Evaluation of Efficacy of Lemon Juice Extract (Citrius Lemoni Risso) on Wound Healing and Haemostatic Mechanism of Albino Wister Rats

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Abstract: ***The efficacy of Lemon (Citrus lemonirisso) juice on wound healing of albino wistar rat was investigated; also the potential haemostatic mechanism associated with administration of the extract was investigated. Results showed that lemon juice extract decreased haemoglobin concentration, packed cell volume while it has no significant effect on platelet count, white blood cell count and white cell differential counts in albino rats. Furthermore, the bleeding and clotting times were shortened and the period of healing of wound using lemon juice could possess some elements that is affecting the haemostatic mechanism.***

Keywords: Haemostasis, haemoglobin, bleeding time, lemon juice, platelet count, wound healing.

1. Introduction

Crude extracts of some plants could play roles in hastening the haemostatic activities in a damaged tissue or vessels when applied topically on the wound or cut, to arrest bleeding and hasten healing of wound. This study on wound healing and haemostatic mechanism of albino wister rats is to ascertain the acclaimed facts by ethnomedical practitioners of its healing ability and to ascertain its safe usage in humans. This study also evaluated the effect of this lemon juice extracts on bleeding time, clotting time, platelet count, haemoglobin level, packed cell volume, white blood cell count and white blood cell differentials of albino rats.

Wound healing is the process of repair following injury to the skin and other soft tissue. Initial stages of wound healing involve an acute inflammatory phase followed by synthesis of collagen and other extracellular matrix which are later remodeled to form a scar (Ligha et al 2008). Wound healing is influenced by many factors including the kind of medicine is 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 cells 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).

Lemon tree is a perennial tree of the citrus family-rutaceae. It grows up to 3m. it has toothed, ellipitical or lanceolate leaves, pointed. It flowers are with inside, rosy at the argin of the petals. The fruit is a herperidum till 12.5cm wide, with a thick rind, dark yellow when fully ripe. It is cultivated because of its fruits and as a garden tree in warm Meriditerranian places next to the sea. It probably descends from the species *Citrus medica* L, "native from India". It grows successfully in the Eastern part of Nigeria W. Africa.

2. Medicinal Value of Lemon (C*itrus Limonium Risso*)

Lemon is an antioxidant which deactivates the free radicals preventing many dangerous diseases like stroke, cardiovascular disease and cancers. It also fights against infection. It helps in production of WBC's and antibodies in blood which attacks the invading micro organisms and prevents infection. Its healing ability could be due to its bacteria ridding and astringent properties. Lemon juice when applied in the site of bites and stings of certain insects relieves its poison and pain. It relieves chilblains and itchy skin. Its application to acne (pimples) dries the existing ones and prevent from getting more. Chemical contents of citrus limonum risso: its main active ingredients are flavenoids, Ascorbic acid (vitamin c), essential oil (Lignan 2005), caffeine, pectin, minerals especially potassium and calcium, water fibers and sucrose, (Wikipedia 2013).

3. Materials and Method

The phytochemical analysis for the constituents of the plant extract $LD_{50}$of the extracts were studied before the haematological and haemostatic experiments were done Animals: Sixty healthy adult male and female albino rats of wistar strain weighing between 180-200g were used in the study. The animals were housed separately, keeping males and females apart under standard conditions of temperature (23 ± 2^0 c) and humidity, receiving 12h light (7:00a.m-7:00pm). They were kept in wire meshed cages and fed with commercial rat pellets and drinking water ad libitum. The animals were handled in accordance with national and institutional guidelines for the protection of animal welfare.

4. Experimental Design

The animals were randomly assigned into three groups of 20 rats each. Group A served as control and was treated with normal saline. Group B (males) and C (females) was the test groups treated with lemon juice extracts orally and topically respectively.

4.1 Preparation of Extract

Fresh fruits of lemon were selected, weighed, washed, cut and their juice contents pressed out in a clinical glass beaker. There after 100mls of the juice filtered out using Whatman no.1 filter paper: the juice was dried by evaporation to dryness 0.5g of the dried extract was dissolved in 100ml of sterile water to give a concentration of 5mg/kg. This was given to the animals with blunt sterile needle while giving them their normal rat pellets and drinking water ad libitum.

4.2 Phytochemical Analysis of Lemon Juice

The pressed out lemon juice was screened for the presence or absence of various secondary metabolites that could be of therapeutic values using standard phytochemical screening procedures described by Harbourne (1973) Trease and Evans (1996). The extract was tested for Glycosides, Flavenoids, Alkaloids, Tannins, Reducing sugar, Calcium, Saponins, Acidic compounds, Resins, Fat and Oil, Carbohydrates and steroids.

4.3 Toxicity Study

The LD_{50} of the extracts in albino rats was determined by Lorke's (1981), The procedure of determining the lethal done is by increasing the concentration of extracts administered into rats (after weighing them), in each group consisting of eight (8 rats) per group for five (5) days. The concentration was given at the rate of 100ml/kg, 1,500mg/kg, and 2000 mg/kg. The percentage rate of their death and survival is noted and a graph plotted to determine the LD_{50}. The Haemoglobin estimation was determined by Baker et al (1985). The packed cell volume estimation was determined by simple method of microhaematocrit centrifugation method. The total white blood cell count and differentials were also determined by method of Baker 1985. The platelet count estimation was done by method of Brecher (1950). The whole blood clotting time was estimated by the method of Lee and White (1985). The bleeding time was determined by the method of Dejana et al (1982).

5. Inflicting Wounds on Albino Rats

The animals were made to acclimatize to housing condition in animal house for one week and were fed very well. Prior to the commencement of the experiment, the test rats were injected with 0.4ml of thiopentine injection to anaesthesize them. The area for wound infliction was chosen preferable on the back. The hairs were shaved off with surgical blade and lancet was used to cut the skin. The wounds was in form of square (the length and width) of the wounds were measured and the result expressed in centimeter. Equal area of wounds was given to both the control and the test rats. Measurement was taken on the first day, the wound was inflicted.

- The control rats were rubbed with normal saline while lemon juice extract was rubbed on the test rats.
- The wounds were treated every day and the areas measured to check difference in size in all the animals.

6. Collection of Blood Sample and Duration of Study

2.0ml of blood sample were collected from each rat in all the groups into EDTA bottle to determine the initial blood pictures before feeding them on the extracts. Their weights, full blood count (FBC), including platelet count, bleeding time, clotting time were measured. At the end of the acute study (28 days) of feeding on the extracts, blood sample were again obtained by cardiac puncture for the haemostatic and hematological analysis as was done initially.

6.1 Statistical Analysis

The data obtained from the study were expressed as mean and standard deviation (mean $\pm$ S.D) while student's t-test was used to compact the result of the control and the test. A p-value of less than or equivalent to ($p<0.05$) or $p=0.5$) was noted statically significant.

7. Results

Table 1: Indicates the photochemical study

	Alkal - oids	Acidic Compound	Reducing Sugar	Flaven - oids	Calcium	Potassium	Steroid, tannins, saponins, resin, terprnoids, fats and oils, glycosides.
Degree of Concen t-ration	+	++	++	++	+++	+++	-

-Negative (absent)
+ Present in small concentrations
++ Present in moderately high concentrations
+++ present in very high concentration

Table 2: shows the effect of lemon juice extract on duration of healing in albino rats. Lemon juice extracts fastened healing more than normal saline. ($p<0.05$)

Groups	*Extract*	*Days Mean ± S.D*	*P.value*
Control (n=20)	N/Saline	15±0.2	-
Test Rats Male (n=20)	Lemon Juice	9±0.5	P<0.05
Female (n=20)	Lemon Juice	8±0.7	P<0.05

Table 3: shows the effect of lemon juice extract on complete blood count, platelet count, bleeding and clotting times of albino wister rats

Extracts	*Hbg/dl*	*PCVL/L*	*Platelet coun x 10^9/l*	*Bleeding time Min ± S.D*	*Clotting Time Min ± S.D*
Control rat (extract free)	13.4 ± 0.5	40 ± 0.1	170 ± 16.0	2.7 ± 0.8	5.6 ± 0.5
Day 1 before lemon juice Male (n=20)	13.8 ± 0.7	41 ± 0.3	160 ± 42	2.8 ± 0.2	5.4 ± 0.7
Female (n=20)	13.7 ± 0.4	41 ± 0.5	180 ± 30	2. ± 0.2	5.4 ± 0.6
28 days administration of Lemon juice Male (n=20)	9.4 ± 0.6	27 ± 0.4	150 ± 10.0	1.5 ± 0.2	3.7 ± 0.8
Female (n=20)	9.1 ± 0.4	27 ± 0.2	152 ± 8.0	1.3 ± 0.3	3.4 ± 0.6
Significance	P<0.05	P<0.05	P<0.05	P<0.05	P<0.05

Table 4: White blood cell counts and differentials in albino rats before the administration of lemon juice and 28 days after the administration of lemon

EXTRACTS	*WBC/MM^3 ± S.D*	*N%± S.D*	*L%± S.D*	*M%± S.D*	*E%± S.D*	*B%± S.D*
Control rat (n=20) Extract free	4,120±650	52±1.5	44±0.7	3±0.4	1±0.6	0±0
Day 1 before Lemon juice Male (n=20)	4,200±420	50±0.8	47±1.7	2±0.6	1±0.5	0±0
Female (n=20)	4,400±250	53±1.2	45±1.4	1±0.4	1±0.2	0±0
28 days after administration of Lemon juice Male (n=20)	3,100±140	50±1.2	48±1.0	1±0	1±0.2	0±0
Female (n=20)	3000±260	51±1.6	47±1.5	1±0	1±0	0±0
Significance	P<0.05	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05

N=Neutrophils, L=Lymphocytes, M=Monocytes, E=Eosinophils, B=Basophils.

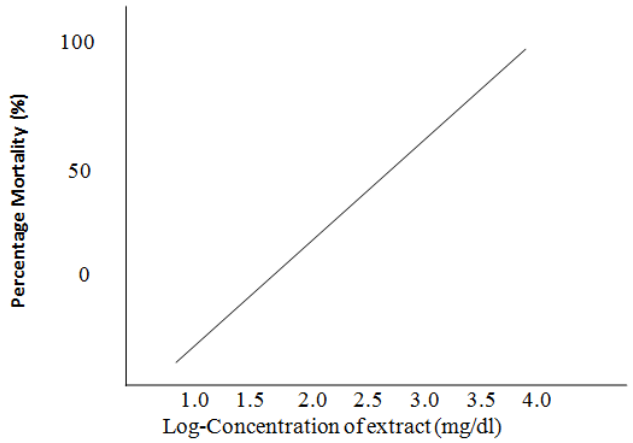

Figure 1: Lethality studies showing the effects of administering graded doses (1,000-4,000 mg/kg IP rat) of the lemon juice against the percentage mortality

8. Discussion

The efficacy of lemon juice extract on wound healing haemostatic mechanism has been evaluated. Most times plant extracts are given to humans in excessive doses by the ethnomedical practioners. Toxicity may affect the result of this study and so acute toxicity studies using LD_{50}were carried out before the extract was administered to the animals. The result of lethality studies showed that the LD_{50} in rats using lemon extract was 3,000mg/kg (fig. 1). The dose 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. Local medicinal herbs have been employed in the management of various diseases and their protective effect on the body from damage due to free radical and lipid peroxidation has been reported (Wikipedia, 2010). Evaluation of efficacy of lemon juice extracts on wound healing and haemostasis activity provides physiological information on a proper blood assessment in the body (Ita et al, 2007). In this study the test rats fed with lemon juice recorded significance decrease in Haemoglobin concentration and packed cell volume ($p<0.05$) Table 3.

The reported decrease in HB in rats treated in lemon juice by earlier workers (constable, 1963) indicates that the indiscriminate consumption of lemon juice could predispose to anaemia to susceptible individuals. Anaemia by definition is a state of lower than normal concentration of haemoglobin which can also result from low packed cell volume below 30% have been reported as indicative of anaemia (chen et al, 1998).On healing of wounds, it was observed that rats whose wounds were treated with lemon juice had their days shortened in wound healing (Table 1) compared with their corresponding control whose healing lasted longer (15±0.20 days). I imagine that it's due to its bacteria ridding and astringent properties. The presences of vitamin c in the citrus plant could also play role in the healing of wounds.

The bleeding and clotting times in the test rats were shortened ($p<0.05$) compared to their corresponding control. This could be due to moderate presence of calcium and potassium in the juice. Calcium ions are physiologically active in coagulation mechanics calcium ions are essential for the conversion of prothrombin to thrombin and for the normal action of the heart muscle and for neuromuscular conduction. The presence of calcium in the lemon juice might have contributed to the shortening of bleeding and clotting times respectively in albino rats. Calcium ions present in the extracts probably act in the intrinsic and extrinsic coagulation mechanic to convert prothrombin to thrombin, which in turn converts fibrinogen to fibrin strand (Seegers, 1950). Haemostasis involves the spontaneous arrest of bleeding from damaged blood (Okoli et al, 2007) vessels and the prevention of tissue death through haemorrage.

References

[1] Baker F.J and Silverton E (1985): investigation for haemostatic abnormalities. Introduction to medical Laboratory Technology pp 348. Butter Worths publication Woburn London

[2] Brecher and Cronkite (1950). Platelet count in Dacie J.V ed. Basic Haematological Techniques, Practical Melbourne.

[3] Chen L.T and Chang P.E (1998). Intrasplenic induced Anaemic Rats. Am. J. haematol 11: 403-407.

[4] Constable B.J (1963). Changes in blood volume and prothrombin time rat and Gunea pigs from Birth to Maturity. J physiology (London 197:229-238.
[5] Dejana E, Villa S de Galactano G (1982). Bledding time of rats. A comparison of different experimental conditions. Thromb. Haemostat 48:108-111.
[6] Ita S.O, Etim O.E, Ben E.E, Ekpo O.F (2007). Haematopoetic properties of ethanoic leaf extract of Ageratum conyzoides (Goat weed) in albino rats. Nigerian Journal of physiological sciences 22 (1-2) 83-87.
[7] Lawrence W.T, DiegeImann R.F (1994). Growth factors in wound healing clin dermatol 12: 157.
[8] Lee and White (1985): investigation for haem ostaticabnormalities". Introduction to Med. Lab Technology 6th Edition Butterworths publication, Woburn londonb pp 346.
[9] Ligha A.E, FAwehinin H.B (2008). Histological observations of the wound healing properties of plant exudates of Jatropha Curcas linn. Journal of Experimental and Clinical Anatomyy 7(1) 17-21.
[10] Lignan contents of Ducth plant foods; a database 1, British Journal of Nutrition 2005-Pubmed Result.
[11] Lorke D (1985): A new approach to practical acute toxicity testing. Arch. Toxical 54:275-278.
[12] Okoli C.O, Akph P.A, Okoli A.S (2007). Potentials of leaves of AspillaAfricana (compositae) in wound care: an experimental evaluation.
[13] Prockop D.J, Kivirikko K.J (1995). Collagen, molecular biology, diseases and potentials form therapy. Annual Rev. Biochem64:403.
[14] Seegers W.H, 1950. Blood coagulation and the practical significance of recent advance in knowledge of Prothrombin and A.C globulin circulation 1:2.
[15] The Roselle plant; Wikipedia 2010.
[16] Trease G.F, Evans W.C (1996). Textbook of pharmacology, 14th Ed. W.B Sauders, London pp 46-47, 832.