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Histological and Haemostatic Effect of Aqueous Extract of *Lycopersicon esculentum* (Tomatoes) On Wound Healing and Liver Cells of Male Albino Wistar Rats.

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ABSTRACT

It is often believed that most plants in ethno-medical practices possess medical values, hence for thousands of years, people have resorted to medicinal plants as source of healing. The histological and haemostatic effect of aqueous extract of *Lycopersicon esculentum* (Tomato) on wound healing and the liver cells of male albino wistar rats was investigated using seventy (70) rats weighing 150-180g. The control group was fed normal rat feed and drinking water ad libitum while the test rats were given normal rat feed and water ad libitum in addition to administration of 1.0ml once daily of the fruit extract of *Lycopersicon esculentum.* Blood samples were obtained from the tails of the animals into EDTA bottles and sodium citrate bottles for analysis, later the animals were stunned and their liver extracted and placed in formalin for tissue processing and examination. The blood samples were examined for haematological and haemostatic test. Result indicate that the extract of *Lycopersicon esculentum* has no significant effect on haemoglobin concentration, packed cell volume and white blood cell count, while there was a significant effect on the platelet count (P<0.01), Bleeding and Clotting time (P<0.05) resulting to shortening of their time. The histological result showed no distortion in the cyto- architecture of the livercells. It could be deduced from this work that *Lycopersicon esculentum* contains biological principle that can affect haemostasis.

INTRODUCTION

Crude extracts of some plants could play roles in hastening the haemostatic activities in a damaged tissue when applied topically on the wound or cut to arrest bleeding and hasten healing of wound. Such plant is *Lycopersicon esculentum* (Tomato). This study is to know the histological and haemostatic effect of aqueous extract of *Lycopersicon esculentum* on wound healing and liver cells of male albino wistar rats.

Wound healing is the process of repair following injury to the skin and other soft tissues. Initial stages of wound healing involve an acute inflammatory phase followed by synthesis of collagen and other extracellular matrix which are later remolded to for a scar (Ligha et al, 2008).

Wound healing is influenced by many factors including the kind of medicine used to accelerate the wound healing process and to prevent infection (Prockop et al, 1995). The healing response is characterized by the movement of specialized cells into the wound site. Platelets and inflammatory cells are the first to arrive at the site of injury and they provide key functions and "signals" needed for the influx of connective tissue cells and a new blood supply. These chemical signals are known as cytokines or growth factors (Lawrence et al, 1994).

Lycopersicon esculentum belongs to the same family with eggplant and potato, solanacae (Ogundipe 2004). It is one of the most widely grown vegetable crop in the world and also one of the common vegetables grown in home gardens and in every garden in Nigeria.

Chemical contents and Medicinal value:

Lycopersicon esculentum contains calories, protein, carbohydrates, fat, sodium, potassium, dietary fiber, vitamin A (20%), vitamin C (40%), potassium (10%), Iron (2%) (Peralta et al, 2001). They are rich in calcium and phosphorus.

Being rich source of vitamin A, *Lycopersicon esculentum* are dependably preventive against night blindness, short sightedness and other diseases of the eye.

The liver is the largest organ in the body (Baker et al, 1998) and from metabolic stand point is the most complex. Tests of its many functions have been devised in the hope that they will serve as diagnostic aids when a metabolic process has been disturbed. The adult liver weighs about 1500g is located beneath the diaphragm. It produces coagulation factors.

Homeostasis is a process of arrest of blood loss (Guyton, 2006). Whenever a tissue is damaged, haemostatic activity occurs by successive mechanisms which include vascular spasm, formation of blood clot, platelet aggregation and growth of tissues into the blood clot.

MATERIALS AND METHODS

Selection of Animals:

For the haemostatic and histological studies, seventy (70) rats were used, weighing 150-180g. Male rats were kept separately for 30 days feeding.

Preparation of Extracts:

The method used here is as described by Samuelson et al 1992.Fresh fruits *Lycopersicon esculentum* were collected, weighed, washed, cut and blended ina mortar with a pestle. Thereafter, 50mls of the extract was filtered out using Whatman No.1 filter paper. The extract was dried by evaporation to dryness .0.5g of the dried extract was dissolved in 1000 ml of the sterile water to give to the animals. They also received their normal rat pellets and drinking water along with the control rats ad libitum.

- **Phytochemical analysis of the fruits**

Toxicity study:

The LD_{50} of the extract in albino Wister rats was determined using Lorke's method. The procedure of determining the lethal dose is by increasing the concentration of the extracts administered to the rats (after weighing them) ineach of the groups consisting of eight (8) rats per group for five (5) days.
The concentration given was at the rate of 1000mg/kg, 1500mg/kg, 2000mg/kg, 2500 mg/kg, 3000mg/kgand 4000 mg/kg. The percentage rate of their death and survival was noted and a graph was plotted to determine the LD_{50}.

Haemostatic mechanism test:

- The haemoglobin, packed cell volume and WBC counts were done using the method described by Baker et al, (1998).
- Platelet count estimated was done by the method of Brecher and Cronkite (1950).
- The whole blood clotting time was estimated by the method of Lee and White.
- The bleeding time was carried out by the method of Dejana.

Histological studies:

The histological sections of the liver were stained with Eosin and haematoxylin (H&E staining) as described in Baker et al 1985. These studies were later examined by a histologist.

Phytochemical analysis of fruits was done using standard phytochemical screening method described by Trease and Evans (1985), Harban (1972).

Experimental Designs:

This study was divided into two (2) different studies namely: Study A and Study B.

Study A: It is the oral administration of the aqueous extracts of *L. esculentum* to the test rats Group B (50), while the control rats Group A (20) received rat feed. This lasted for 30 days.

Study B: in this study, prothrombin time (PT) and partial thromboplastin time with kaolin test (PTTK) were done to establish the possible pathway of the action of the extracts. The concentration of extracts used was 5mg/kg.

Collection of samples for study:

2.0ml of blood sample was collected from each rat in all the groups (A&B) into EDTA bottle to determine the initial blood pictures before feeding them on the extract. Their weight and full blood count (FBC) including platelet count, bleeding time, clotting time, prothrombin time and partial thromboplastin time kaolin were monitored. At the end of 30 days of feeding on the extract, blood samples were again obtained from the tails of the animals for the various tests as monitored earlier. The liver of the test rats and control rats were extracted by stunning the animals and using sterile blood lancets to remove them, they were kept in a formalin container and later used for tissue processing and staining.

Statistical Analysis:

The result obtained in the study for haemoglobin concentration (mg/dl), packed cell volume, WBC count, platelet counts, bleeding time prothrombin time test, partial thromboplastin time kaolin and clotting time test were represented as mean and standard deviation (Mean ± S.D), while students-t-test was used to compare the result of the control and tests a P value of less than or equivalent to P<0.05 or P=0.05) was considered statistically significant.

Results

Table 1: Phytochemical study

	Alkaloids	**Glycosides.**	**Calcium.**	**Carbohydrate.**	**Flavonoids, Steroids, Tannins, Fats & Oil, Saponins, Terpenoids.**
Degree of concentration	+++	++	+++	-	-

Key: - (Negative) + (Present in small concentration) ++ (Present in moderate concentration, +++ (Present in large concentration).

Table 2: Haematologicalindicies of albino wistar rats before the administration of extracts and 30 days following the administration of *L. esculentum.*

Groups	**Hbg/dl ± S.D**	**PCV L/L ± S.D**	**Platelets count * 10^9 ± S.D**	**WBC count/mm^3 ± S.D**	**Bleeding time sec± S.D**	**Clotting time sec± S.**
Group A (control) n=10, Extract free	12.3± 0.4	36.9 ±0.3	174 ± 402	5,246 ± 0.5	2.5 ± 0.8	5.8 ± 0.5
Day 1 before *L.esculentum*	12.0± 0.5	36.0± 0.5	168 ± 315	5,830± 0.3	2.7 ± 0.5	5.7± 0.6
30 days after administration of *L.esculentum* Group B (50)	11.2 ± 0.7	33.6± 0.9	172±418	5,190±1.6	1.6 ± 0.5	3.7 ± 0.5
Significance	P>0.05	P>0.05	P>0.01	P>0.05	P<0.01	P<0.01

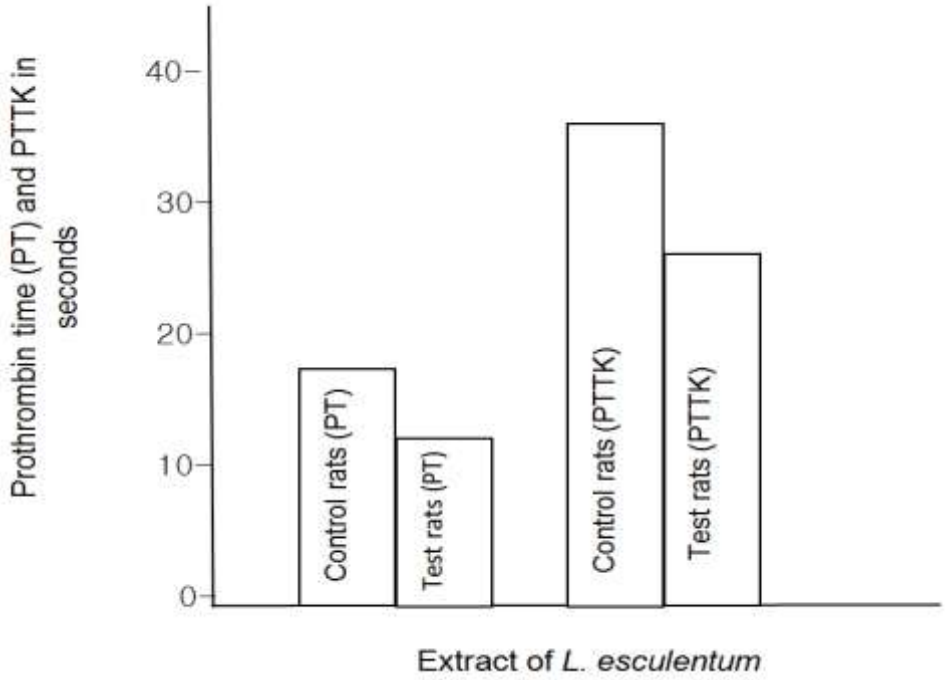

Figure 1: The effect of *L.esculentum* on the prothrombin time and partial thromboplastin time kaolin of albino wistar rat

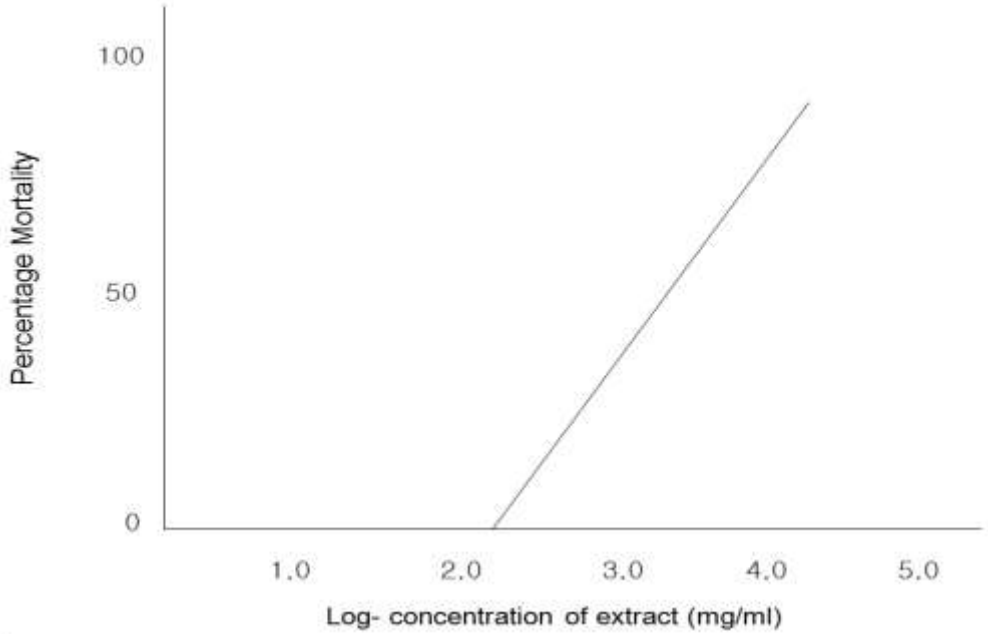

Fig 2: Lethality studies indicating the effects of administering graded doses (1,000mg/kg I.P rat) of the fruit extract of *L. esculentum* (tomatoes) against the percentage mortalities. LD_{50}= 2,500mg/kg body weight.

DISCUSSION

The histological and haemastatic effect of aqueous extract of *Lycopersicon esculentum* (Tomatoes) on wound extract healing and liver cells of male albino wistar rats has been studied.

The result of the lethal studies showed that the LD_{50} in rats using extract of *L. esculentum* was 2,500mg/kg (Fig.2). The dose used in this study (5mg/kg) was far below the lethal dose and so was considered safe to the animals used throughout the period of study. This therefore shows that the result obtained with the plant material was authentic and nontoxic. The phytochemical analysis of the extract indicates the presence of typical plant constituents (Okoli et al, 2007) such as alkaloids, calcium, saponins, steroids, terpenoids, carbohydrates, glycosides, tannins, acidic compounds in the extract.

The alkaloids (protein precipitants) and calcium contained in this plant extract are known to be involved in the precipitation of coagulation factors (Taofeeq et al, 2005) for clotting to occur fast.

On red cell indices, there is no significant change in both the Hb concentration PCV in rats fed with the extracts of *L. esculentum*. This agreed with the rich vitamin C, protein, and iron contents earlier reported (Peralta et al, 2001). *L. esculentum* extract has no effect on white blood cell count but has a little effect on increasing platelet count (Table 2). The extract arrested bleeding time hence demonstrating haemostatic activity. The prothrombin time and partial thromboplastin time were shortened by the extracts of *L. esculentum*. The high concentration of calcium and alkaloids in *L. esculentum* may have contributed to its ability in shortening bleeding and clotting time. On the histological results of the state of the liver cells, it could be noticed that there was high improvement of the performance of liver cells in the test rats than in the control rats. There is no distortion of the cyto-architecture of the liver cells. This tallies with the haematological and haemostatic results hence the reduction in the bleeding and clotting time of test rats.

The liver plays an important role in the formation and destruction of red cells (Oguwike 2013). The liver also forms a large portion of the bloodsubstances utilized in the coagulation process. They are namely fibrinogen, prothrombin accelerator, prothrombin, accelerator globulin (factor V), factor VII and several other less important coagulation factors. Vitamin K is required by the metabolic process of the liver for the formation of prothrombin and factors VII. IX and X. It could be deduced from this work that the presence of high quantity of calcium and alkaloids in *L. esculentum* and high performance of liver cells in producing coagulation factors during the intake of *L. esculentum* extracts could have contributed to rapid healing of wounds in the rats.

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