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Evaluation of the Systemic Effects of Topical Application of *Jatropha Curcas* Crude Latex, on Intact Skin and Wounds in Wistar Rats.

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ABSTRACT
Jatropha curcas is a flowering plant used locally for treatment of skin lesions, wounds, oral candidiasis and as a purgative. This study is aimed at evaluating the systemic effects of Jatropha curcas sap on the full blood count parameters and renal function following topical application on intact and wounded skin in Wistar rats. These rats were placed in four groups (A, B, C, D) of 6 wistar rat each. A 3cm long incision was inflicted at the back of rats in group A of and were treated with the latex of Jatropha curcas. Group C had the crude sap of Jatropha curcas applied on the shaven intact skin. These were compared with groups B that had same size of wound and was treated with 0.9% normal saline and the last group (group D) had no wound nor treatment. The animals were sacrificed after four weeks. Blood samples were collected for biochemical assays. Following application of the latex of Jatropha curcas to the wound, there was formation of blood clots in 5 seconds. There was a significant decrease in the total white blood cell count in the groups treated with Jatropha curcas. There was increased neutrophil count in the groups treated with Jatropha curcas with decreased lymphocyte count when compared to the control. There was no significant difference ($p<0.05$) in the results of the electrolyte urea and creatinine. There was no statistical difference in the renal function parameters following topical application of Jatropha curcas latex on intact and wounded skin when compared to the control. The result shows that application of Jatropha curcas latex decreased total white cell count with no effect on the renal function and serum electrolytes.

Keywords: Wound, Jatropha cucas, blood clots, anaesthesia, incision.

INTRODUCTION
Jatropha curcas is a species of flowering plant in the genus Jatropha in the spurge family; Euphorbiaceae. The plant is found in American tropics mainly[1]. It is cultivated in tropical and subtropical regions of the world[1]. It is a semi- evergreen shrub reaching a height of 6 metres[1]. The leaves are green to pale green, alternate to sub opposite and three to five-lobed with a spiral phyllotaxis[2]. Male and female flowers are produced on same inflorescence with a ratio of about 20 male flowers to each female flower[3]. The petiole length ranges from 0.6cm to 2.3cm. The seeds are usually produced in the later part of the year and changes from green to yellow and later brown when dry[2].

It has been shown that leaf extract of *Jatropha curcas* formulated into ointment base applied topically to wound in albino rats had no effect on the liver function test parameters[4]. There was no significant difference in the levels of total protein, albumin, and globulin content, when incised wounds in rats were treated with leaf extract of this plant. Histopathological examinations of the kidney and liver cells of the rats showed that they had normal histological features[4].

The effect of *Jatropha curcas* on the kidney functions of broiler chicks were assessed using levels of serum creatinine and urea. The birds were fed with grounded seed of *Jatropha curcas* for four weeks after which the level of serum urea and creatinine assayed for. There was significant elevation of serum creatinine and urea levels at $p<0.05$, when compared with the control[5].

Jatropha curcas is also a poisonous plant. All parts of the plant are poisonous when taken in large quantity via oral route. Symptoms are largely those associated with gastrointestinal irritation. There is acute abdominal pain and burning sensation in the throat about half an hour after ingestion of the seeds, followed by nausea, vomiting and diarrhoea. The vomitus and faeces may contain blood[6]. In severe intoxication, dehydration and haemorrhagic gastroenteritis can occur. There may be Central Nervous System and cardiovascular depression and collapse. Children are more susceptible[6].

The fertility regulatory effect of the fruit of *Jatropha curcas* was investigated by oral administration of different extracts to pregnant rats for varying periods of time. Foetal resorption was observed with methanol, petroleum ether and dichloromethane extracts indicating the abortifacient properties of the fruit. The results also suggest that the interruption of pregnancy

occurred at an early stage after implantation. This effect could be accomplished even when the extracts were given from the 6th to the 8th day of pregnancy[7]. Loss of body weight during the dosing period, ranging from slight to severe was seen in the treated animals[7].

This plant is commonly used among farmers in Edo state of Nigeria and other part of Nigeria as purgative, treatment of superficial wounds and treatment of some skin lesions. Hence the need for this study to evaluate the effect of topical application of the latex of Jatropha curcas. This will give an insight into the effect of the latex of this drug on the renal function and some haematological parameters.

MATERIALS AND METHODS

Consent was obtained from the Ethics and Research Committee of the College of Medicine, University of Benin, Benin City.

Materials: Surgical blade, dissecting set, sterile universal bottles, EDTA bottles, lithium heparin bottles.

Experimental design

This study was carried out in the Anatomy Department of the University of Benin, Benin City. The plant, was identified at the Botany Department of the University of Benin, Benin City. The sap of the plant was collected by making a cut on the stem of the plant thus allowing the sap to run freely into a sterile universal bottle. The sap was covered and placed in an ice pack and used fresh. It was transported to the laboratory in an ice pack. The latex allowed to warm up to room temperature before use.

This study involved the use of 24 Wistar rats which were randomly placed in 4 groups of 6 rats each:

Group A: The group consisted of 6 Wistar rats with wound inflicted on their back. This involved the creation of a surgical wound at the back of the rat. The rat was anaesthetized with chloroform. The skin at the back of the rats was prepared by first shaving the hair. The skin was then cleaned with chlorhexidine and then 70% alcohol. After which the rat was placed on a clean board which was also cleaned with chlorhexidine and 70% alcohol. An incision was made at the back of the rat which was 3cm long by cutting through the full depth of the skin and subcutaneous tissue. This group had their wound treated with the latex of *Jatropha curcas*, every 48 hours by applying it topically on the wound.

Group B: This group consisted of 6 Wistar rats. This involved the creation of a surgical wound at the back of the rat. The rat was anaesthetized with chloroform. The skin at the back of the rats was prepared by first shaving the hair. The skin was then cleaned with chlorhexidine and then 70% alcohol. After which the rat was placed on a clean board which was also cleaned with chlorhexidine and 70% alcohol. An incision was made at the back of the rat which was 3cm long by cutting through the full depth of the skin and subcutaneous tissues. These wounds were treated with 0.9% normal saline, every 48 hours by applying it topically on the wound.

Group C: This group consisted of 6 Wistar rats which had a portion of their skin over the back shaven and delineated with and indelible ink. The latex of Jatropha curcas was applied on the skin of the rat once every 48 hours for 4 weeks, to the shaven portion of the back.
Group D: this group is the control consisting of 6 Wistar rats. This group has no wound on their skin. They were fed with growers mash for 4 weeks. The rats were kept in separate cages (one rat per cage). They were fed with growers mash (Top feeds).

Dissection

After 4 weeks the experimental animals were all sacrificed and dissected. This involved anaesthetizing the animals with chloroform, and the animals were sacrificed. A midline abdominal incision was made to access the abdominal cavity. The blood was collected from the abdominal aorta with the aid of a sterile 5ml syringe and needle (18G). This blood was placed in Ethylenediaminetetraacetic acid (EDTA) bottle and Lithium heparin bottle, 2.5ml each.

Biochemical analysis

The Electrolyte urea and creatinine were assayed, using a spectrophotometer two hours after collection of the samples.

Haematological analysis

The full blood count was also done using the haemocytometer. This was done an hour after collection.

Statistical analysis

Statistical analysis was done using Analysis of Variance (ANOVA). P values of 0.05 or less were taken as significant and mean differences were compared using a multiple comparison test[8]. The mean and standard error of mean for each of the groups of animals were calculated and recorded.

RESULTS

There was formation of blood clot over the wounds within 5 seconds of application of the crude latex of *Jatropha curcas*. This sealed up the wound surface and prevented bleeding. (Plate 1). The wound treated with normal saline showed minimal blood clot after 2 minutes of application. (Plate 2).

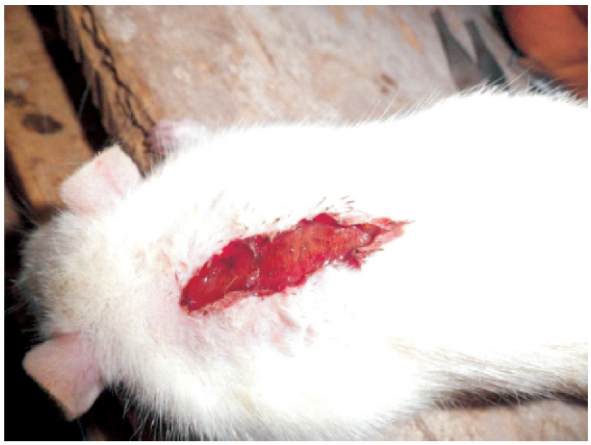

Figure 1: Wound treated with Jatropha curcas showing blood clot five seconds after application of the extract (Group A)

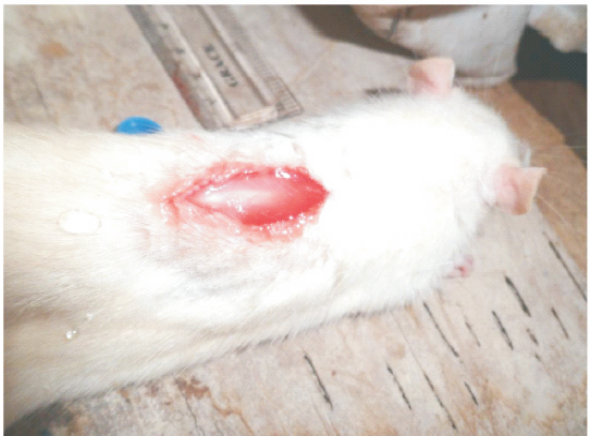

Figure 2: Wound treated with normal saline showing minimal clot formation after two minutes of application (Group B)

Full blood count

The values of white blood cells count (/UL) for the experimental groups: A, B and C were 6850±321.2, 7000±516.4 and 7650±394.8 respectively and that of control was 7783.3±357.2. There was statistically significant decrease in the experimental groups when compared to that of the control-Group D (figure 3).

The values of Neutrophils in percentage for the experimental groups (A, B and C) were 30±2.4, 40.5±3.8 and 27±2.3% respectively while that of the control group was 27.2±2.3. There was statistically significant increase in the experimental groups treated with normal saline (group B) and that treated with *Jatropha curcas* latex (group A). The experimental group C showed no statistically significant difference when compared to that of the control group D (figure 3).

The values of Lymphocyte in percentage for the experimental groups (A, B and C) were 70±2.4, 61.5±4.0 and 72.8±2.3 respectively while that of the control group was 72.7±2.3. There was a statistically significant decrease in the experimental group A and group B but the experimental group C showed no statistically significant difference when compared to that of the control group D (figure 3).

The value of platelets (/UL) for the experimental groups (A, B and C) were 176X103±4497.5, 191X103±4805.7and 194X103±11448.4 respectively while that of the control group was 209X103±7551.7. There was statistically significant decrease in the experimental groups when compared to that of the control group D (figure 4).

Haemoglobin concentration (g/dl) for groups A, B and C were 15.9±0.6, 16.4±0.4, and 15.7±0.4 respectively. The value for the control (group D) was 15.6±1.1. There was no statistically significant difference between the experimental groups when compared to the control group D (figure 2)

Biochemical findings

There was no statistical significant difference in the values of serum urea, creatinine, sodium potassium, chloride and bicarbonate at $p<0.05$ for groups A, B and C, when compared to the control group D (figure 1)

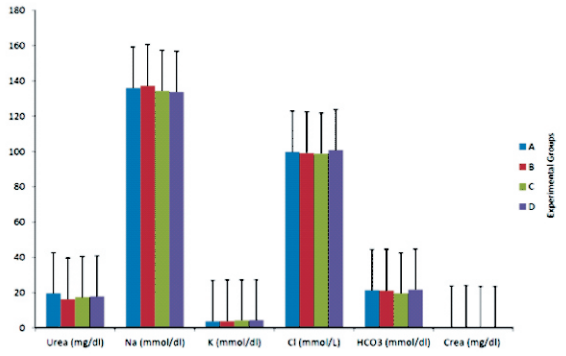

Figure 3: The chart above shows the values of electrolytes, urea and creatinine in each of the groups with no statistically significant differences at $p \leq 0.05$

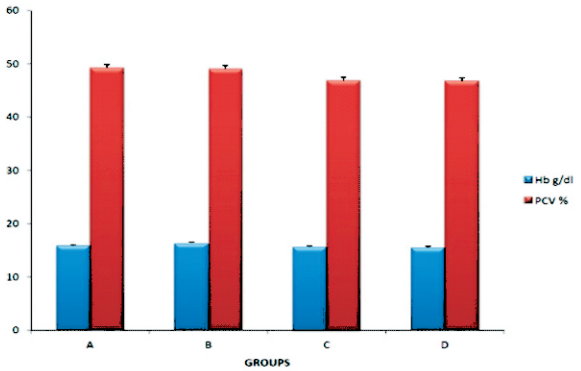

Figure 4: Shows the values of haemoglobin (g/dl) and packed cell volume (%) in the control and experimental groups with no statistically significant difference at p value ≤ 0.05.

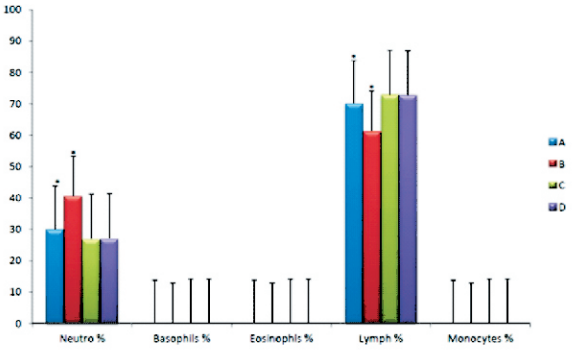

Figure 5: Shows there was a statistically significant difference in neutrophil and lymphocyte values in A and B experimental groups compared to the control group D.

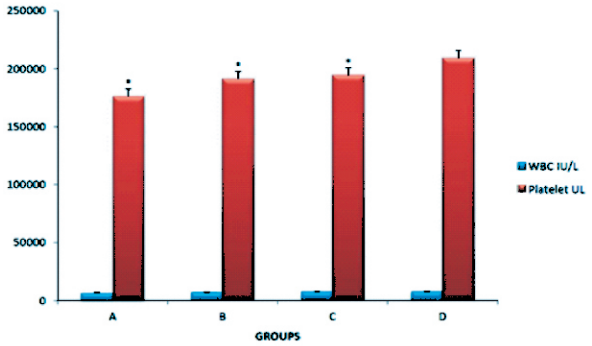

Figure 6: The figure above shows statistically significant difference in the value of white blood cells and platelets values in all the experimental groups at p value $\leq$0.05 when compared to the control groups

DISCUSSION

The haemoglobin concentration and packed cell volume of the experimental groups showed that there was no significant statistical difference when compared to the control as shown in figure 2. This corroborated a study by Sarkiyayi *et al*, 2016 in which the packed cell volume of rats infected with malaria parasites and treated with aqueous stem bark extract of Jatropha curcas had improved pack cell volume[9]. This was contrary to a study by Ojo et al 2003 in which broiler chicks were fed with the crushed seed of this plant and it resulted in decreased packed cell volume after 4 weeks. This suggests that topical application of this latex have no effect on the haemoglobin concentration. The latex of this plant may have little or no effects on suppression of haematopoiesis[5].

Following injury there is infiltration of the wound with polymorpho-nuclear leukocyte which peaks at 24-48 hours. Their main function is phagocytosis of bacteria and debris[10]. In this study the Neutrophil count in groups A and B (the groups that had wound inflicted on them) shows significant increase at $p< 0.05$. This is comparable to the work done by Sarkiyayi et al, 2016 in which elevated white blood cell were observed in malaria infected rats treated with aqueous stem bark extract of *Jatropha curcas*[9]. The increase in neutrophils may be a metabolic response to the injury as well as a stimulatory effect of the latex of *Jatropha curcas* on the immune system, which is necessary for wound healing.

Lymphocyte usually invade wound after injury and peak in about a week. It aid wound healing, but it function is not fully understood[11]. In this study there was a statistically significant decrease in the lymphocyte count of the animals in the groups treated with *Jatropha curcas* when compared to the control group.

There was statistically significant decrease in the platelet count experimental groups when compared to that of the control group. The platelets count was significantly decreased in groups A and B. This is comparable to the works of Igbinosa et al 2013 in which higher doses of methanol leaves extracts of *Jatropha curcas* resulted in decreased levels of platelet count[12]. This decrease may be attributed to depletion of platelets following response to the injury by aggregating platelets to the site of injury for blood clot[12]

The electrolytes urea and creatinine of the experimental groups showed no significant statistical difference at $p<0.05$ as shown in figure 1. This shows that topical application of the latex of Jatropha curcas has no effects on serum sodium, potassium bicarbonate and chloride concentration. The serum creatinine and urea were normal which a reflection of good renal function. Previous study showed the effects of *Jatropha curcas* on the kidney functions of broiler chicks. The levels of serum creatinine and urea were assessed after feeding the birds for four weeks with grounded seed of Jatropha curcas. The level of serum urea and creatinine assayed for. There was significant elevation of creatinine and urea levels at $p<0.05$ compared with the control[5]. This suggested that the latex may not be harmful and the dose required for nephrotoxicity may not be achieved via topical application.

CONCLUSION

This study showed that topical application of Jatropha curcas has no deleterious effect on the renal electrolyte urea and creatinine level and the packed cell volume. This suggest that topical application of this latex has no deleterious effect on renal function. There was a significant increase in the neutrophil count and a statistically significant decrease in the platelets count.

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