Effects of *Irvingia Grandifolia* on the Plasma Concentration of Urea, Creatinine and White Blood Cells Count (Total & Differential) in Normal Rabbits.

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ABSTRACT

The hypoglycemic effects of *Irvingia grandifolia* is documented. While medicinal plant may have recognizable therapeutic effect, it may also have few toxic side-effects. The use of *Irvingia grandifolia* has been found to be efficacious in the management of diabetes mellitus but the effect of this herb on plasma urea, creatinine concentrations and white blood cell count has not been characterized so far. The effect was investigated and found out that there is no pronounced effect of the herb on plasma creatinine concentration based on student distribution (t-test) on the treated animals. Also, there is no significant effect of the herb extract on plasma urea concentration, white blood cell count (total and differential) also found from student distribution (t-test) after comparing treated animals with control animals.

Key Words; Diabetes mellitus, Medicinal plants, *Irvingia grandifolia*, Anti-diabetic, Plasma Urea, Plasma Creatinine, White cell count (total), White cell count (differential).

1.0 INTRODUCTION

Focus on medicinal plants research has significantly increased in recent times and a large body of evidence has been collected to show the immense potentials of medicinal plants. *Irvingia grandifolia* has been studied using modern scientific approaches. Generally, the use of medicinal plants in the management of diabetes mellitus proves to be useful and *Irvingia grandifolia* is one that has been found to be efficacious (Onoagbe et al, 1999); but the effect of this herb on plasma urea, creatinine concentrations, and white blood cell count has not been evaluated. Diabetes mellitus is a syndrome that is characterized by hyperglycemia, change in the metabolism of lipids, carbohydrates, and proteins, and in the long term, with eye, kidney, cardiovascular, and neurological complications. Medicinal plants have a long history of usage and today, they are being extensively used for various diseases. There are several reasons for increasing the use of medicinal plants. Many plants from different parts of the world have been investigated for antidiabetic effects (Rao et al, 2010).

Irvingia is a genus of African and Southeast Asian trees in the family Irvingiaceae, sometimes known by the common names wild mango, African mango, bush mango, dika or ogbono. They bear edible mango like fruits and are especially valued for their fat and protein rich nuts. The fruit is a large drupe with fibrous flesh. The subtly aromatic nuts are typically dried in the sun for preservation and are sold whole or in a powder form. They may be ground to a paste known variously as dika or Gabon chocolate. Their high content of mucilage enables them to be used as thickening agents for dishes such as ogbono soup which is popular in Nigeria as a menu. The nuts may also be pressed for vegetable oil (Harris, 1999).

Irvingia grandifolia is a large deciduous tree with spreading branches and a hemispherical crown. The plant is sometimes gathered from the wild for local use as a food, medicine and source of wood. The bark has a number of medicinal uses which include: A decoction is taken internally for stomach & kidney complaints and to treat menstrual and vaginal affections, macerated in palmwine, it is taken as an aphrodisiac, and externally a decoction is used for bathing children with fever and as an eyewash for ophthalmia and treatment of diabetes mellitus. An ointment is made of the crushed bark combined with palmoil for topical application in treating muscular pain, arthritis, rheumatism, sprains, fractures, oedema etc (Burkil, 2004). Onoagbe, et al (1999) equally documented the anti-diabetic efficacy of *Irvingia grandifolia* in their work.

Plasma urea concentration reflects the balance between urea production in the liver and urea elimination by the kidneys in urine, so increased plasma urea can be caused by increased urea production, decreased urea elimination or a combination of the two. By far the highest levels occur in the context of reduced urinary elimination of urea due to advanced renal disease and associated marked reduction in glomerular filtration rate (GFR). GFR is a parameter of prime clinical significance because it defines kidney function. All those with reduced kidney function whatever its cause have reduced GFR and there is a good correlation between GFR and severity of kidney disease. The rate of decline in GFR distinguishes chronic kidney disease (CKD) and acute kidney injury (AKI). CKD is associated with irreversible slow decline in GFR over a period of many months, years, or even decades; whereas AKI is associated with precipitous decline in GFR over a period of hours or days; AKI is potentially reversible. The value of urea as a test of renal function depends on the observation that plasma urea concentration reflects GFR: as GFR declines, plasma urea rises (Chris, 2016).

Creatinine, also a non-protein nitrogenous waste product, is produced from the breakdown of creatine and phosphocreatine and can also serve as an indicator of renal function.2 Creatine is synthesized in the liver, pancreas, and kidneys from the transamination of the amino acids

arginine, glycine, and methionine. Creatine then circulates throughout the body and is converted to phosphocreatine by the process of phosphorylation in the skeletal muscle and brain. The majority of the creatinine is produced in the muscle. As a result, the concentration of plasma creatinine is influenced by the patient's muscle mass. Compared to blood urea nitrogen, creatinine is less affected by diet and more suitable as an indicator of renal function (Jose H, 2014).

White blood cells fight infections through a process known as phagocytosis. In response to an acute infection, trauma or inflammation, white blood cell release a substance called colony-stimulating factor (C.S.F.). Colony stimulating factor stimulates the bone marrow to increase white blood cell production.

Blood glucose concentrations at any time is determined by the balance between the amounts leaving the blood into tissues. Under normal conditions, the blood glucose concentration is strictly regulated and the blood glucose concentration is maintained between 80-120 mg/dl (4.4-6.7) mMol/L in between meals.
Impaired insulin action result in chronic metabolic disorder of carbohydrate, protein and fat metabolism of which death may result. Long standing metabolic derangement has been associated with permanent irreversible functional and structural changes in the cells of the body. The structural and functional changes lead to the development of well- designed clinical presentation referred to as complications of diabetes mellitus; which includes: hypertension, neuropathy, retinopathy, nephropathy, cardiomyopathy, etc. (Barkis, 1993).
Hyperglycemia results when insulin level is low and there is lower transport of glucose into cells and reduced extracellular utilization of glucose. Increased glucose in the blood increases the osmotic pressure of the extracellular fluid causing withdrawal of water from cells. There is no curative treatment for diabetes mellitus but can be managed.

1.1 LITERATURE REVIEW

Diabetes mellitus is a chronic condition associated with abnormally high levels of glucose in the blood. This is a major endocrine disorder affecting nearly 10% of the population all over the world. This is a disorder of carbohydrate metabolism caused by inadequate production or utilization of insulin, the hormone secreted by beta-cells of the pancreas needed to convert sugar, starches and other foods into energy needed for daily life. As a result, glucose builds up in

the bloodstream. Despite the introduction of hypoglycemic agents, diabetes and related complications, continue to be a major medical problem (Hers, 1999).

1.2 MEDICINAL PLANTS USED IN THE MANAGEMENT OF DIABETES MELLITUS

Several plant species have been studied as potential therapeutic agents in the management of diabetes and its related complications, its efficacy as a hypoglycemic agents.

Irvingia Grandifolia (Epo Oro)

This medicinal plant have anti-diabetic effect by lowering the level of blood glucose in experimental animals. The efficacy of this plant as an anti-diabetic agent has been established for long by the past scientist who had research on it. It was found that *irvingia grandifolia* decrease basal blood glucose by approximately 50% in normal rabbits and rats (Onoagbe, et al, 1999). Studies also shows that *Irvingia gradifolia* might enhance the rate of synthesis or secretion of insulin from the beta cells of the pancreas of diabetic animals since only mild diabetics was established in the animals.

The anti-diabetic activity of this plant can also be extended to treatment of human diabetes since they can effectively lower blood glucose in the treated animals.

1.3 TOXICITY OF DRUGS USED IN THE MANAGEMENT OF DIABETES MELLITUS

The hypoglycaemic effect of *Irvingia grandifolia* have been established but its effects on kidney, white blood cells and other organs has not been characterized so far therefore the aim of this study. Toxicology is a science that involves a complex interrelationship among dose, absorption, distribution, metabolism and elimination. All substance have a potential toxicity. All herbs can therefore be harmful but most would have to be ingested in impossible amounts to cause harm. The primary determinate of the safety of a substance is the dose (Timbrell, 1995).

1.3 THE TOXICITY OF A SUBSTANCE

Every substance has potential toxicity from the most benign to the most obvious. Even so, the same dose will not affect every person in the same way. It is not enough to say that a substance is toxic. There are a myriad of factors which may make the substance more or less toxic to a particular individual.

1.4 Dose-Response Relationship

Toxicity depends not only on the dose of the substance but also on the toxic properties of the substance. The relationship between these two factors are important in the assessment of therapeutic dosage.

Lethal dose and LD_{50}

Lethal dose is the term used to describe the acute toxicity of a substance while LD_{50} is used to show that 50% of the animals exposed to a specific amount of a substance died as a result. The LD_{50} values on animal experiments are used to estimate the lethal dose of substances for humans. LD_{50} is expressed as milligram (mg) of chemical per kilogram (kg) of body weight thus mg/kg (Ottoboni, 1991). LD_{50} was not calculated out from this study but was taken from literature as reported by Onoagbe, et al 1999.

Acute and Chronic Toxicity

Acute toxicity refers to the ability of the substance to do systemic damage as a result of a one-time exposure to relatively large amounts of a substance. Effects are noticed fairly quickly after exposure. Chronic toxicity refers to the ability of a substance to cause similar or different damage as the result of repeated exposures of smaller amounts of the substance. Effects may not be evident until days or months later. Substances can be either acutely toxic or chronically toxic or both.

Markers of toxic response are evidenced by presence in tissues or fluids of metabolites of the compound and/or conjugates. There is a wide range of markers of effect such as enzymes released into the blood by damaged tissues, induction of enzyme synthesis or stress proteins or increased release of intermediary metabolites. Indirect markers include changes in the immune system or in reproductive cycle of the organism (Ottoboni, 1991).

Acute and Chronic toxicity was toxicity was not calculated out from this study but was guided by what was documented by Onoagbe, et al 1999.

1.5 THE PROCESS OF POISONING

Site of Absorption

Cell membranes differ throughout the tissues in the body but they are mainly composed of phospholipids and proteins. The cell membranes are selectively permeable. The processes which cell membranes use to allow foreign substances to pass through them are:

- Filtration through pores
- Passive diffusion through the membrane phospholipid
- Activated transport
- Facilitated diffusion
- Phago/ pinocytosis.

These are the same processes which are used by the cells to absorb and excrete endogenous substances as well as sodium, neurotransmitters and hormones. Toxicants can interrupt metabolism of carbohydrates, lipids and protein and alter synthesis, release, and storage of hormones.

Enzyme Systems

Enzymes are proteins produced by the body to facilitate the elimination of both endogenous and exogenous substances. Enzyme catalyzes such essential metabolic processes such as respiration, synthesis of Adenosine Triphosphate (ATP) or the synthesis of lipids. Many of these enzymes sit in the lipid layer of the cell membrane and carry important roles in intercellular communication. Enzymes are induced in response to the ingestion of a foreign substance. They will try to break these substances down into a less harmful substance or to forms that are more easily excreted. Caffeine for example stimulates the production of monoamine oxygenases (MAO) one type of enzyme system. MAO normally functions to break down neurotransmitter amines and hormones.

By binding to enzymes a toxicant can impair the functioning of the enzyme which is sometimes evidenced by symptoms of poisoning. If the receptor is bound up by the toxicant, it cannot perform its biological functions which may lead to a toxic response (Desmet, et al, 1992).

Some of the important enzymes which detoxicate foreign compounds are glutathione transferases, superoxide dismuthases and monoamine oxidases. These enzyme systems are concentrated in the endoplasmic reticulum of the liver and kidney cells. These enzymes put foreign substances through specific biochemical processes such as oxidation, hydroxylation, hydrolysis, dealkylation, dehalogenation, epoxidation, reduction, a n d conjugation.

Conjugation

Conjugation reactions are important detoxication mechanisms. The process of conjugation links an endogenous molecule with the foreign substance to make it more water soluble and therefore easier to excrete. However, sometimes conjugation can produce free radicals that may attach to cells and damage them. Many cancer- causing agents are activated through conjugation. Other enzyme such as superoxide dismutase can detoxicate and neutralize free radicals. Dietary antioxidants such as vitamins C and E, beta-carotene flavonoids play a protective role in detoxifying free radical and other toxins (Desmet, et al, 1992).

Oxidation

The one enzyme system responsible for this reaction is the cytochrome P_{450} mono-oxygenase system which is found in the smooth endoplasmic reticulum of the cell. The liver has the highest concentration of these enzymes, although they can be found in most other tissues. Cytochrome P_{450} proteins make foreign substances more reactive. Certain oxidation reactions are catalyzed by other enzymes such as alcohol dehydrogenases, xanthine oxidase, monoamine and diamine oxidases. The enzyme processes of oxidation, reduction, and hydrolysis rearrange and change fat-soluble compounds so that they are more easily excreted.

Serum Binding Protein

The amount of carrier proteins present in the plasma can greatly affect the metabolism of both endogenous and exogenous substances. The binding of foreign compounds with blood protein can alter their distribution and excretion and hence their toxicity.

Route of Exposure

In order to be poisoned by a medicinal plant one must first be exposed. Herbs are used in suppositories (direct mucus membrane contact), smoking mixtures, steam treatment and essential oils (inhalation), teas, tinctures, syrups (oral), salves (dermal) and as intravenous injections. The way in which the individual is exposed is crucial in a toxic response (Desmet, et al, 1992).

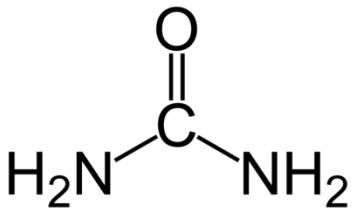
1.6 ENZYMES AND METABOLITES

This is conducted because most enzymes and metabolic waste are specific to some tissues and when the concentrations in the blood exceed the normal value shows that there is a problem with the tissue or organ.

UREA

This is the end product of protein metabolism in the liver and cleared by the renal glomerular of the kidney in urine. When an animal lives on a high protein diet, the urea concentrations in the blood will increase and mount a pressure on the kidney for clearance, and once the integrity of the kidney to clear urea is partially or totally lost, the urea concentration in the blood will rise over time. It can be concluded that blood urea nitrogen levels are therefore related to protein metabolism (intake and catabolism) and to liver and kidney function.

STRUCTURE OF UREA



(Urea)

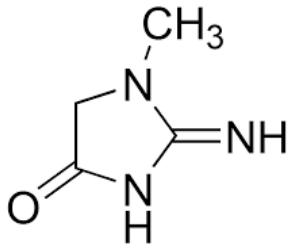
Principle of assay: Urea is hydrolyzed by urease to produce ammonia. The rate of absorbance decrease or increase is directly proportional to the amount of urea present in the sample (Henry et al, 1974) .

$$\text{Urea} + 2H_20 \xrightarrow{\text{Urease}} 2NH_3 + H_2C0_3$$

CREATININE

Creatinine concentration is most commonly used as an indirect indicator of renal glomerular filtration rate and to thereby estimate renal function. Creatinine i s a byproduct of the breakdown phosphocreatine an energy storage compound in the muscle. Creatinine in actuality has poor sensitivity in diagnosing renal disease but creatinine clearance is a reliable test of renal glomerular filtration rate due to specific elimination of creatinine by the kidney and general lack of metabolism elsewhere in the body.

STRUCTURE OF CREATI NINE



Creatinine is water soluble and distributes throughout the body water equilibrating between various fluid regions in approximately 4 hours with the help of Na/cl transporters on cell membranes. The equilibration time of creatinine is longer than that for urea which is within 1$\frac{1}{2}$ hours. Elevations in creatinine concentrations do not cause uremia. Uremia is the clinical signs of illness due to renal failure. The serum/plasma creatinine concentration can vary based on a number of factors including an animal's diet, muscle mass and gender (Watson, et al, 2002). Serum creatinine values also depend on the kidneys ability to excrete creatinine. An elevation of creatinine is

called azotemia and usually occurs simultaneously with an increase in blood urea nitrogen, a compound that is also freely filtered by the glomerulus. There are other non-renal diseases that can create an elevation in serum creatinine concentrations from secondary renal involvement. Examples is leptospira spp. which can increase creatinine concentration that may mistakenly indicate the kidney as part of the disease process. Disease process like gastric torsion, diabetes mellitus also can create secondary renal injury and elevate serum creatinine levels (Braun, et al, 2003).

When there is impaired formation or elimination of urine, there is an increase in several compounds present in the blood plasma. These compounds are relatively nitrogen containing molecules that are collectively called the non-protein constituents of plasma or serum. The compounds whose serum concentrations are of great significance in kidney disease are creatinine and urea. Circulating levels of creatinine is used primarily as an index of renal function. High plasma creatinine concentrations are encountered in nephritis and renal obstruction, reflecting the degree of impairment (Bishop, et al, 2000).

WHITE BLOOD CELL COUNT

TOTAL AND DIFFERENTIAL

White blood cells or leukocytes are classified into two main groups: granulocytes and non-granulocytes (also known as agranulocytes). The granulocytes which include neutrophils, eosinophils and basophils have granules in their cell cytoplasm. They also have a multi-lobed nucleus. As a result they are called polymorphonuclear leukocytes. The non-granulocytes white blood cells, lymphocytes and monocytes do not have granules and have non-lobular nuclei and sometimes referred to as mononuclear leukocytes.

The life span of white blood cells ranges from 13-20 days after which they are destroyed in the lymphatic system. They are produced in the bone marrow and released into the peripheral blood. Leukocytes fight infection through a process known as phagocytosis. During phagocytosis, the leukocytes surround and destroy foreign organisms. Leukocytes also produce, transport and distribute antibodies as part of the body's immune response.

In a complete blood count two measurements of white blood cells are commonly done:

- The total number of white blood cells in a micro liter of blood reported as an absolute number of "X" thousands of white blood cells.
- The percentage of each of the five types of white blood cells and known as differential. Each differential always add up to 100%.

The numbers of leukocytes changes with age and during pregnancy.

- On the day of birth, a new born has a high white blood cell count ranging from 9000-30,000 leukocytes but falls to adult levels within two weeks.
- The percentage of neutrophils is high for the first few weeks after birth but then lymphocyte predominate.
- In the adult stage, neutrophil are more predominant than lymphocytes.
- In the elderly, the total white blood cell decrease slightly.
- Pregnancy results in a leucocytosis primarily due to an increase in neutrophils with a slight increase in lymphocytes.

  Leucocytosis is due to an increase in white blood cell (WBC) above normal value and the increase may be increase in one of the white blood cells and is given the name of the cell that shows the primary increase.

- Neutrophilic leucocytosis – Neutrophilia
- Lymphocytic leucocytosis – lymphocytosis
- Eosinophilic leucocytosis – eosinophilia
- Monocytic leucocytosis – Monocytosis
- Basophilic leucocytosis – Basophilia

In response to acute infection, trauma or inflammation, white blood cell release a substance called colony-stimulating factor (CSF). Colony-stimulating factor stimulate the bone marrow to increase white blood cell production.

In some disease such as measles, pertussis and sepsis, the increase in white blood cells is so dramatic that the picture resembles leukaemia.

Therapy with steroids modifies the leucocytosis response. When corticosteroids are given to healthy person the WBC count rises. However, when corticosteroid are given to a person with a severe infection, the infection can spread significantly without producing an expected WBC rise. Leucocytosis as a sign of infection can be masked in a patient taking corticosteroids.

Leukopenia occurs when the WBC falls below 4000. Viral infections, overwhelming bacterial infections and bone marrow disorders can all cause leukopenia. Patients with severe leukopenia should be protected from anything that interrupts skin integrity. Drug that may produce leukopenia include: Antimetabolites, Barbiturates, Antibiotics, Anticonvulsants, Anti-thyroid drugs, Arsenicals, Anti-neoplastics, cardiovascular drugs, Diuretics, Analgesics, anti-inflammatory drugs, and heavy metal intoxication.

Leukocytes: Critical low and high values;

- A WBC of less than 500, places the patients at risk for a fatal infection.

- A WBC over 30,000 indicates massive infection or a serious disease such as leukaemia (Struat, 1996).

MATERIALS AND METHODS

EXPERIMENTAL ANIMALS

The animals used for this research work are white albino rabbits of both sexes New Zeeland breed obtained from Lagos State University of Technology (LUTH). The animal's weight ranges from 1.30 – 1.80 kg respectively and were kept under normal animal house (metal cage), allowed free access to food (grower's mash) and water. The Rabbits were not induced by any diabetic agent since it has been established by Onoagbe, et al, that *Irvingia grandifolia* have a hypoglycaemic effect on their experimental animals.

MEDICINAL PLANT

The herb used is the bark of *Irvingia grandifolia* known in Yoruba as *epo oro* and Ogbono in Igbo. The herb was obtained at Oyingbo Market in Lagos State from medicinal plant dealers. The herb was identified in the Forestry and Wild-life Department of University of Benin, Benin City.

PREPARATION OF HERB EXTRACT

The bark of the herb was properly dried in oven, cut into pieces and crushed into powder using mortar and pestle, and electric grinding machine (Puveriza) in Pharmacognosy Laboratory, University of Benin and weighed. The weighed powder was boiled in distilled water (10g herb powder/100ml of distilled water) for 10 hours over a period of 3 days; 1st day-5 hours, 2nd day-3 hours, 3rd day-2 hours. The water level in the pot was maintained throughout the period of boiling. The boiled herb solution was allowed to cool to room temperature after which it was filtered with sintered glass funnel to remove debris. The filtrate was collected and concentrated using rotary evaporator in Pharmaceutical Chemistry Laboratory, University of Benin. The concentrated extract was collected and weighed where a concentration of 250mg/ml was prepared and ready for administration to the experimental animals (Rabbits). From Literature the acute toxicity of *Irvingia grandifolia* was found to be above 5000mg/kg body weight as reported by Onoagbe, et al 1999.

ADMINISTRATION OF HERB EXTRACT

The herb extract was administered on the basis of body weight (300mg/kg body weight) for a period of 14 days on the 5 experimental rabbits while the 4 control used were not given. The extract was given to the animals by oral administration (Orotracheal intubation) on a daily basis (Benumof, 2007). Excess plant extract was stored in a working fridge until the next day.

PREPRATION OF A CONCENTRATED ACTIVE HERB EXTRACT

5000mg of the active extract was dissolved in 20ml of distilled water.

5000mg ⟶ 20ml

x mg ⟶ 1 ml

Therefore: $\frac{5000X1}{20}$ = *x mg*=250 *mg*

A concentration of 250mg/ml of the herb extract was made, but a concentration of 300mg/kg body weight was given.

Example: 300mg ⟶ 1 kg body weight

Y mg ⟶ 1.3 kg

This equals 300 x 1.3 = Y mg = 390 mg

And 1ml contains 250mg, how many mls is in 390 mg = 1.6ml (approx.)

VOLUME OF EXTRACT TAKEN BY EACH RABBITS DAILY FOR 2 WEEKS

WEEK ONE

Body weight (kg)	mls given
R_1 = 1.308	1.6 ml
R_2 = 1.395	1.7 ml
R_3 = 1.345	1.6 ml
R_4 = 1.790	2.1 ml
R_5 = 1.490	1.8 ml

WEEK TWO

Body weight (kg)	mls given
R_1 = 1.300	1.6 ml
R_2 = 1.400	1.7 ml
R_3 = 1.350	1.6 ml
R_4 = 1.800	2.2 ml
R_5 = 1.500	1.8 ml

COLLECTION OF BLOOD SAMPLES

Blood was drawn from the large vein at the back of the rabbit ear. Before collection of blood, the blood vessels were dialated and the ear sterilized by rubbing each rabbit's ear with cotton wool soaked with methylated spirit. Blood was drawn from the large vein using sterile needle and syringe into anticoagulated tubes (Heparin and EDTA). The collected samples in heparin tubes centrifuged at 3000 rpm for 10 minutes to obtain the plasma (supernatant) which was transferred into a clean plain tube prior to analysis. The whole blood in EDTA tubes were used for white blood cell count (WBC).

EXPERIMENTAL DESIGN

The basal levels of plasma urea, creatinine concentration (levels), and white blood cell count (total and differential) of the experimental animals (test and control) were measured 4 times at an interval of 3days except for the last day that was 5 days interval, for a total period of 14 days; after which the herb extract was administered to the test animals only on a daily basis for a period of 14 days. During the herb administration to the treated animals, the plasma urea, creatinine levels, white blood cell count of both test and control animals were measured again for, 4 times at an interval of 3days except for the last day that was 5 days interval, for a period of 14 days of herb administration.

ASSAY PROCEDURE

UREA

Principle: Urea in plasma is hydrolysed to ammonia in the presence of urease. The ammonia is then measured spectrophotometrically by Berthelot's reaction

$$\text{Urea} + H_2O \xrightarrow{\text{Urease}} 2NH_3 + CO_2$$

$$NH_3 + \text{hypochlorite} + \text{phenol} \longrightarrow \text{Indophenol (Blue compound)}$$

Sample: Plasma

Reagent Composition Content

Initial Conc. Of Solution

1+2 EDTA	116mMol/L
Sodium nitroprusside	6mMol/L
Urease	1g/L
3. Phenol (diluted)	120mMol/L
4. Sodium hypochlorite (diluted)	27mMol/L
Sodium hydroxide	0.14N
Standard	13.3mMol/L (80mg/dl)

Stability and preparation of reagents 1+2. Sodium nitroprusside and urease. Transfer the contents of vial 1 into bottle 2 and mix gently. Stable for 2 months at $+2^0$C to $+8^0$C.

3. Phenol. Dilute content of bottle 3 with 660ml of distilled water. Rinse bottle thoroughly and mix. Store in a dark bottle stable for 2 months at $+2^0$C to $+8^0$C.

4. Sodium hypochlorite. Dilute the contents of bottle with 750 ml of distilled water, Rinse bottle thoroughly and mix. Store in dark bottle. Stable for 3 months at 2^0C to $+8^0$C.

PROCEDURE

Measurement against reagent blank

Pipette into test tubes

	Blank	Standard	Sample
Sample	-	-	10µL
Standard	-	10µL	-
Distilled H_2O	10µL	-	-
Solution	100µL	100µL	100µL
Mix and incubate at 37^0C for 10 minutes			
Solution 3	2.50ml	2.50ml	2.50ml
Solution 4	2.50ml	2.50ml	2.50ml

Mix and incubate at 37^0C for 15 minutes.

Read absorbance of the sample and standard against the blank at 546nm.

Calculations:

$$\frac{SamplesAbs}{Standard\ Abs}$$ x 80 mg/ dL

Abs = Absorbance

CREATININE

Principle: Creatinine is a by-product of the breakdown of creatinine and phosphocreatine an energy storage compound in muscle. The concentration of creatinine produced is then measured spectrophotometrically by creatinine modified Jaffe's method.

Sample: Plasma.

Reagent Composition Content

Initial Conc. of Solutions

1. Reagent A
   Picric Acid 55mMol/L
2. Reagent B

Sodium hydroxide (NaOH) 0.4M
Sodium Carbonate 50mMol/L

PROCEDURE:

Measurement against reagent blank

Pipette into test tubes

	Blank	Standard	Sample
Reagent A	1.0ml	1.0ml	1.0ml
Reagent B	1.0ml	1.0ml	1.0ml
Standard	-	0.1ml	-
Sample	-	-	0.1ml
Distilled water	0.1ml	-	-

Mix immediately and Read at 510nm after 15 mins incubation at room temperature

Read absorbance of sample and standard against the blank:

Calculation

$$\frac{Sample\ Abs}{Standard\ Abs}\ \text{X 3mg/dL}$$

Abs = Absorbance.

WHITE BLOOD CELL COUNT

(Total and Differential)

Principle: Distilled water haemolyse the red blood cells, glacial acetic acid destroys the white blood cells and gentian violet stains the white blood cells and give colour to the solution.

Sample: Whole blood

Turks Solution Reagent

Gentian violet (GV) 2ml, glacial acetic acid (1%) 2ml,

Distilled water 2ml,

PROCEDURE:

Pipette 0.38ml of Turk's solution into a test tube + 0.02ml of whole blood and allow to stand for 3 mins to lyse the cells.

Charge the counting chamber and the appearance of Newton ring will indicate that the chamber is well charged.

Load the chamber using Pasteur pipette. View under microscope using x 40 to count the cell

CALCULATIONS:

Total count is expressed in µL.

Differential count is expressed in percentage (%).

STATISTICAL ANALYSIS

The student t-distribution (t-test) was used for the statistical analysis of the result. Results were expressed as Mean ± S.E.M. for the number of experiments done.

Mean: $\Sigma\frac{x}{n} = \chi$

Where Σ = summation of values.

x= observed value of parameter

n= number of observations.

STANDARD DERIVATION S.D. = $\frac{\sqrt{\Sigma(x-\chi)2}}{n-1}$

Where x = observed value of parameter

χ = mean of values obtained

n = number of observations.

STANDARD ERROR MEAN Sχ= $\frac{S.D}{\sqrt{n}}$

Where S.D. = standard deviation

n = number of observations

t-test= $\frac{observed\ difference\ between\ mean}{standard\ error\ of\ difference\ between\ mean}$

t-test=$\frac{X1-X2}{\frac{S1}{n1}+\frac{S2}{n2}}$

Where X_1 = mean of control

X_2 = mean of treated

n_1 = number of control animals

n_2 = number of treated animals

S_1 = Standard deviations of control animals

S_2 = Standard deviation of treated animal

The P value obtained being greater than 0.05 were considered to be non-significant.

RESULTS

The mean and standard deviation of all parameters for different days after plant administration to the test animals were calculated and recorded. The results were expressed in mg/dl, per micro litre (/µL) and percentage (%). The parameters were calculated for both control and treated animals as presented in the table below where 5 animals served as test and 4 animals as control.

CONTROL ANIMALS

Basal concentrations of plasma urea, creatinine and white blood cell count (total and differential) in normal rabbits

Parameters	Day 3	Day 6	Day 9	Day 14
Urea mg/dL	39.25 ±02.28	37.25 ±01.64	39.50 ±01.48	42.25 ±04.74
Creatinine mg/dL	0.90 ±0.09	0.85 ±0.16	0.83 ±0.8	0.85 ±0.15
WBC total x 10^3 µL	4.70 ±0.597	6.15 ±0.280	5.83 ±0.792	5.83 ±1.134
WBC differential %				
a. Neutrophils	49.25 ±04.56	43.25 ±07.40	52.00 ±08.60	42.50 ±03.20
b. Lymphocytes	49.50 ±05.02	56.25 ±03.21	48.00 ±06.72	54.00 ±04.04
c. Eosinophils	01.25 ±01.88	-	-	01.50 ±02.25
d. Monocytes	-	00.50 ±00.18	-	02.00 ±01.12
e. Basophils	-	-	-	-

Values are mean ±S.D for plasma urea, creatinine, and white blood cell count (total & differential). Values of urea and creatinine are expressed in (mg/dl), WBC total in (µL) and WBC differential in (%). Values indicated were obtained at 3 days interval except the last day that was 5 days interval of extract administration.

TREATED ANIMALS

Effect of *Irvingia grandifolia* on plasma concentrations of urea, creatinine and white blood cell count (total and differential) in normal rabbits**.**

Parameters	Day 3	Day 6	Day 9	Day 14
Urea mg/Dl	36.80±08.01	33.80±08.86	38.60 ±07.28	39.00 ±07.90
Creatinine mg/dL	0.80 ±0.07	0.91 ±0.14	0.82 ±0.10	0.78 ±0.11
WBC total x 10^3 µL	4.70 ±0.206	5.22 ±0.673	5.40 ±0.381	5.74 ±1.140
WBC differential %				
a. Neutrophils	53.00 ±06.69	46.80 ±06.10	45.00 ±04.97	47.00 ±08.75
b. Lymphocytes	45.00 ±08.02	57.00 ±08.75	54.60 ±04.21	50.40 ±06.70
c. Eosinophils	02.00 ±01.58	-	00.40±00.72	01.80 ±01.74

d. Monocytes	-	00.20 ±00.36	-	00.80 ±01.43
e. Basophils	-	-	-	-

Values are mean ±S.D for plasma urea, creatinine, and white blood cell count (total & differential). Values of urea and creatinine are expressed in (mg/dl), WBC total in (µL) and WBC differential in (%). Values indicated were obtained at 3 days interval except the last day that was 5 days interval of extract administration.

DISCUSSION

Irvingia grandifolia has been found to be very effective as anti-diabetic agent. The effect of this herb on plasma urea, creatinine concentrations and white blood cell count has not been investigated so far. The current investigation that was conducted shows that the herb produces no significant effect (P>0.05) from students t-test, on plasma urea concentration on the treated animals (37.05 ± 03.65) mg/dL as against the control (39.56 ± 02.54) mg/dL. The Laboratory reference range for plasma urea is 10-55mg/dl. Plasma urea concentration is an end product of protein metabolism in the liver and cleared by renal glomerular of the kidney. Once an animal continues to live on a protein containing diet, plasma urea will be continuously produced by the liver and any metabolites that interfere with the clearance of this urea impairs kidney function (Henry, et al, 1974).

From this study, it has been found that *Irvingia grandifolia* does not interfere with urea clearance. There is also no significant effect (P>0.05) from students t-test, of this herb on white blood cell count (WBC) total and differential, as found out from the result obtained from this study. This value of white blood cell count (WBC) total was (5.27 ± 0.341 x 10^3) for treated animals as against (5.63 ± 0.351 x 10^3) µL for control animals. The laboratory reference range for total white cell count is 3.5-10.0 X 10^3/L. There is no significant effect (P>0.05) from students t-test, of this herb on white blood cell (differentials) neutrophil of (47.95 ± 02.77) % for treated animals as against (46.75 ± 03.21) % for control animals, the Laboratory reference range for neutrophil is 45-65 %. Lymphocytes of (50.75 ± 02.77) % for treated animals as against (51.94 ± 03.20) % for control animals, Eosinophils of (01.67 ± 00.69) % for treated animals as against (02.75 ± 00.99) % for control animals, monocytes of (01.25 ± 0069) % for treated animals as against (01.25 ± 00.69)% for control animals.

White blood cell fights infections through a process known as phagocytosis. White blood cells or Leukocytes also produce, transport and distribute antibodies as part of the body's immune response (Struat, 1996). Any substance that causes a reduction in the normal value for white blood cell count (WBC) total and differential will as well impair WBC to fight infections, produce, transport and distribute antibodies, so that in a serious disease state such as diabetes, will allow the disease to overpower the individual's immune system that can eventually lead to death. As

obtained from this study *Irvingia grandifolia* does not alter the normal values of WBC and therefore allows WBC to carry out its physiological functions of fighting diseases. This finding seems to be one of the greatest advantages *Irvingia grandifolia* enjoys as a local herb in the treatment of diabetes mellitus.

The herb also produced no significant effect (P>0.05) from students t-test, on plasma creatinine level on the treated animals (0.83 ± 0.09) mg/dL as against (0.86 ± 0.12) mg/dL for control animals. The Laboratory reference range for plasma creatinine is 0.6-1.2mg/dl. All the parameters comparison was determined using students distribution (t-test).

CONCLUSION

Though plasma creatinine concentration can vary based on a number of factors including an animal's diet, muscle mass and gender (Watson, et al, 2002). Circulating levels of creatinine are used primarily as an index of renal function; high plasma creatinine concentration are encountered in nephritis and renal obstruction reflecting the degree of impairment (Bishop, et al, 2000). It can therefore be concluded that *Irvingia grandifolia* has no effects on kidneys clearance ability which is a clinical sign of renal failure that would produce elevations in both plasma urea and creatinine concentrations that was not observed in this study.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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