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THE EFFECT OF ETHINYL ESTRADIOL CONTAINING ORAL CONTRACEPTIVE PILL ON THE HEMOSTATIC FUNCTION OF PLATELET OF WOMEN

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BACKGROUND OF STUDY

Hematological parameters are linked with health status and are important in the clinical evaluation of the state of health (Saliu et al 2012). It is known that some acute and chronic diseases adversely affect blood cells; this therefore makes the analysis of blood parameters very relevant in estimating alterations in human hematological system (Olson et al. 2000).Glucose is the most important monosaccharide in the body and monosaccharide is the final product of carbohydrate digestion. Glucose is the human body's key source of energy, through aerobic respiration, provides energy for cellular functions. Through glycolysis and later in the reactions of the citric acid cycle and oxidative phosphorylation, glucose is oxidized to eventually form CO2 and water, yielding energy mostly in the form of ATP(Adenosine triphosphate). The insulin reaction, and other mechanisms, regulates the concentration of glucose in the blood. Glucose is a primary source of energy for the brain, so its availability influences psychological processes. When glucose is low, psychological processes requiring mental effort (e.g., self-control, effortful decision-making) are impaired (fairclough 2003 and gailliot 2007). Optimium level of glucose in the blood is required for normal body function. Cases of hyperglycemia(i.e diabeties mellitus) has been reported to be a treat to the entire globe while hypoglycemia is a condition of reduced blood glucose concentration below the basal level poses also great threat to the body physiology. Therefore, studies on the blood glucose level and the hematological parameter gives important insight and information about the physiological status of individuals.

Plants has been of immersed benefit to mankind ranging from its utilization as food, Therapeutic agents, recreational activity, construction e.t.c. Fruit and vegetable consumption have been shown by wide epidemiological studies to reduce the risk diseases such as cancer, heart disease and stroke (Block et al., 1992) among others. A diet rich in fruits and vegetables has recently been found to positively affect serum antioxidant capacity and protect against lipid per oxidation. Free radicals may cause disruption of membrane fluidity, protein denaturation, lipid per oxidation and alteration of platelet functions, which may be associated with many chronic health problems. Vegetable are very important for health optimisation (Ibrahim, 2011) as such inadequate intake may increases the risk of illness and several haematological disorders from contagious diseases because of lowered defence system which in turn compromise the normal body physiology (Black, 2003).

Solanum species (eggplants) belong to the family of Solanaceae and the plant genus Solanum with over 1,000 species worldwide (Eze 2014). It is represented in Nigeria by about 25 species including those domesticated; with their leaves, fruits or both eaten as vegetables or used in traditional medicine (Bonsu et al., 2008: Manoko & van der Weerden, 2004). They are known as garden eggs in Nigeria and called gauta in hausa, Anara in Igbo or Igba in Yoruba. They are highly valued constituents of the Nigerian foods and indigenous medicines that are either eaten raw or cooked, very popular in mixed and rich dishes such as stews and soups (Edem et al, 2009), especially in the southern and western parts of Nigeria, although, they are highly cultivated in the north (Chinedu et al, 2011). Eggplants come in different species and varieties. They also vary in fruit color, shape, and size (Akanitapichat et al., 2010; Chinedu et al., 2011).

Solanum aethiopicum leaf is a vegetable that is very popular in the eastern part of Nigeria. It is commonly known as garden egg leaf and locally called ”Akwokwo Anara” in igbo, ”Ewe-Igba” in Yoruba, and “Ganyen Yaro” in Hausa. The leaves of most species of garden egg found in Nigeria looks alike but there is one notable difference among them : The leaf of solanum aethiopicum is usually hairless with its tiny fruits on its highly branched stems. The fruit of the shum group of S. Aethiopicum is usually very small and bitter. This S. Aethiopicum leaves is the predominant or the most popula species of garden egg leaf which is widely consumed especially among the populance of the eartern part of Nigeria in preparing their so called ”African salad” and yam dishes.

Solanum aethiopicum is one of the species of solanum or garden egg. It is commonly known as ‘African eggplant’ and grown in West Africa for its immature fruit (garden egg) and leaves. S. aethiopicum is a highly valued constituents of the Nigerian foods and indigenous medicines and commonly consumed almost on a daily basis by both rural and urban

families (Akoroda, 1990).It is widely cultivated across west Africa especially for its nutritional, medicinal and economic values of the leaves and fruits. It is one of the most important vegetable crops in West Africa as it is consumed daily and remains a source of income for many rural dwellers (Anosike 2012). In indigenous medicine, S. aethiopicum has a wide range of utilization form weight reduction to treatment of several ailments including asthma, allergic disease, swollen joint pains, gastro-esophageal reflux disease, constipation and dyspepsia. Scientific studies have supported the traditional use of this plant in treating inflammation, asthma, glaucoma, diabetes and excessive weight gain (Anosike 2012).Adetutu et al 2013 has reported the antioxidant activity and antibacterial activity of the leaf of this plant, but, Nothing has been known about its effect on the blood glucose level and the haematological system.

Therefore, this study is designed to find out the effect of aqueous extract of solanum Aethopicum leaf on the blood glucose level and some haematological indices using a young adult male albino rat.

MATERIALS AND METHOD

3.1.0 MATERIALS

The following materials were used for this study:

- Male albino wister rat
- Standard cages
- Vital feed
- Syringe(2mls and 5mls)
- Garden egg leaf
- Cotton wool
- A distiller
- Distilled water
- Glucometer and a test strip
- Optical microscope
- An electric oven
- haemocytometer
- 7o% alcohol
- Ether
- A chloroform
- Ammonium oxalate solution
- White blood cell diluting fluid(1% gentain violet in 0.5% acetic acid)
- Drabkin's cyanide- ferricyanide solution
- Sphectrophotometer
- Capillary tube
- EDTA(Ethylenediamine tetra-acetic acid) bottle
- Refegerator
- Electronic scale

3.1.1 STUDY SETTING : This research where carried out in the college of health science Nnamdi azikiwe university, Nnewi campus and in the school of pharmacy Nnamdi azikiwe university, Agulu campus, both in Anambra state.

3.1.2 PLANT MATERIAL: The garden egg leaves where obtained from the market in Nnewi north L.G.A Anambra state Nigeria called Ekeamobi and They were spread to dry under the air at room temperature. The plant sample was taken to the school of pharmacy at Agulu in Anambra state, Nigeria, for aqueous extraction and phytochemical screening.

3.1.3 EXTRACTION OF THE PLANT RESIDUE

The plant leaves were allowed to dry under room temperature. The dry leaves where grounded to fine powder using a grinder. the leaves where dissolved distilled water for 24 hours and was filtered using a white transparent fibric material and a cotton wool. The filterate were concentrated using an electric oven at a temperature range of $40℃ - 50℃$.

3.1.4 PHYTOCHEMICAL SCREENING OF THE PLANT RESIDUE

The phytochemical screening of the powdered leaves was carried out to identify some natural occurring chemicals that are present in the plant using some chemical test as was assisted by a pharmacognosist at the faculty of pharmacy Nnamdi Azikiwe University in Agulu, Anambra state, Nigeria. The phytochemical test for the following chemicals were carried out:

- PHYTOCHEMICAL TEST FOR ALKALIOD:

To test for the presence of alkaloids in a plant material, about 5g of the powdered leaves were placed in a test tube and 20ml of methanol were poured into the test tube.The mixture is allowed to boil for 2minutes in a water bath, cooled and filtered. To 2ml of the filterate,2 drops dragendoff's reagent (solution of potassium bismuth iodide) were added. To another 2ml of the filtrate, 2 drops of meyer's reagent (potassium mercuric iodide solution) were added. To a 5ml portion of the filtrate, 2 drops of the wager's reagent (solution of iodine and potassium iodide). Also, to another 5ml portion of the filtrate, 2 drops of hager' reagent were added which is a saturated solution of picric acid.

The alkaloids are precipitated and have the following colours:

Dragendoff's reagent	reddish brown
meyer 's reagent	cream
wagner's reagent	reddish brown
hager's reagent	yellow

➢ PHYTOCHEMICAL TEST FOR FLAVONIODS

To test for the presence of flavoniods,10ml of ethyl acetate was added to about 0.2g of the powered plant materials and heated on a water bath for 3 minutes. The mixture was cooled, filtered and the filtrate used for the following test:

- ✓ AMMONIUM TEST: A 4ml volume of the filtrate is shaken with 1ml of dilute ammonium solution.The layers are allowed to separate and the yellow colour in the ammonical layer indicates the presence of flavoniods ALLUMINIUM CHLORIDE SOLUTION TEST:
- ✓ Another 4ml portion of the filtrate is shaken with 1ml of 1% aluminium chloride solution. The layers were allowed to separate. A yellow colours in the aluminium chloride layer indicates the presence of flavoniod.

➢ PHYTOCHEMICAL TEST FOR GLYCOSIDES

Two general test was employ in this study to test for the presence of glycosides. Fehling 's test and hydrolysis test.

Fehling 's test: five millimeter of a mixture of equal parts of fehling's solution s is added to 5ml of aqueous extract of the plant materials. The mixture eas heated in a water bath for 5 minutes. a brick red precipitate at the botton of the test tube indicates the presence of reducing surgar.

Hydrolysis test: Five milliliters of dilute sulphuric acid were added to about 0.1g of the powder in a test tube and boiled for 15 minutes on a water bath, then cooled and neutralized with 20% potassium hydroxide solution. volume of a mixture of fehling's solution was added and boiled for 5 minutes. a picrick red precipitate indicates the presence of glycoside.

➢ PHYTOCHEMICAL TEST FOR TANNINS

1g of the powdered material were boiled with 50ml of water and used for the following test.

- ✓ **ferric chloride test** :To 3ml of the filtrate, few drops of ferric chloride were added. a greenish black precipitate were observed which indicates the presence of tannins.
- ✓ **lead subacetate test**: A drop of lead subacetate is added to 3ml of the filtrate. a cream precipitate showed the presence of tannins.

➢ PHYTOCHEMICAL TEST FOR TERPENIOD:

A 9ml portion of ethanol were added to lg of the powdered leaves. This was refluxed for a few minutes and filtered. The filtrate was concentrated to 2.5ml on a boiling water bath and 5ml of the water were added. The mixture was allowed to stand for 1hr and the waxy matter filtered off. The filtrate was extracted with 2.5 ml of chloroform using a separating funnel. A 0.5ml of the chloroform extract is evaporated to dryness on a water bath. The residue was reconstituted with 3ml of concentrated sulphuric acid and heated for 10minutes on a water bath. A grey colour was observed and this indicates the presence of terpeniod.

3.1.5 EXPERIMENTAL ANIMALS: Young adult male albino rats weighing between 150g-310g were use for the study. The rats were obtained from a private farm in okofia, in Nnewi north L.G.A Anambra state and transported to the research site at college of health science Okofia in Nnewi north L.G.A Anambra State.

3.1.6 MANAGEMENT OF THE EXPERIMENTAL ANIMALS: The animals were allowed to acclimatize for 2weeks under a standard animal house. They were maintained on the standard poultry feed and potable water.

3.1.7 EXPERIMENTAL DESIGN: A total of 24 adult male rats were use for the study which lasted for 4weeks (one mouth).They were randomly distributed into three group labelled group A-C comprising of 8 rats per group. Group A is the control group while group B and C is the major treatment group for the experiment. Each of the treatments group were given eggplant leaves extract by dose 300mg/kg/day for group B and 600mg /kg/day for group C.

3.1.8 BLOOD SAMPLE COLLECTION: On day 15 after the administration of the leaf extract, while under chloroform anaesthesia, blood where collected from each rat in all the group through the occular puncture and transferred into an EDTA bottle using a capillary tube.

3.1.9 DETERMINATION OF BLOOD GLUCOSE LEVEL

The blood glucose levels of the blood sample in all the groups where measured by GLUCOSE OXIDASE METHOD using a standard glucometer. A glucose meter (or glucometer) is an electronic medical device for determining the approximate concentration of glucose in the blood. It is a rapid and non –invasive method of blood glucose measurement requiring a very small amount of blood. This method involves the use of a glucometer sensor called a test strip, the sensor used has an electroenzymatic approach, which means that it takes advantage of glucose oxidation with a glucose oxidase enzyme. The presence of glucose oxidase catalyzes the chemical reaction of glucose with oxygen, which causes an increase in pH, decrease in the partial pressure of oxygen, an increase of hydrogen peroxide because of the oxidation of glucose to gluconic acid:

The test strip measures changes in one or several of this component to determine the concentration of glucose. The strips used in this design have three terminals or electrodes. • reference electrode • working electrode • trigger electrode a negative voltage of –0.4 v is applied at the reference electrode. When blood or a glucose solution is placed in the strip, a chemical reaction occurs inside it, generating a small electrical current proportional to the glucose concentration. This current is constantly monitored while the strip is in place, allowing the device to monitor when blood is placed.

3.2.0 DETERMINATION OF THE HAEMOGLOBIN CONCENTRATION

The haemoglobin concentration was estimated in this study using the haemiglobincyanide (HICN) technique.This technique is also called cyanmethaemoglobin method.

➢ **PRINCIPLE OF TEST:**

Whole blood is diluted in a modified drabkin's solution which contains potassium ferricyanide and potassium cyanide.The red cell are haemolylized and the haemoglobin is oxidized by ferricyanide to methaemoglobin.This is converted by the cyanide to a stable haemiglobincyanide (hicn).Absorbance of this is read in a spectrophotometer at a wavelength of 540nm or in a filter coulorimeter using a yellow- green filter paper.The absorbance obtained is compared with that of a reference HICN standard solution.Haemoglobin values are obtained from tables prepared from a calibration graph.

Drabkin solution is a diluting fliud with a pH of 7.0 -7.4.The fluid contain the following :

- ✓ Potassium ferricyanide (hexacyanoferrate III)
- ✓ Potassium cyanide
- ✓ Potassium dihydrogen phosphate
- ✓ Non- ionic detergent (e.g nonidet,triton-x-100)

➢ **Distilled or deionised water**

PROCEDURE:

1. 0.02ml of blood were added to 5ml of the diluents in a test tube.
2. The tube was sealed with the thumb and inverted several times
3. The solution in the test tube were kept at room temperature for 2-4 minutes to ensure the completion of the reaction.
4. A black drabkin solution is used to zero colourimeter.
5. The optical density of the solution was read in a colourimeter (sphectrophotometer).

NORMAL REFERENCE RANGE OF HAEMOGLOBIN CONCENTRATION(GUIDELINE FIGURE):

Children at birth …….13.5-19.5g/dl

Children 2 years-3 years……..11.o-14.0g/dl

Children 6-12 years……11.5-15.5g/dl

Adult men……13.0-18.0 g/dl

Adult women……..12.0-15.0g/dl

(pregnant women)……..…11-13.8g/dl

Figures are taken from practical haematology,8th edition,1994.churchill living stone.

3.2.1DETERMINATION OF PLATELET COUNT

The platelet count is a screening test that assess the integrity of the primary haemostasis (Sharish 2013).Primary hemostasis is the process of platelet plug formation and this is the second mechanism of hemostasis.platelet plug is formed by the processs of platelet aggregation upon the adhesion of platelet to a damaged endothelium(i.e blood vessel). However, platelet count was investigated in this study using manual method. This method involve the use of the following equipment:

1. Ammonium oxalate (a diluent)
2. Optical microscope
3. Haemocytometer

➢ PRINCIPLE OF TEST:

Blood is diluted in a filtered solution of ammonium oxalate reagent which lyzes the red cells.Platelet are counted microscopically using an improved neubauer ruled counting chamber and the number of platelets per liter of blood calculated.

➢ PROCEDURE:

1 0.38ml of filtred ammonium oxalate diluting fliud were measured and was dispensed into a small container.

2 0.02ml of an anticoagulated blood was added to the container and was mix.

3 The counting chamber were assembled and were filled with the well-mix blood

4 The chamber was left undisturbed for 20 minutes.

The underside of the chamber was dryed and placed on a microscope stage. Using the 10x objective,focus the ruling of the grid and bring the central square of the chamber into view. The objectives was changed to 40x and focus and the small platelet was brought in to focus the platelet was counted in the small square.

3.2.2 DETERMINATION OF THE TOTAL WHITE BLOOD CELL

This was done to estimate the number of white blood cell (WBC) in a unit volume of blood the following apparatus and reagents were used:

- ✓ Haemocytometer (pipette with a small bulb and white bead and original neubauer counting slide)
- ✓ Cotton gause
- ✓ 70% alcohol
- ✓ Ether
- ✓ Wbc-diluting fluid (1%gentian violet in 0.5 to 1%acetic acid)
- ✓ Optical microscope.

➢ PRINCIPLE OF TEST:

Whole blood is diluted in the acid reagent to haemolyzed the red cell, leaving behind the white cells to be counted.White blood cells are counted microscopically using an improved ruled counting chamber (haemocytometer) and the number of WBCs per liter of blood calculated. The gentian violet is added which stains the nucleus of the white cell.

PROCEDURE:

1 0.38ml of the diluents were added to a test tube.

2 0.02ml of anti-coagulated blood was added to the test tube and mix.

3 The counting chamber was assembled :

- ✓ Make sure the centralgrid areas of the chamber and the special haemocytometer cover glass are completely clean and dry.
- ✓ slide the cover glass into position over the grid areas and press down on each side until rainbow colour are seen prior moistening of the chamber surface on each side of the grid areas will help the cover glass to adhere to the chamber.

4 The diluted blood sample was re-mix using a pastuer pipette held at an angle of about 45%, fill one of the grids of the chamber with the sample, taking care not to overfill the area.

5 The chamber was left undisturbed for 2 minutes to allow time for the white cells to settle.

6 The underside of the chamber was dried and was placed on the microscope stage using the 10x objective with the condenser iris closed sufficiently to give good contrast, focus cells until they appear as small black dots.

7 The cells in the four large corner of the chamber was counted.

WBC REFERENCE RANGE

These are guidelines figures which are usually checked while performing test on the total white blood cell.

Children at one year……..……..6.0-18x109/l

Children at 4-7 years……..……5.0-15x10x109/l

Adult ……..….4.0-10.0x109/l

Pregnant women…….. ….. Upto 15x10/l

3.2.4 STATISTICAL ANALYSIS

The result were evaluated using analysis of variance (ANOVA) and were presentated as the mean value ± SEM(STANTARD ERROR OF MEAN) FOR THE CONTROL and the experiment rats. Differences among the means for the groups were assessed using the duncan's multiple range test to determine which mean values were significantly difference at $p<0.05$(sokal and rohif,1969). All statiscal comparison and tests were performed using spss soft ware application (version 21).$p<0.05$ was accepted as statistically significant.

RESULT

4.1.0 EFFECT OF AQUEOUS EXTRACT OF SOLANUM AETHIOPICUM LEAF ON BLOOD GLUCOSE LEVEL.

The mean blood glucose level was significantly decreased across the test group when compared to the control($p<0.05$)

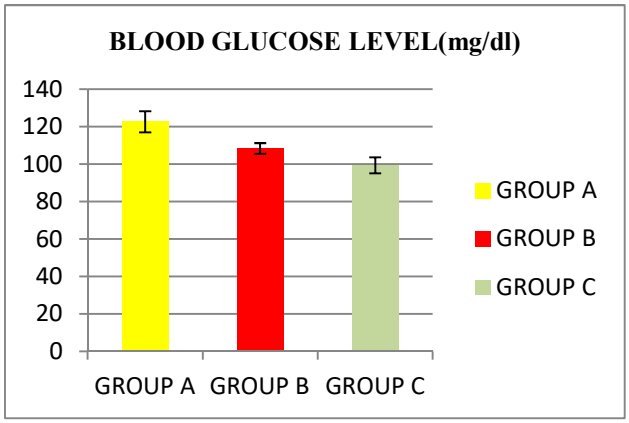

Fig 1.1 Bar chart presentation of the effect of aqueous extract of S. Aethiopicum leaf on the blood glucose level after 14 days of treatment at *$p<0.05$ was significant when compared with the control group for n=24.Where n is the number of experimental animal used for the research.

5.1.0 DISCUSSION

The result of this investigation showed that the aqueous leaf extract of S.Aethipicum stimulate an increase in the haemoglobin concentration of blood. This could be as a result of iron, high protein content and ascorbic acid that the leaves contained. These are the important nutrient that are required by the body for haemoglobin synthesis. It has been reported by (Guyton and Hall 2011) that when any of this substances is in short supply to the body, there will be impaired haemoglobin synthesis leading to anemia. Protein is used by the body for globin synthesis, iron is used for haem synthesis while ascorbic acid stimulates an increase in iron absorption from the alimentary canal of humans(Chetterjea 2012).

The significant decrease in the platelet concentration at increased dosage of the plant extract could be as a result of the increase activity of tannin which was found to be present in this leaf. Tannin has been reported by dora et al 2005 to have anti-platelet effect, though the mechanism of action was not clearly stated.

Also, in this study, the blood glucose concentration was found to be decreased significantly across all the test groups following the administration of this leaf extract when compared to the control group that receives water and food throughout the period of the experiment. This therefore suggest that garden egg leaf stimulates a decrease in the blood

glucose level judging from my result. However, this could be as a result of hormonal changes induces by some of the phytochemical present in the leavy vegetable such as certain flavoniods.

The role of flavoniod to enhance the immune system has been noted. This study reveal a significant increase in the total white blood cell count on a dose dependent manner across the test groups, when compared to the control. This could be attributed to flavoniod found in the leaves.

5.1.1 CONCLUSION

- ✓ Considering the result of this study, S.Athiopicum leavy vegeteble can find its usefulness traditionally as possible therapeutic agent in cases associated with hyperglycemia such as in diabetics mellitus. Also, individual who are hypoglycemic as a result of reduced activity of the anterior pituitary gland, cretinism, or in severe liver disease should consumed the leaves in reduced quantity or it should not be included in their meal.
- ✓ Because of its effect to increase haemoglobin concentration, chronic diseases that adversely affect the red blood cells such as in malaria, It can find its use.
- ✓ Finally, S. Aethiopicum leavy vegetable boasts the immune system, it is therefore, recommended for every individuals who wishes to keep his or her immune system strong and avoid deterioting effect of infectious diseases.

Also, In regards to my finding on platelet concentration following the administration of this leaf extract, It can find its usefulness in disorders like thrombocytosis, also individuals who are thrombocytopenia should consumed the leaves in a reduced quantity since the observed decrease was more effective at the treatment group receiving a higher dose of the leaf extract.

5.1.2 RECOMMENDATION FOR FURTHER STUDY

- ✓ Further study on this topic is recommended to understand or to find out in detail the molecular basis of the observed effect of garden egg leaves on the blood glucose level, hemoglobin concentration, platelet count and the total WBC count.
- ✓ It is also recommended that the duration of this experiment be shorten to about 7-3 days in further study in order to known If the observe effect could be immediate.

Studies should also be carried out on other blood parameters such as the lipid profile, humoral parameters of the immune system and the hepato protective activity using this same garden egg leaf leaf (solanum Aethiopicum leaf).

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