethylacetate extract,125 mg/kg, 250 mg/kg, 500 mg/kg, 1000 mg/kg and DMSO on pregnant rats Table 4). The mean values of the litters at term were 5.00±0.71, 5.00±1.00, 5.00±1.58, 5.20±1.64 and 4.40±1.67 in treated with 125, 250, 500, 1000mgkg$^{-1}$ body weight. The control (DMSO) had 4.40±1.67. The results of the second Phase II (Result of the effect of ethylacetate extract 250 mg/kg, oxytocin 2 IU/kg and DMSO) were also presented in the Table 4. The results shows that there was no significant ($p>0.05$) different between the treated groups and control. There mean values were 5.40±1.35 and 4.90±1.52 respectively in groups treated with 250 mgkg$^{-1}$ body weight of ethyl acetate extract and 2 IU/kg body weight oxytocin control drug while the control diluents solvent (DMSO) had 5.30±1.16 litters at term.

**Table. 4:** Effect of ethyl acetate leaf extract on pregnant rats/ number of litters

| *Phase I (N = 5)* | |
|---|---|
| *Dose of EAE (mg/kg)* | *Mean ± SD of Litters* |
| 125 | 5.00±0.71 |
| 250 | 5.00± 1.00 |
| 500 | 5.00±1.58 |
| 1000 | 5.20±1.64 |
| DMSO (A) | 4.40±1.67 |
| *Phase II (N = 10)* | |
| *Dose* | *Litters* |
| 250 mgkg of EAE | 5.40±1.35 |
| OXYTOCIN 2IU/kg (B) | 4.90±1.52 |
| DMSO (A) | 5.30±1.16 |

DMSO = Dimethylsulphoxide, A = Negative control,
B = Positive control, EAE = Ethylacetate extract

**Effect of ethylacetate leaf extract of *Byrsocarpus coccineus* on hematological parameters**

Treatment of rat orally with 250mg/kg extract shows significant ($P<0.01$) increase in haemoglobin concentrations when compared with the control. The mean values were 12.38±0.44g/dl while the control value was 10.60± 0.30 g/dl (Table 5). The packed cell volume showed significant ($P<0.001$) decrease in mean value. The mean value of the treated with the extract was 44.80±1.48%. The control had 47.00±1.87%. The red blood cells count shows no ($p>0.05$) difference between the treated and the control. There mean value was $6.81\pm0.29^{6}/mm^{3}$ while the control mean value was $6.32\pm0.12^{6}/mm^{3}$ of blood. The white blood cell counts also showed no significant ($p>0.05$) difference between extract treated and the control. There mean values were $10.60\pm0.75^{3}/mm^{3}$ and the control had $9.84\pm0.30^{3}/mm^{3}$ of blood. The haematological indices mean corpuscular volume shows no significant ($p>0.05$) difference between the treated group and the control. There mean corpuscular values were 6.59±0.37 Fl of the treated and 7.44±0.25 Fl (Table 5). Mean corpuscular haemoglobin shows slight significant ($p<0.05$) increase compared to the control. The mean value of the treated rats was 18.22±1.24 pg while the control had 16.77±0.21 pg. The mean corpuscular haemoglobin concentration significant ($p<0.001$) increase compared to the

control. There mean values were 27.66±1.23% of the treated and 22.57±0.85% of the control (Table 5).

**Table. 5:** Effect of one week administration of ethylacetate leaf extract of *Byrsocarpus coccineus* (250 mg/kg) on hematological parameters in wistar albino rats.

| *Haematological parameters* | *Mean levels of haematological parameters (N = 10)* | |
|---|---|---|
| | *Control (DMSO)* | *Extract* |
| Hb (g/dl) | 10.60±0.30 | 12.38±0.44** |
| PCV (%) | 47.00±1.87 | 44.80±1.48*** |
| RBC (x $10^6$/$mm^3$) | 6.32±0.12 | 6.81±0.29 |
| WBC (x $10^3$/$mm^3$) | 9.84±0.30 | 10.60±0.75 |
| MCV (FI) | 7.44±0.25 | 6.59±0.37 |
| MCH (pg) | 16.77±0.21 | 18.22±1.24* |
| MCHC (%) | 22.57±0.85 | 27.66±1.23*** |

Mean ±SD, N=10
*P<0.05 significant difference compared with the control
**P<0.01 moderately significant compared with the control
***P<0.001 highly significant compared with the control

### Effect of ethylacetate leaf extract of *Byrsocarpus coccineus* on biochemical parameters

Following treatment of rats with the ethylacetate extract on some serum biochemical parameters, the results were shown on Table 6. The results shows that there was significant (p<0.05) increase in the activity of aspartate aminotransferase (ASAT) when compared with the control. The mean activity of ASAT of the extract treated was 93.80±3.19 IU/L and the control had 87.40±6.47 IU/L. The activity of the alanine aminotransferase (ALAT) shows no significant (p>0.05) difference between the control and the treated rats. There mean values were 26.80±2.95 IU/L and the control had 24.00±1.58 IU/L.

**Table. 6:** Effect of ethylacetate leaf extract of *Byrsocarpus coccineus* (250 mg/kg) on serum biochemical parameter in wistar albino rats.

| *Biochemical parameters* | *Mean levels of serum biochemical parameters (N = 10)* | |
|---|---|---|
| | *Control (DMSO)* | *Extract* |
| ASAT (IU/L) | 87.40±6.47 | 93.80±3.19* |
| ALAT (IU/L) | 24.00±1.58 | 26.80±2.95 |
| Alk. Phos. (IU/L) | 156.20±4.60 | 180.20±7.56*** |
| T/P (g/L) | 40.40±2.70 | 35.40±4.93 |
| Urea (mmol/L) | 5.74±1.16 | 2.92±0.58 |
| Creatinine (mmol/L) | 61.60±4.10 | 53.80±7.40** |
| $Na^+$ (mmol/L) | 140.80±2.39 | 149.00±7.42** |
| $Cl^-$ (mmol/L) | 101.80±1.79 | 111.80±1.79*** |
| $K^+$ (mmol/L) | 6.82±0.78 | 8.10±0.72 |
| $HCO_3^-$ (mmol/L) | 18.00±3.32 | 22.80±1.79 |

Mean ±SD, n=10
*P<0.05 significant difference compared with the control (ANOVA)
**P<0.05 Moderately significant compared with the control (ANOVA)
***P<0.05 Highly significant compared with the control (ANOVA)

The activity of the alkaline phosphotase shows significant increase (p<0.05) in rats treated with the extract when compared with the control. The treated rats had their mean values to be 180.20±7.56 IU/L while the control had 156.20±4.60 IU/L. The total protein and urea value shows no significant (p>0.05) difference when compared with the control (Table 6). Creatinine values of the treated shows significant (p<0.05) decrease compared with the control. The mean of the treated was 53.80±7.40 mmol/L and the control, 61.60±4.10 mmol/L. The sodium and chloride electrolyte shows significant (p<0.05) increase in the treated than the control. The mean value was 149.00±7.42mmol/L and 111.80±1.79mmol/L, while the control 140.80 mmol/L and 101.80±1.79 mmol/L (Table 6). Potassium and bicarbonate ion shows no significant (p>0.05) difference between the treated and control. There mean values of the treated were 8.10±0.72 and 22.80±1.79 mmol/L and the control values were 6.82±0.78 and 18.00±1.79 mmol/L.

## DISCUSSION

In this study the $LD_{50}$ for ethylacetate extract in both rats and mice that was found to be non toxic, while ethanol leaf extract that was found to be slightly toxic in mice using intraperitoneal (IP) route have not been reported elsewhere. However, the $LD_{50}$ by oral route for the ethylacetate extract that was found to be 3.3 g/kg in rats showed this plant needs to be consumed with caution especially when higher therapeutic doses are required. The N-butanol leaf extract that was found to be relatively toxic in rats and mice using IP route have not been reported to the best of our knowledge. The relative non toxicity of aqueous leaf extract of *Byrsocarpus coccineus* after IP administration observed in this present study did not quite agree with the report of Adeyemi and his colleaques (2010) in which the aqueous leaf extract of was found to be slightly toxic in mice. However, the aqueous leaf extract that was found to be practically non toxic orally by Adeyemi et al (2010) was not carried out in this study. Ethylacetate leaf extract of *Byrsocarpus coccineus* appeared to have potentiated the delivery of pregnant rats on days 21 of pregnancy, when compared with those rats that took only the negative control (dimethyl sulfoxide) that led to their delivery on days 24 and 25 of pregnancy. On the other hand, 2 IU/kg of Oxytocin was able to induce abortion in all the rats, while the group given ethylacetate leaf extract had normal delivery on day 21 and 22 of pregnancy. The effect of ethylacetate leaf extract on the pregnant rat uterus supports the traditional use of this plant in gynaeco-obstetrics to induce labour at terms especially when mothers are at risk of their live (Irvine, 1961).

The administration of ethylacetate leaf extract of *Byrsocarpus coccineus* at a dose of 250 mg/kg to rats for 7 days appears to increase the Hb values when compared with control. This increase may be an indication that the extract has haemolytic effect thereby destroying the red blood cells to allow the haemoglobin to be freely in circulating blood. Wiam *et al* (2006) and Effriam *et al* (1999) reported that some plants have haemolytic properties in rats. The increase in haemoglobin concentration observed in the treated rats may be due to haemolysis which has allowed free haemoglobin in the plasma.

Treatment of rats with the extract at a dose of 250 mg/kg for seven days appeared to have stimulated increased production of WBC and platelets significantly. This could be the result of possible stimulation of immune defense system (Kashinath, 1990). Furthermore, it has been reported that some plant products seem to stimulate body defense mechanism to fight against foreign

substance (Gupta, 1994). Wiam *et al* (2006) also reported that, aqueous leaf extract of *Cassia siamea* suppresses erythropoietin and enhances leucopoietin and thrombopoietin of wistar albino rats. Moreover, results have shown that persistent antigen load in the body would result in increased WBC and platelets count (Schalm *et al*., 1975). Though there were no significant changes in the RBC values, the anaemia may have resulted from development of lesions in the organ of biotransformation and elimination which might have affected the RBC production by the bone marrow. It may also have occurred as a result of haemolysis. The observed increase on day seven of treatment may be due to haem concentration in the cardiovascular system. The measurement of the activity of marker enzymes in the serum plays significant role in the assessment of drug and plant extract for safety/toxicity risk. The enzymes considered in this study, are useful marker enzymes of liver cytolysis and damage to the plasma membrane of the liver cells. Alkaline phosphatase is a marker enzyme for the plasma membrane and endoplasmic reticulum of the tissue studied. It is often employed to assess the integrity of the plasma membrane, since it is localized predominantly in the microvilli of the bile canaliculi located in the plasma membrane. Since alkaline phosphatase hydrolyses phosphate monoesters, the enzyme hyperproduction within the 7 days of treatment could constitute a threat to the life of the cells that are dependent on a variety of phosphate esters for their vital process, as it may lead to indiscriminate hydrolysis of the phosphate ester metabolites of the liver. Serum and urea concentrations are used for the assessment of renal sufficiency (Smith *et al*., 2006). Higher than normal levels of serum creatinine and urea are indications of deficiency in renal function (Whelton *et al*., 1994). Thus, the decrease in serum creatinine concentrations with concomitant decrease in the serum urea concentration in the treated rats suggests that functioning of the kidney is normal. Alanine aminotransferase (ALAT) or Glutamic pyruvic transaminase (SGPT) is found in serum and variuos bodily tissues, but is most commonly associated with the liver. Significant elevated levels of ALAT often suggest the existence of medical problems such as viral hepatitis, diabetes, congestive heart failure, liver damage, bile duct problems, infectious mononucleosis, or myopathy. For this reason, ALAT is commonly used as a way of screening for liver problems. Elevated ALAT may also be caused by dietary choline deficiency. However, elevated levels of ALAT do not automatically mean that medical problems exist. A fluctuation of ALAT levels is normal over the course of the day, and ALAT levels can also increase in response to strenuous physical exercise. In this study, there was no significant difference observed in the levels of ALAT between the treated group and the control group, suggesting that the extract may not be toxic to the liver at the dose given to the rats for the period of seven days. Aspartate aminotransferase (ASAT) or serum glutamic oxaloacetic transaminase (SGOT) is a pyridoxical phosphate dependant transaminase enzyme. It catalyzes the reversible transfer of an α-amino group between aspartate and glutamate and as such is an important enzyme in amino acid metabolism. ASAT is found in the liver, heart, skeletal muscles, kidney, brain and red blood cells, and it is commonly measured clinically as a marker for liver health. ALAT is more specific indicator of liver inflammation than ASAT. In this study, it has been observed that there was significant difference of ASAT levels between the treated groups and the control. This again suggests that the extract may not be toxic to the liver since ASAT is not predominantly in the liver. Alkaline phosphotase is a hydrolase enzyme responsible for removing phosphate group form many types of molecules including nucleotide, proteins, and alkaloids. High levels can show that the bile ducts are blocked. Levels are significantly higher in children and pregnant women. Elevated levels also indicate that there could be active bone formation occurring as it is a byproduct of osteoblast activity. Levels are also elevated in people with untreated Celiac Disease. Lower levels of alkaline phosphotase are less common than elevated levels. In this study, there was an elevation of alkaline phosphotase which indicates that probably the extract might have caused blockade of the bile duct and or active bone formation in the rats.

## CONCLUSION

*Byrsocarpus coccineus* has been found to contain a lot of bioactive phytochemical compounds which may be responsible for the observed uterotonic effects of the ethyl acetate leaf extract on pregnant uterus of albino rat. This amply justifies the traditional use of this plant in augmenting delivery when women are at terms.

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