Greener Journal of Medical Sciences

ISSN: 2276-7797 ICV: 5.98

Submitted: 13/04/2016
Accepted: 19/04/2016
Published: 01/05/2016

DOI: http://doi.org/10.15580/GJMS.2016.2.041316077

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Research Article (DOI: http://doi.org/10.15580/GJMS.2016.2.041316077)

Evaluation of Activities of Aquoepus Extract of M Avocado (*Persea americana*) on Wound Healing and Biochemical Indices of Albino Wistar Rats

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ABSTRACT

The aqueous crude extracts of some plants could play a helpful role in hastening the coagulation system in a damaged tissue or vessels at the same time restore order in a diseased tissue, hence this research evaluated the activities of aqueous crude extract of Avocado (*Persae Americana*) on wound healing and biochemical profile of male albino wistar rats using Sixty rats. The animals weighing 180-200g were randomly selected and placed into five (5) groups group A (control), group B (Skin ulcered rats on extract), group C (rats with skin ulcers on normal saline), group D (wound incised animals on extract)and group E(incised rats on normal saline).All the test rats were 12 animals in each group. They were gavaged with 1ml of aqueous extracts of Avocado for twenty eight days (acute study) before blood samples were collected from them for investigations.

The results showed that avocado extract decreased haemoglobin concentration, Packed cell volume and White blood cell ($p<0.05$).It has no effect on platelet counts ($p>0.05$), it shortened days of healing in rats inflicted with wounds and those with skin ulcers. The bleeding and clotting times were reduced as well as the cholesterol level ($p<0.05$), again the LD_{50} of Avocado is zero in albino wistar rats.

It can be deduced from the study that Avocado (*Persae Americana*) possess some active principles that hastens wound healing and reduces cholesterol level.

Key words: Wound healing, avocado, bleeding time, clotting time, cholesterol hemoglobin.

INTRODUCTION

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 remodeled to form a scar (Ligha et al, 2008). Wound healing is influenced by many factors including the kind of medicine or agent used. The use 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 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 new blood supply. These chemical signals are known as cytokines or growth factors (Lawrence et al, 1994).

Some crude aqueous extracts from avocado (*Persae Americana*) could play role in hastening haemostatic activities in a damaged tissue, diseased tissue or vessel when ingested orally or applied topically on the wound. The activities of aqueous extract of avocado in albino wistar rats is studied with the curiosity to know its effect on the clotting time, bleeding time, hemoglobin and packed cell volume levels, WBC count, platelet count, duration of healing of diseased tissue and to know its effect on cholesterol level in the body.

Avocado (*Persae Americana*) is a tree native to central Mexico (Index fresh com, 2007). The tree is partially self-pollinating and often is propagated through grafting to maintain a predictable quality of the fruits. The tree grows to 20m (69ft) with alternatively arranged leaves. An average avocado tree produces about 500 avocados annually. It does not tolerate freezing temperatures and can be grown only in subtropical or tropical climates. The avocado is a climacteric fruit. It matures on the tree and falls off from the tree and ripen on the ground. Avocado ripens in a few days at room temperatures. The fruits are consumed as part of the human diets.

CHEMICAL CONTENTS OF AVOCADO

Avocado fruit is high in nutrients and low in calories, It contains sodium and fats, carotenes, folate, vitamin E (water soluble vitamins), ascorbic acid, mono-unsaturated fats and glutathione, luteine, flavoids coumarin, alkanols and oleic aci.

MEDICINAL VALUE OF AVOCADO

Avocado, like olive oil is high in oleic acid which has been found to prevent breast cancer. It inhibits prostate cancer, oral cancer and good eye sight. The presence of more of the carotenoid especially lutein protect against macular degeneration and cataracts. The high levels of folate in avocado are protective against strokes. People who can eat diet rich in folate have much lower risk of stroke than those who do not and also folate is good for heart health.

PHYTOCHEMICAL ANALYSIS OF AVOCADO

The emulsified fluid 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 flavenoids, alkaloids, tannins, saponins, reducing sugars, fats and oil, carbohydrate, calcium and steroids.

TOXICITY STUDY

The toxicity of the extract of avocado in albino rats was determined using Lorke's method (1981), the procedure of the determining the lethal dose is by increasing the concentration (after weighing them) in each group consisting eight (8 rats) per groups for five (5)days. The concentration given is at the rats of 100mg/kg, 1500mg/kg, 2500mg/kg, 3500mg/kg and 4500mg/kg, the percentage rate of their death and survival is noted a graph plotted to determine the LD_{50}.

Other investigations carried out are;

- Bleeding time as described by Dejana et al (1982).
- The whole blood clotting time was estimated by the method of Lee and White (1985).
- The haemoglobin concentration was determined by the method of Baker et al, 1985.
- The total white blood cell count was estimated according to the visual method of Dacie and Lewis (1975). The percentage packed cell volume was determined according to the haematocrit method of Alxander and Griffiths (1993). The platelet count estimation was done by the method describe by Baker et al, 1985.
- Inflicting wound 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 anesthetize them. The area for the wound infliction was chosen preferably on the back. The hairs were shaved off with surgical blade and lancet was used to cut the skin, thereby making an incisional wound in the animals. The wound was in form of square (the length and width of the wound were measured and the result expressed in centimeter. Equal area of wound 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 avocado extract was rubbed with normal saline, while avocado extract was reubbed on the test rats and also administered to them orally (1.0ml). the wounds were treated everyday and the areas measured to check difference in size in all the animals.

COLLECTION OF BLOOD SAMPLE AND DURATION OF STUDY

2.0ml of the blood sample were collected from each rat in all the group by cardiac puncture into EDTA bottle to determine the initial blood picture before feeding them on the extract. Their weight, complete blood count (CBC) including platelet count, bleeding time and clotting times were estimated. At the end of the acute feeding (28 days) on the extract blood samples were again obtained from the animals for the hemostatic, haematological and biochemical analysis as was done initially. Lastly, cardiac blood samples for cholesterol estimation were kept in plain tubes so as to allow them to clot and the serum expressed for the test.

STATISTICAL ANALYSIS

The results obtained from the study were expressed as Mean and Standard deviation (Mean ± S.D) while students' t-test was used to compare the result of the control and test rats. A p-value of less than or equivalent to ($p<0.05$) or ($p=0.05$) was considered statistically significant.

RESULTS

Table 1: Indicates the phytochemical analysis of Avocado pear

CONSTITUENTS IN EXTRACT OF AVOCADO						
	Fats and Oils	Acidic compounds	Flavenoids	Calcium	Alkaloids	Glycosides, Steroids, Resins, Terpenoids, Tanins and saponins
Degree of Concentration	++	-	+++	+	+	-

- Negative
+ Present in small concentration
++ Present in moderately high concentration
+++ Present in High concentration.

Table 2: Indicates the effects of Avocado pear extraction duration of healing in albino rats. Avocado extracts fastened healing more than normal saline ($p<0.05$)

Groups	Extracts	Days Mean ± S.D	P. value
Group A Control (n=12) Males on avocado extract	Placebo	-	-
Test Rats Group B n=12 Skin ulcered animals on extract.	Avocado	21±3.19	P<0.05
Group C (n=12) skin ulcered animals on normal saline.	Normal saline	35± 4.86	-
Group D (n=12) wound incised animals on extract	Avocado	12 ± 1.7	P<0.05
Group E (n=12) wound incised animals on normal saline	Normal saline	17 ± 2.05	

Table 3: Shows the effect of avocado on the haematological and biochemical profile of albino rats

Groups	Extracts	Hbg/dl mean ± S.D	Pcv% Mean ± S.D	Platelet count X 10^9/l mean ±S.D	WBC per mm^3 mean ± S.D	Bleeding time (min) ± S.D	Clotting time (min) mean ± S.D	Cholesterol mg/dl mean ± S.D
Group A control (n=12)	-	14.4 ± 2.15	43 ±1.6	170 ± 26	5700 ± 0.5	2.6 ± 0.5	5.2 ± 0.6	170 ± 2.4
Test rats group B n=12 Skin ulcered rats on normal saline	Normal saline	13.8 ± 0.62	41 ± 1.5	172 ± 32	5,280 ± 215	2.5 ± 13.0	5.0 ± 0.7	172 ± 18
Group C (n=12) Skin ulcered rats 28 day after Avocado Extract	Avocado	9.86 ± 1.02	28 ± 0.5	165 ± 12	2800 ± 17	2.0 ± 0.6	4.2 ± 0.5	135 ± 5.8
Group D incissed wounded rats on normal saline.	Nomal saline	13.5 ± 0.84	40 ± 1.72	180 ± 3.6	5100 ± 104	2.4 ± 0.8	5.0 ± 0.8	170 ± 58
Group E n=12 incissed wounded rats 28 days after avocado extract	Avocado	10.4 ± 2.15	30 ± 3.6	168 ±16	3000 ± 302	1.8 ± 0.4	3.1 ± 0.7	140±16
P. value		P<0.05	P<0.05	P<0.05	P<0.05	P<0.05	P<0.05	P<0.05

DISCUSSION

The activities of aqueous extract of Avocado (*Persae Americana)* on wound healing and biochemical index in male albino wistar rats have been investigated and evaluated. Before this study was carried out, the toxicity study on aqueous extract of Avocado pear was carried out using LD_{50}. This is because toxicity may affect the result of the study. The result of the lethality studies showed that the LD_{50} in rats using Avocado pear extract was zero. The doses (1.0ml) used in this study was considered safe to the animals used in the experiment throughout the period of the research.

Local medicinal herbs and foods have been employed in the management of various diseases, and their protective effect in the body from damage due to free radicals and lipid peroxidation has been reported (Wikipedia, 2010). Evaluation of efficacy of Avocado extract on wound healing and biochemical profile provides physiological information on a proper blood assessment in the blood (Ita et al, 2007). In this research study, the test rats fed with Avocado extract recorded 25% significant decrease ($p<0.05$) in white blood cell count (WBC/mm^3), bleeding and clotting times, cholesterol estimation, haemoglobin concentration and packed cell volume (Table 3). The repeated decrease in Hb concentration in rats treated with pear extract by earlier workers (Constable 1963) indicate that the indiscriminate consumption of avocado pear could predispose to anaemia in susceptible individuals. Anemia by definition is a state of lower than the normal concentration of haemoglobi which can also result from low packed cell volume Results below 30% have been reported as indicative of anaemia (chen et al, 1998).

It could be observed from the study there is lowering of cholesterol level ($p<0.05$) in Table 3 using avacado pear. This indicates that the extract of avocado may contain some active principle that acts like simvastatin tablet which is used in cutting down and controlling high cholesterol level in human beings.

On wound healing and repair of ulcered skin and incised treated wounds, it is observed that rats whose wounds were treated with aquoues extract of avocado and also recieved orally had their days of wound healing shortened by (12±1.17 days) in wound healing compared with their corresponding controls (17±2.05 days) ($p<0.05$), table2.

The incisional wounds inflicted on the test rats were kept in asceptic environment by injecting tetanus toxoid (0.1ml) to the animals.

According to researchers(Ding 2011), avocado (*P. America*) fruits are consumed as part of human diet and the extract have been shown to have growth inhibitory effectiveness of individual component and their underlying mechanism are poorly understood. Using activity guided fractionation of flesh of avocado fruits, a chloroform-soluble extracts (D003) was identified that exhibited high efficacy towards pre-malignant and malignant human oral cancer cell lines. From their research, they extracted from avocado 2 aliphatic acetogenins previously known as structure, viz compounds: 1(25, 45);- 2.4-dihydroxyheptadec-16-enylactate and 2(25, 45), -2,4-dihydroxyheptadec -16-ynylanceted. The growth inhibitory efficacy of this chloroform extract (ding 2011) is due to blocking the phosphorylation of EGFR (Tyr1173), C-RAF (Scr 338), and ERK ½ (Thr 202/Tyr 204) in EGFR/RAS/RAF/MEK/ERK ½) cancer pathway.

Avocado pear extract has been shown to contain folate. Those that eat it have much lower incidence of heart disease than those who don't (Zeides 2010). The Vitamin E (water soluble vitamin) or ascorbic acid, mono-unsaturated fats and glutathione in avocado are also great for heart.

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. Calcium ions are physiologically active in coagulation mechanism. Calcium ions are essential for the conversion of prothrombin to thrombin for the action of heart muscle and for neuromuscular conduction.

Wound healing which begins from the inside is also brought about by the help of collagen. It is an organic matrix of connective tissue unto which is deposited as a complex salt of calcium and phosphate hydroxyapatite and calcium carbonate that assist in wound healing.

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Cite this Article: Onubeze DPM and Oguwike FN (2016). Evaluation of Activities of Aquoepus Extract of M Avocado (Persea americana) on Wound Healing and Biochemical Indices of Albino Wistar Rats. Greener Journal of Medical Sciences, 6 (2): 030-035, http://doi.org/10.15580/GJMS.2016.2.041316077