

The Potential of Ceylon Spinach (*Talinum triangulare Jacq. Willd.*) on Selected Haematological, Liver and Kidney Parameters

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Abstract: *The study was conducted to determine the potential of Talinum triangulare Jacq. Willd.) for selected haematological, liver and kidney parameters. The data obtained were statistically analyzed using three-way variance analysis with Tukey's Honestly Significant Difference test, post-hoc tests to assess the level of significance between control and experimental groups. The conversion of data to percentages is compared and expressed to quantify the change, and the different parameters tested before and after oral administration have been increased and decreased. Results showed that the leaves of Ceylon Spinach contained an appropriate amount of saponins, tannins, sterols, triterpenes, glycosides, flavonoids and alkaloids. Among the three treatments of the crude extract the 150 mg/kg was consistent and effective to have the highest improvement in terms of increasing the level of RBCs and PCVs which can contribute significantly in the treatment of anaemia, but not in persons with kidney and liver diseases without medical supervisions.*

Keywords: Ceylon spinach, haematological, phytochemical analysis

1. Introduction

For decades the screening of medicinal plant materials has continued to represent potential sources of new effective medicines for their therapeutic values. Man has derived enormous benefits from the use of medicinal herbs in disease management, as they are relatively safer, more affordable and sometimes offer better therapeutic value than synthetic drugs. Increasing the discovery of more medical plants has required increased scientific scrutiny of their bioactivity to provide data that will help doctors and patients make wise decisions prior to their use.

The Department of Health (DOH) has already seen a dramatic increase in the number of people either starting or undergoing dialysis since reaching those figures, with 23,000 going through the process due to renal failure in 2013 compared to only 4,000 in 2004. Since then, the DOH has reported an increase of 10 to 15 percent in cases of kidney disease per year.

Dialysis and transplantation are under such enormous financial pressure that the majority of patients seeking kidney care were those living in Germany, the United States, Japan, Brazil and Italy [1].

The liver is an organ about the size of a football located on the right side of your abdomen, just below your rib cage. The liver is essential for digesting food and for getting rid of toxic substances in your body. Liver disease can be hereditary (genetically) or caused by a number of adverse factors in the liver, such as viruses and alcohol use. Obesity has to do with liver damage as well. Over time, liver damage leads to scarring (cirrhosis), which may lead to life-threatening liver failure [2].

Sometimes, variations in the pattern of liver blood tests give an indication of the type of liver disease. For example, an AST to ALT ratio of more than two (as long as both are less than nine times normal) suggests some form of alcoholic hepatitis or cirrhosis [3].

More Filipinos are diagnosed with liver disease, yet it remains a "silent epidemic." According to experts, lifestyle, which is primarily influenced by western practices, is said to be among the factors leading to rising numbers [1]. If not stopped now, the number of liver cancer deaths in the next 20 years will be doubled as to the rate for the current 20 Filipinos per day. This prediction is based on the latest projections of the International Cancer Research Agency (IARC) for the Philippines. Hepatic cancer is said to be the third leading cause of cancer death in the country, the second among Filipino men, and the fifth among women [4].

There are various blood cancers, some of them benign or non-cancerous, while others can be known as blood cancer types. Blood disorders can affect any of the three major components of your blood: (1) red blood cells that carry oxygen to the body's tissues; (2) white blood cells that fight infection; and (3) platelets that allow blood to clot. To ensure that the kidneys, liver and blood remain healthy, be sure to eat nutrient-rich foods.

Nature has given man the most effective remedies for any disease. Every day, scientific science advances the limits of nature's healing capacity by researching medicine in the form of Ceylon spinach.

The Ceylon Spinach, or locally known as Talinum, is adaptable throughout the Philippines. It is best suited to a tropical climate which is partially shaded and humid. However, according to the record of the Bureau of

Agricultural Statistics (BAS), there are no data recorded regarding production area and volume of talinum production.

According to a study Ceylon Spinach has an anti-hyperglycemic effect on diabetics. These leaves are a good candidate as a complementary medication to regulate diabetes. Throughout India these leaves are used as anti-diabetic agents throughout ayurvedic medicinal goods. It complements a balanced lifestyle and diet, which is important for proper management of blood glucose levels [5].

Ceylon Spinach leaves contain a phytochemical called alkaloid and are a potent stimulant to the central nervous system, i.e. improving physical activity, mental alertness and length of attention, and use Central Nervous System (CNS) stimulants to treat Attention Deficit Hyperactivity Disorder (ADHD). The phytochemical also interacts well with pain relievers.

Acute oral toxicity studies provide preliminary information on the toxicity of plant content about which there is no specific toxicological information. LD50 (dose known to be lethal, causing death to 50 percent of the group of animals tested). This is usually the first step in determining and measuring the toxic properties of a substance. It is an initial assessment of the toxic nature, providing information about the health hazards likely to occur in the short term from drug exposure [6]. The LD50 for a particular substance is the amount that can be predicted to cause death in half (i.e. 50 %) of a population of certain species of animals, generally rats or mice, when delivered by a specific route [7]. This is generally expressed as the amount of chemical (e.g., milligrams) administered per 100 g (for small animals) or per kilogram (for larger subjects) of the body weight of the test animal [8].

Furthermore, researchers continue to stress that Ceylon Spinach or water leaf, due to its high antioxidant content and medicinal properties, is not only exceptionally nutritious, but also safe against chronic and degenerative diseases. The ability of Ceylon Spinach (*Talinum triangulare Jacq. Willd.*) has, therefore, been tested on selected hematological, liver and kidney parameters of the Sprague Dawley rat model.

Statement of Objectives

The goal of this study was to identify the potential of Ceylon Spinach (*Talinum triangulare Jacq. Willd.*) on selected parameters of haematology, liver and kidneys.

Specifically, this study aimed to (1) Determine the phytochemical constituents of the leaf extract of the Ceylon Spinach (*Talinum triangulare Jacq. Willd.*) using ethanol as solvent; (2) Determine the amount of (PCV) Packed Cell Volume / Haematocrit, Red Blood Cells, Creatinine, (SGOT) Serum Glutamic-oxaloacetic transaminase/(AST) Aspartate aminotransferase, (SGPT) Serum Glutamic-pyruvic transaminase/ (ALT) Alanine transaminase of the various male and female experimental rat weight treatments; (3) Determine the significant difference in efficacy of Ceylon Spinach at different concentrations (*Talinum triangulare Jacq. Willd.*); (4) Determine the extent of

Ceylon Spinach toxicity (*Talinum triangulare Jacq. Willd.*); (5) Develop a validated Information and Education Communication (IEC) Materials on Ceylon Spinach (*Talinum triangulare Jacq. Willd.*).

2. Materials and Methods

2.1 Research Design

In the conduct of this study, descriptive and experimental research methods were used to understand the purpose of the research. Descriptive method has been used to respond to concerns about plant material authentication and verification; phytochemical analysis to predict biologically active secondary metabolites present in plants including alkaloids, sterols, triterpenes, flavonoids, saponins, tannins and glycosides; acute oral toxicity test (LD50) to determine the level or predicted dose that causes mortality on ICR mice; and to determine the level of validity of the material produced for Information Education Communication.

Experimental research method has been used to respond to concerns regarding the potential of Ceylon Spinach (*Talinum triangulare Jacq. Willd.*) on selected haematological, liver and kidney parameters. Packed Cell Volume (PCV)/Haematocrit, Red Blood Cells (RBC), Creatinine, Serum glutamic oxaloacetic transaminase (SGOT)/Aspartate transaminase (AST), Serum glutamic pyruvic transaminase (SGPT)/Alanine aminotransferase (ALT) and the weight of laboratory rats.

The experimental rats were selected at random and allocated to two groups: control group and treatment group. Thirty-two (32) Sprague Dawley rats of both sexes, weighing between 100-150 grams (16 male rats and 16 female rats) were used and divided randomly into four (4) different treatments. Animals were grouped into two (2) according to their genders in each treatment. Four (4) heads for the male and four (4) heads for the female. There are 4 treatments. Treatment 0: Standard diet; Treatment 1: 100 mg/kg body weight of the ethanol crude extract of *T. triangulare* leaves; Treatment 2: 150mg/kg body weight of the ethanol crude extract *T. triangulare* leaves; Treatment 3: 200 mg/kg body weight of the ethanol crude extract *T. triangulare* leaves

The study consisted of six (6) phases: Phase I authentication preparation of plant material; preparation of Ceylon spinach leaves for phytochemical analysis and acute oral toxicity test; Acquisition of experimental rats; Preparation of plant extract for oral administration; Experimental process (In-vivo study of Animal model; and Development of an Information Education Communication (IEC) material.

2.2. Time and Place of the Study

Ceylon Spinach (*Talinum triangulare Jacq. Willd.*) leaves were submitted for phytochemical analysis and toxicological studies (LD50) to the Department of Science and Technology-ITDI (Institute for Industrial Technology Development), Standards and Testing Division, Gen. Santos Ave., Bicutan, Taguig City, Metro Manila.

Authorization for scientific procedures using animals has been submitted to the Regulatory Division, Department of Agriculture, City of San Fernando, La Union and an Animal Research Permit has been granted by the Department of Agriculture, Bureau of Animal Industry, Visayas Avenue, Diliman, Quezon City, pursuant to the provisions of RA 8485 (Animal Welfare Act, 1998) as amended by RA 10631 and DA-Administrative Order (AO) No. 40, series of 1999, on the Rules and Regulations on the Scientific Procedure Using Animals.

The research was conducted at Don Mariano Marcos Memorial State University Animal Laboratory, Campus North La Union, Bacnotan, La Union.

Determination of Packed Cell Volume (PCV)/Haematocrit, Red Blood Cells (RBCs), Creatinine Assay, SGOT (AST) and SGPT (ALT). Those 32 SD rats of both sexes weighing between 100-150 grams were brought for testing to High-PRECISION Diagnostics, City of San Fernando, La Union.

2.3. Materials and Procedures

Phase I. Preparation of Plant Material for Authentication

Matured healthy Ceylon Spinach (*Talinum triangulare Jacq. Willd.*) plants were collected, air dried and properly labeled in an herbarium sheet and submitted for authentication / verification to Dr. Analyn V. Sagun, Agriculturist / Horticulturer at Don Mariano Marcos Memorial State University- Sapilang Campus, Bacnotan, La Union.

Phase II. Phytochemical Analysis and Acute Oral Toxicity Test

For the preparation of plant material for Phytochemical Analysis, three kilos of fresh, matured leaves of Ceylon Spinach (*Talinum triangulare Jacq. Willd.*) were gathered at Caburao, Santiago, Ilocos Sur. They were thoroughly washed with water to remove sticking dirt. The leaves were cut into small pieces and placed in a separate container. These were submitted to Department of Science and Technology- ITDI (Industrial Technology Development Institute), Standards and Testing Division, Gen. Santos Ave., Bicutan, Taguig City, Metro Manila for qualitative phytochemical screening employing the standard methods.

Preparation of the Plant Material for Acute Oral Toxicity Test (LD50)

Five kilos of matured and fresh leaves of Ceylon Spinach (*Talinum triangulare Jacq. Willd.*) were gathered at Caburao, Santiago, Ilocos Sur and washed thoroughly to remove adhering dirt. The leaves were cut into small pieces and were placed in a separate container. These were submitted to Department of Science and Technology- ITDI (Industrial Technology Development Institute), Standards and Testing Division, Gen. Santos Ave., Bicutan, Taguig City, Metro Manila for LD50 Oral Toxicity Test screening employing the standard methods.

30 male ICR mice (25-37 grams and 100 grams of greenish-black crude extract) were used for the acute oral toxicity test. The sample's median lethal dose (LD50), given orally to

ICR mice using Mice Adjusted Acute Oral Toxicity; OECD 401. Toxidrome ranged from lower motor and respiratory activity, grooming, hyperemia, ptosis, piloerection, loss of grip resistance, pine and remedial reflexes, defecation, urination, passivity, ataxia, tremor, paralysis, and death of mice.

Preliminary dosing was conducted to evaluate the predicted dose which will cause the experimental animals to die by 50 percent. Three (3) the log doses of the test substance were administered orally to the animals on group three (3) of ten (10). The number of deaths and other adverse pathological signs and symptoms was closely monitored and recorded during the first two (2) hours after the test samples were administered. This happened within the next 24 to 48 hours, up to 14 days average.

Phase III. Procurement of Experimental rats (SD rats)

Fifty (50) heads of Sprague Dawley Rats were procured from Frankie Pet Shop of Agtas, San Juan, San Carlos, Pangasinan, a registered PETSHP with BAI registration No. PTS-0031 (New System) dated November 10, 2016 valid until November 2019 in compliance with RA No. 8485 (Animal Welfare Act of 1998) as amended by RA No. 10631, at the Animal Health and Welfare Division (AHWD), Bureau of Animal Industry.

Of the fifty (50) Sprague Dawley (SD) rats, thirty-two (32) rats were used in the analysis whereas the remaining eighteen (18) Sprague Dawley (SD) rats acted as reserves in the event of death.

Phase IV. Preparation of Plant Extracts for oral administration

For the preparation of the Plant Material for Crude Extraction, half a kilogram of Ceylon Spinach (*Talinum triangulare Jacq. Willd.*) freshly matured leaves were collected in Caburao, Santiago, Ilocos Sur and washed thoroughly to avoid the adhering soil. The leaves were cut into small parts, placed in a separate container. These were sent to LORMA, College of Pharmacy for Crude Extraction, where it undergoes 48 hours of cold maceration in 1400 ml of ethanol. The filtrate was collected for solvent removal in the water bath and stored in the fridge until it was needed.

Phase V. Experimental Process (In vivo study on animal model)

a) Test Animals

In-vivo study with 32 Sprague Dawley rats of both sexes (16 male rats and 16 female rats) weighing between 100-150 grams, was distributed randomly for approximately 4-5 weeks and divided into four (4) different treatments. In each treatment, the animals were grouped into two (2) heads according to their sexes. Four (4) heads for the male and four (4) heads for the female.

The experimental rats were utilized at the Animal Laboratory of Don Mariano Marcos Memorial State University- North La union Campus, Bacnotan, La union. The laboratory animals were acclimatized at 25oC room temperature, 45-55 percent humidity, and 10-12 hrs for at least six (6) days/light day. The animals were given sufficient feed and were regularly cleaned. Each treatment

group was locked in trays of plastic hole. Wood shavings were used as a base for the bedding. The bedding had the principal purpose to remove urine and feces. The rats were kept under stable conditions at temperature ($22 \pm 20^{\circ}\text{C}$), humidity (55 per cent) and light (12 h light/dark condition).

Oral administration was performed the day after the first blood extraction in order for experimental rats to gain energy before using a gavage tube. Oral administration was given once a day for 10 days.

The rat was restrained and anesthetized. The hind leg was immobilized in the extended position by applying gentle downward pressure right above the knee joint. Blood was extracted from the jugular vein using a 22-gauge needle.

After six (6) days of acclimatization, blood was collected through the jugular vein for the determination of PCV (haematocrit), RBC, creatinine, SGOT/AST and

SGPT/ALT. The blood samples obtained for the SGOT/AST, SGPT/ALT and Creatinine tests were placed in a microtainer blood collection tube to assist in the collection of a satisfactory capillary blood sample. While blood collected for RBC and PCV (Haematocrit) tests has been placed in the BD Vacutainer blood collection tubes for the best possible results.

After ten (10) days of oral administration of the ethanol crude extract of Ceylon Spinach (*Talinum triangulare Jacq. Willd.*) the blood was collected again by cardiac puncture to determine if there is a significant difference and impact on the PCV (Packed Cell Volume), RBC, Creatinine, SGOT/AST and SGPT/ALT levels in different experimental rat treatments. The rat was anesthetized, and a 22-gauge needle is used to collect blood through the left ventricle. Blood was slowly withdrawn so the heart could not collapse.

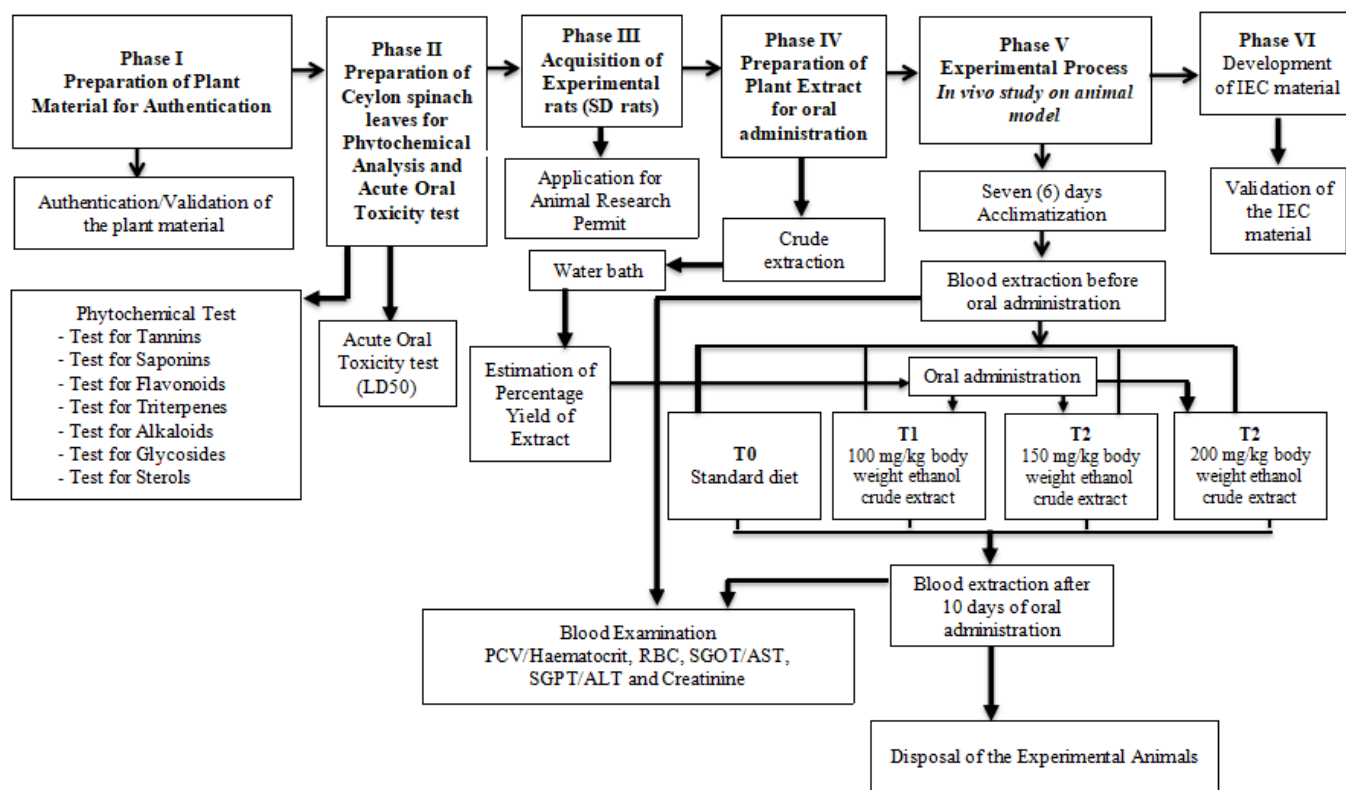


Figure 1: Flow chart of procedure

2.4. Procedure

a) Qualitative Phytochemical Analysis

Phytochemical tests of the collected Ceylon spinach leaves were performed as per standard procedure. The qualitative phytochemical analysis was carried out from the leaves of the plant material and collected using the process of distillation. Specific solvent systems were used to classify important natural chemical groups such as tannins, saponins, flavonoids, phenols, triterpenes, alkaloids, glycosides, and sterols.

Determination of Packed Cell Volume (Haematocrit)

The PCV was obtained by using a microhematocrit reader to measure the height of the RBC column for each tube and expressed this as a ratio of the total blood column height.

$$PCV (\%) = \frac{\text{Height of cell column} \times 100}{\text{Height of total blood column}}$$

b) Determination of Red Blood Cells counts (RBCs)

Dilution factor: 0.5 parts of blood mixed in at least 100 parts of the mixture (99.5 parts of diluting fluid); fluid present in the stem (1.0 part) is not involved in mixing; therefore, 2-3 drops of fluid (present in the stem) are discarded before charging the chamber.

The RBCs (in mm)

= cells counted \times correction for volume \times correction for dilution

= RBC counted in 5 small squares \times 200 \times 1.0/0.2 (or 50) n

= Number of RBC counted in five squares \times 104

c) Creatinine Assay

Using 30 μ L of the samples. The creatinine detection limit of 0.10 mg/dL (8 μ M) for 96-well plate assay. The procedure involved injecting a single working reagent and an incubation period of 5 minutes. It can be automated for thousands of samples per day as a high-throughput assay. The optimized formulation has greatly enhanced reagent stability and signal stability. Tests can be performed with a 96-well plate or cup [9].

d) SGOT/AST

Duplicates have been set up to determine the blank and the sample test tubes. The sample tubes have been pipetted with 0.1 ml of serum. Reagent 1 pipetted 0.5 ml into both the sample and the blank tubes. The mixtures were thoroughly mixed and incubated at 37°C ml and pH 7.4 for exactly 30 minutes. In all test tubes, 0.5 ml of Reagent 2 containing 2, 4-dinitrophenylhydrazine was added, followed by 0.1 ml of sample in blank tubes. The tubes were thoroughly mixed and incubated at 25°C. 5.0 ml of sodium hydroxide solution for exactly 20 minutes, then inserted and pressed into each tube. After 5 minutes at 546 nm, the absorbance was read against the blank. Calculated the change in absorbance per minute. (Δ Abs./30 seconds \times 2). Activity of GOT (AST) in IU/l = Δ Abs./min. \times 3339 [10].

e) SGPT/ALT

The test tubes for blank and sample were mounted in duplicates. The sample tube was pipetted with 0.1ml of serum. This 0.5ml buffer solution containing phosphate buffer, L-alanine and a-oxoglutarate has been added. For exactly 30 minutes the mixtures were thoroughly mixed and incubated at 37°C ml and pH 7.4. In both tubes, 0.5ml of the 2,4-dinitrophenylhydrazine reagent was later added, while 0.1ml of the sample was applied to the blank tube. The tubes were thoroughly mixed and incubated for precisely 20 minutes at 25°C. 5.0ml of sodium hydroxide solution, then inserted into each tube and mixed in. The absorbance was read against the blank after 5 minutes, at 540 nm. The change in absorbance is measured every minute. (Δ Abs / 30 seconds \times 2) GPT (IU/L) = Δ Abs / min \times 3339 [10].

f) Disposal of the Experimental Animals

After the test, the in-house veterinarian properly disposed all the experimental animals which were used in the study. Before disposal the laboratory rats were removed by inhalation of carbon dioxide from their cages and euthanasia. Carcasses were labelled (necropsy) and packaged in a non-PVC containing, sealable, transparent plastic bags. It eventually fell into the carcass tub in the animal house's cold room [11].

g) Computation of Mean decrease and Percentage reduction (%)

PCV/haematocrit, SGPT/ALT, SGOT/AST, creatinine, red blood cells (RBCs), and weight before and after ten (10) days of oral treatment. Computed as Mean in the level = Original level before oral administration – New level after

oral administration. For the Percentage Decrease = Mean difference/ Original level before oral administration*100

Phase VI. Develop and Validate Information Education Communication (IEC) material

IEC materials' validity includes the following indicators: (1) attractiveness and organization, (2) content, (3) writing mechanics, (4) graphics / pictures, and (5) quality of research. The validity of the brochure on the weighted mean's descriptive equivalent, the following statistical ranges were used to assess the validity level of the material produced by the IEC [12].

| Overall Rating | Description |
|----------------|-----------------|
| 3.25 – 4.00 | Very high valid |
| 2.50 – 3.25 | Highly valid |
| 1.75 – 2.50 | Slightly valid |
| 1.00 – 1.75 | Not valid |

Five (5) experts in the fields of science, study and technical writing, health services, and agricultural services were selected as validators of the IEC material on Ceylon Spinach. Two (2) medical practitioners, one (1) from the Department of Agriculture, one (1) from the Ilocos Sur Polytechnic State College, and one (1) from Don Mariano Marcos State University. The validators assessed the validity of face and content of the said IEC material.

2.5 Statistical Analysis

Values acquired from the various tests were expressed as mean. The data collected were statistically analyzed using a tri-way variance analysis (ANOVA) with the Honestly Significant Difference (HSD) test by Tukey, post-hoc tests to compare the significance level between control and experimental groups [13].

Levene's Test has also been used to check the conclusion of the test if k samples have equivalent variances. Equal variances across the samples are called variance homogeneity. Many statistical analyses, such as variance analysis, conclude that the variances between groups or samples are equal. All work was carried out at sense point 0.05.

When comparing details where sample sizes or percentages vary, percentage was used. To order to measure the change, translating the results into percentages will accurately compare and articulate values to determine the sum of the various parameters measured before and after oral administration have been increased or decreased.

3. Results

3.1 Phytochemical Constituents of Ceylon Spinach

Phytochemicals are plant-producing constituents, and these compounds have good healthy biological activity. Plant cells contain two types of metabolites: primary metabolites that are directly involved in growth and metabolism (carbohydrates, lipids and proteins), and secondary metabolites that are considered primary metabolism end-products and are not involved in metabolic activity (alkaloids, phenolics, hormones, saponins, and tannins).

The results of the phytochemical analysis of Ceylon Spinach (*Talinum triangulare Jacq. Willd*) leaf using the standard procedure for the extraction and identification of primary and secondary bioactive metabolites are presented in Table 1. Qualitative phytochemical research has shown the concentration of saponins and tannins in the leaves. There were small concentrations of sterols, triterpenes and glycosides while traces of flavonoids and alkaloids were found. This exhibits a high medicinal and dietary value [14].

Preliminary phytochemical studies confirmed the presence in the leaves of carotenoids [15], sterols, alkaloids, glycosides, flavonoids, triterpenes, tannins, and saponins [16] and Ceylon Spinach leaf extract (*Talinum triangulare*).

Table 1: The Phytochemical Analysis of Ceylon spinach (*Talinum triangulare Jacq. Willd*) leaf

| Constituents Detected/tests | Constituent Detected | Result |
|-----------------------------|----------------------|----------|
| Sterols | ++ | Moderate |
| Triterpenes | ++ | Moderate |
| Flavonoids | + | Traces |
| Alkaloids | + | Traces |
| Saponins | +++ | Abundant |
| Glycosides | ++ | Moderate |
| Tannins | +++ | Abundant |

Note: (+) Traces, (++) moderate, (+++) abundant, (-) absence of constituents

Both of these experiments have reported the cumulative concentrations of these compound groups, without clarifying the actual compounds that make them up. An attempt to identify such components by [17], yielded propanoic acid, allantoin, 3-O-bD-glucopyranosyl-sitosterol, 3-O-bD-glucopyranosyl-stigmasterol, (132S, 17R, 18R)-phaeophytin a, 17R,18R-purpurin18 phytol ester, fuscichlorin D acid ester, talichlorin A, 31, 32-didehydro-151-hydroxyrhodochlorin-15-acetic acid d-lactone-152-methyl-173-phytyl ester, and hydroperoxy-fuscichlorin D [17]. Nonetheless, did not quantify the compounds contained. And to measure the quantitative sum of phytochemicals in plant samples due to lack of experimentation. As a result, the researcher was conducting a qualitative phytochemical analysis to assess the phytochemical constituents [17].

Scientific research suggests the following 12 health benefits of triterpenes through inhibiting or halting cancer growth, colon cancer, inflammatory response, hepatoprotective, breast cancer, human immunodeficiency virus (HIV), antioxidant function, oral mucosal disease, antibacterial activity, human T-cell leukemia and anxiolytic behavior. Triterpenes have been shown to be active against leukemia, hepatic cancer and glioma [18].

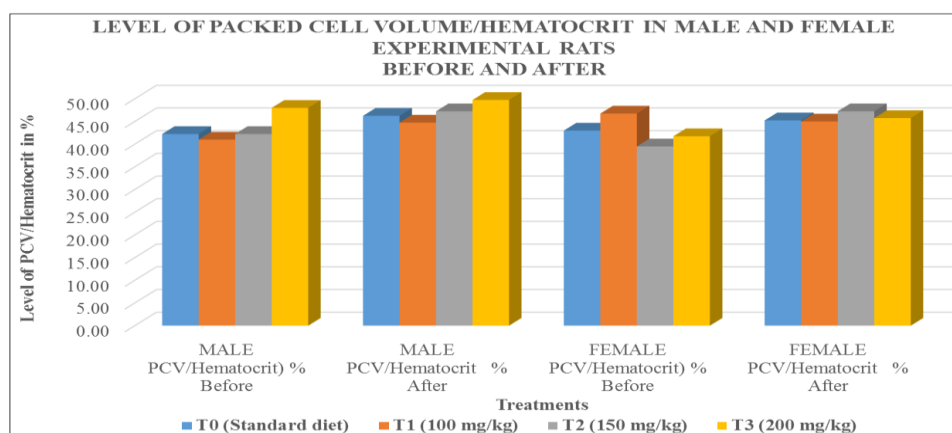
3.2. Level of PCV/Haematocrit, RBCs, Creatinine, SGOT/AST, SGPT/ALT and Weight in Different treatments of Male and Female Experimental Rats

Assessment of possible effects of ethanol crude extract of *T. triangulare* on SGPT/ALT, SGOT/AST, Creatinine, Red Blood Cells (RBCs), PCV/Hematocrit and weight in experimental male and female rats. All these parameters were determined before and after ten (10) days of oral administration.

3.2.1. Packed Cell Volume (PCV)/Haematocrit level in Male and Female Experimental Rats

Figure 2 indicates that after 10 days of routine oral administration of the Ceylon Spinach crude extract (*Talinum triangulare Jacq. Willd.*) in male and female experimental rats. There has been a significant increase in total PCV/Haematocrit between the test groups and the control group. It shows that the plant may be used to treat anaemia or leukaemia, as well as red blood cell loss, excessive hydration, and malnutrition. The result was compatible with [10] research, which found that Ceylon Spinach (*Talinum triangulare Jacq. Willd.*) leaf may be used to combat anaemia and is used by pregnant women and rising children to raise blood pressure.

The increase in body weight experienced by the treated groups could be due to the bioactive components present in Ceylon Spinach (*Talinum triangulare Jacq. Willd.*), which are key players in most metabolic pathways, which could have contributed to their body weight enhancement effect. Ceylon Spinach (*Talinum triangulare Jacq. Willd.*) has been reported as a strong store of nutritionally useful bioactive substances, proteins, vitamins and protective antioxidants [19].



Physiological Data of PCV (Haematocrit) in SD Rats

*Males = 48.3 ± 2.3 % *Females = 51.2 ± 2.6 %

**based on TACONIC Biosciences.com

Figure 2: Level of PCV (Haematocrit) before and After ten (10) Days of Oral Administration in Male and Female Experimental Rats

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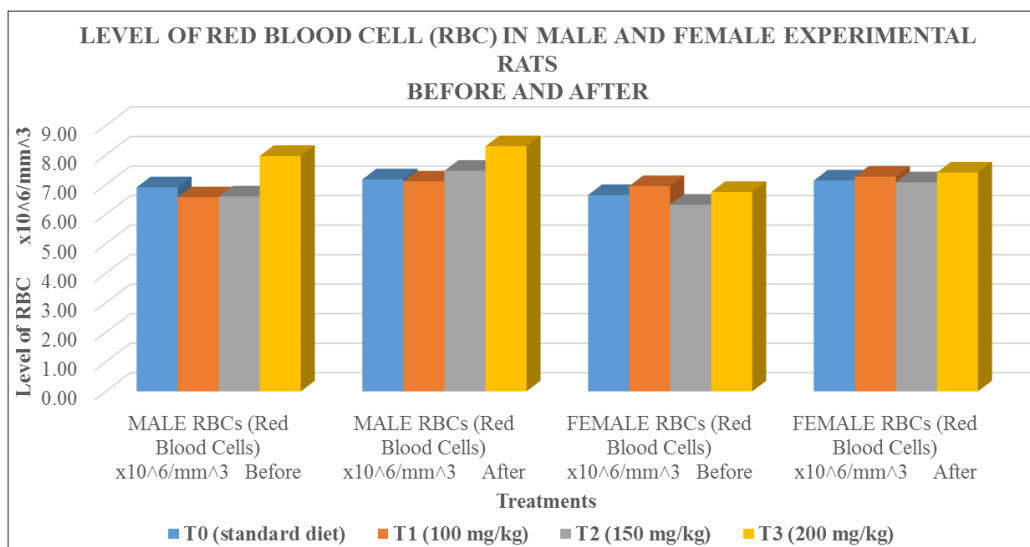
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3.2.2. Red Blood Cells (RBCs) Level in Male and Female Experimental Rats

Following a daily oral administration in male and female experimental rats of Ceylon spinach (*Talinum triangulare Jacq. Willd.*) (100, 150, 200 mg/kg body weight) for ten (10) days, Figure 3 indicates a significant increase in red blood

cells between test groups and control groups. Alkaloids are present in *Talinum triangulare Jacq. Willd.* which are strong analgesics and are responsible for central nervous stimulation and can help anaemic patients relieve anaemia-related pains [20].



Physiological Data of RBC in Sprague Dawley Rat

*Males = $6.9 \pm 0.3 \times 10^6$

*Females = $7.7 \pm 0.3 \times 10^6$

**based on TACONIC Biosciences Info@Taconic.com

Figure 3: Level of Red Blood Cells (RBCs) before and After ten (10) Days of Oral Administration in Male and Female Experimental Rats

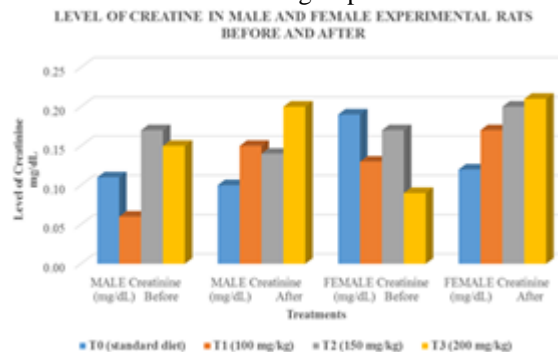
Throughout the case of laboratory female rats, it shows that the *Talinum triangulare* may be used to manage disease conditions such as anaemia, and can even be used to raise blood pressure by pregnant women and rising babies. It was recommended that Ceylon spinach (*Talinum triangulare Jacq. Willd.*) be a part of the diet of pregnant women as the vegetables help to prevent anaemia and increase blood pressure. This is because the plant has the potential to flush bilirubin out of the blood, which ensures it will make the red blood cells live longer and the body use it better [10]. *Talinum triangulare Jacq. Willd.* is an active herbal medicine used to treat gastrointestinal disorders, diarrhea, peptic ulcers, nausea, dysentery, liver disease (like paracetamol), measles, polyuria, edema, diabetes, cancer, schistosomiasis, anemia and high blood pressure [21]. The mineral content, especially iron, may be responsible for its anti-anemic effects, as these minerals are likely to be absorbed directly into the blood with the help of the other components of the plant, thus conferring the medicinal properties of the plant, which is consistent with the previous study on *T. triangulare* [22].

3.2.3. Creatinine Level in Male and Female Experimental Rats.

Creatinine is a product of normal metabolism of the muscle. After it is broken down the chemical enters the bloodstream. Remove it from blood by the kidneys. Then, the creatinine exits the body by urination.

Following the daily oral administration of Ceylon Spinach (*Talinum triangulare Jacq. Willd.*) (100, 150, 200 mg / kg

body weight) for ten (10) days, Figure 4 shows a significant increase in creatinine levels of male and female experimental rats across all test groups.



Physiological Data Creatinine of Sprague Dawley Rat

*Males = 0.3 ± 0.1 mg/dL

*Females = 0.3 ± 0.1 mg/dL **based on TACONIC Biosciences

Info@Taconic.com

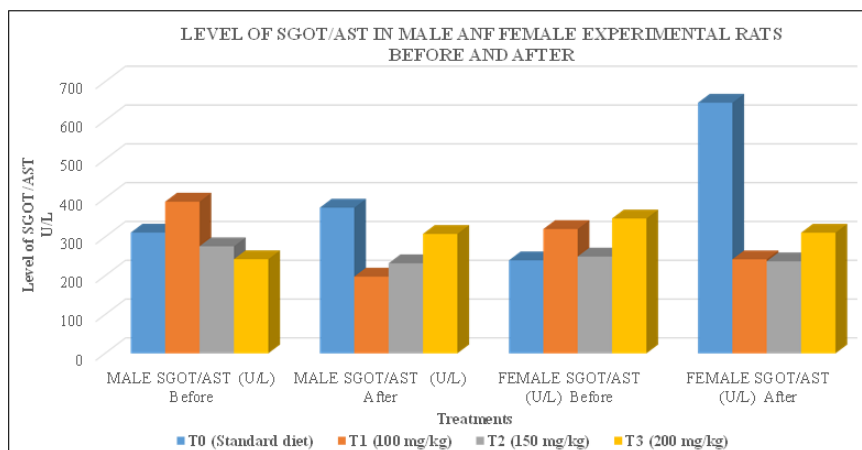
Figure 4: Level of Creatinine Before and After ten (10) Days of Oral Administration in Male and Female Experimental Rats

People with kidney stones, gout and rheumatoid arthritis should not eat ceylon spinach fresh as it contains oxalic acid that could aggravate those conditions. Cooking or blanching Ceylon spinach substantially reduces its oxalic acid, hydrocyanic acid and nitrates [25].

3.2.4. SGOT/AST Level in Male and Female Experimental Rats.

Figure 5 shows that, after the routine oral administration of Ceylon Spinach (*Talinum triangulare Jacq. Willd.*) (100, 150, 200 mg/kg body weight) for ten (10) days, the amount of SGOT / AST in male and female experimental rats in T1 (100 mg/kg) and T2 (150 mg/kg) was significantly reduced compared to T3 (200 mg/kg) and T0 (standard diet) in test groups. This indicates that crude extract of Ceylon Spinach (*Talinum triangulare Jacq. Willd.*) may be used to lower Serum Glutamic-Oxaloacetic Transaminase (SGOT) or Aspartate Aminotransferase (AST) levels in the liver.

The very high level of SGOT / AST as shown in Figure 5 before and after ten (10) days of oral administration based on the normal level. This shows that while T1 (100 mg/kg) and T2 (150 mg/kg) show a decrease in the level of the liver enzyme, Ceylon Spinach (*T. triangulare Jacq. Willd.*) is likely to destroy the liver if consumed in excess of 150 mg/kg because the level of SGOT / AST is above the normal level based on the Sprague Dawley rat clinical biochemistry reference value.



Clinical Biochemistry Reference Value of SGOT (AST) of SD Rats

*Male – 74- 143 U/L

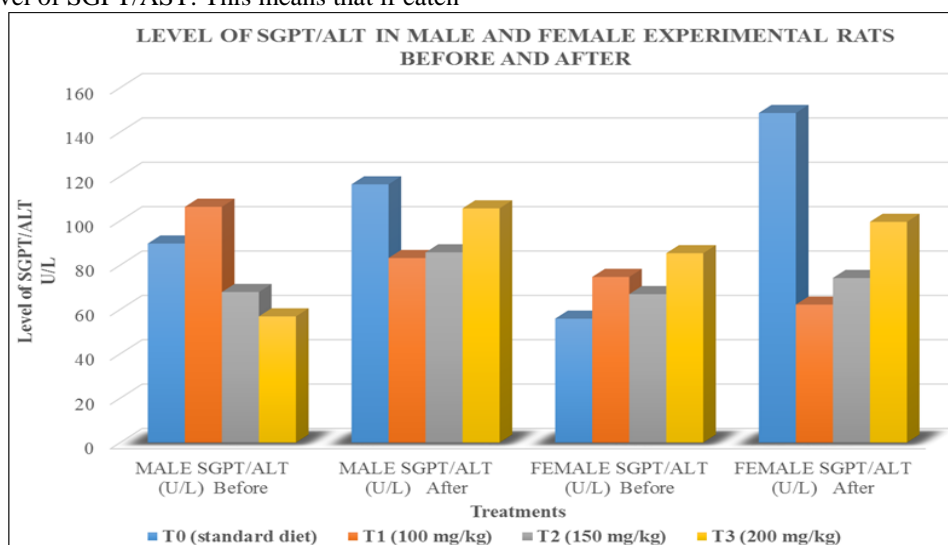
* Female – 65-203 U/L

Figure 5: Level of SGOT/AST Before and After ten (10) Days of Oral Administration in Male Experimental Rats

3.2.5. SGPT/ALT Level in Male and Female Experimental Rats

After a daily oral administration of Ceylon Spinach (*Talinum triangulare Jacq. Willd.*) (100, 150, 200 mg/kg body weight) for ten (10) days, there was a significant increase in the level of SGPT/AST between the test groups and control group in male and female experimental rats wherein T1 (100 mg/kg) shows that there is a significant decrease on the level of SGPT/AST. This means that if eaten

minimally, *T. triangulare* crude extract may be used to lower the level of Serum Glutamic-Pyruvic Transaminase (SGPT)/Alanine Transaminase (ALT) in the hepatic. However, if taken excessively as there has been an increase in liver enzyme rates (SGPT/ALT) in 150 mg/kg and 200 mg/kg *Talinum triangulare Jacq Willd.*, this may cause damage to the liver. Dosage with extract coarse as shown in Figure 6.



Clinical Biochemistry Reference Value of SGPT (ALT) in SD Rats

*Males = 18-45 U/L

*Females = 16-48 U/L

Figure 6: Level of SGPT/ALT Before and After Ten (10) Days of Oral Administration in Male Experimental Rats

Based on Figure 6, T2 (150 mg/kg) and T3 (200 mg/kg) increased the level of SGPT/ALT, which indicates that Ceylon Spinach (*T. triangulare Jacq. Willd.*) causes severe liver damage when ingested at high doses (more than 150 mg/kg to 200 mg/kg) since the level of SGPT/ALT after 10 days of oral administration is higher than the normal level of the enzyme in question. This is confirmed by a report called Biochemical and Antioxidant Effects of *Talinum triangulare* (Ceylon spinach) in Female Sprague -Dawley rats which may trigger liver harm if taken unnecessarily as seen in the present research where the degree of liver enzyme activity increased at (150ml/kg body weight) [26]. The results of the study contradict Afolabi's (2014) research entitled Effects of Aqueous Extract of *Talinum triangulare* (leaves): Evaluation of Enzyme Activities in Tissue Homogenates of Albino Rats in which the higher-dose *Talinum triangulare* aqueous extract may not be hepatotoxic and may be protective to the

organs as indicated in the animals tested. Because there were elevated levels of SGPT/ALT in the blood of treated rats compared to control based on the research on the neurochemical influence of the Aqueous Extract of *Talinum triangulare* on learning and memory in Male Wistar Rats, this could be attributed to potential metabolic stress effects.

3.2.6. Gain in Weight of Male and Female Experimental Rats

After ten (10) days of oral administration of *T. triangulare* in the experimental groups there was a significant increase in the body weight of the male and female experimental rats in the different treatments as shown in Figure 7. This finding is supported by a 2008 Mensah study that reveals the high dietary fiber in Ceylon Spinach that provides a large amount of diet to lower starchy food intake.

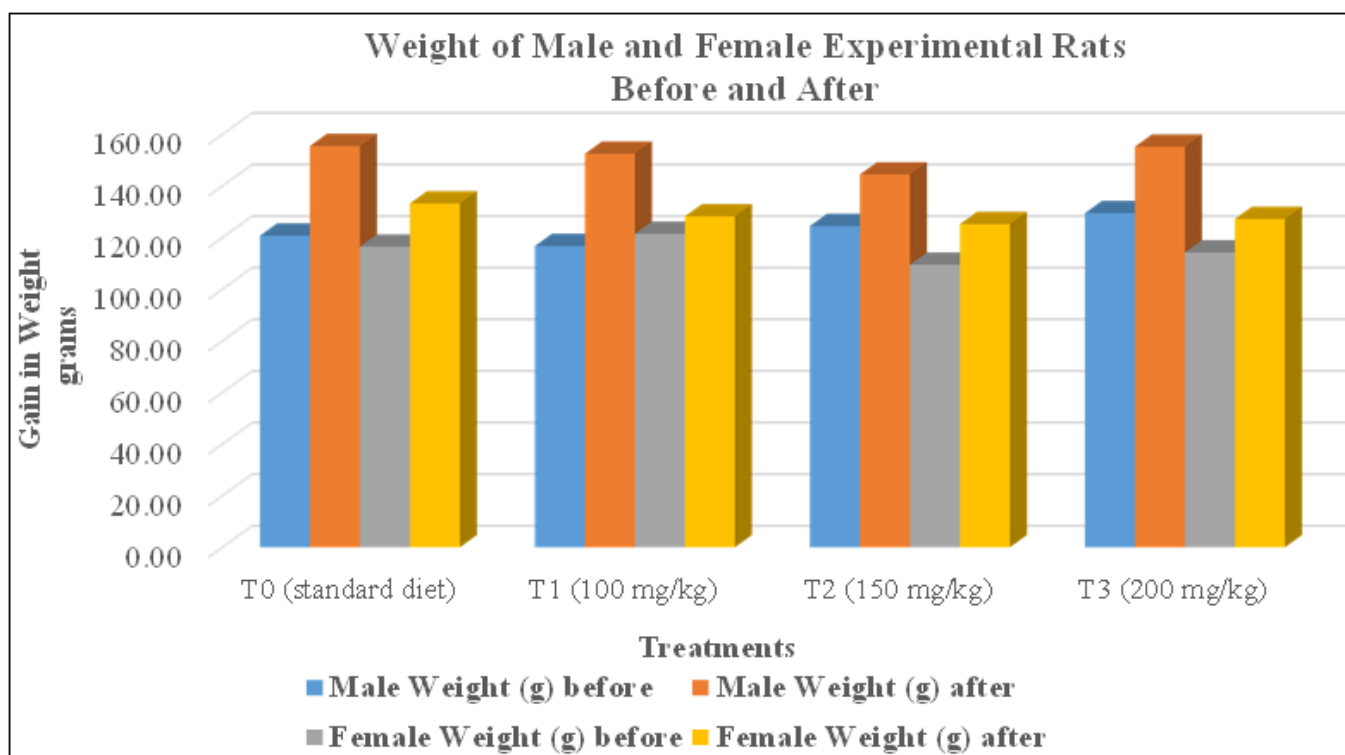


Figure 7: Weight of Male Rats Before and After Ten (10) Days of Oral Administration in Male Experimental Rats

Also supported by a study carried out by Langhout, 2000, in which the increase in weight gain can be attributed to the fact that Ceylon Spinach/water leaf extract has appetizing and digestive stimulating properties reported in his work "New additives for broiler chicken." This digestion and stimulating properties may have led to the use of feed, leading to an increased development.

3.3. Significant Difference and Effectiveness of Ceylon Spinach (*T. triangulare Jacq. Willd.*) Crude Extract in Various Concentrations of Male and Female Experimental Rats

In the study, the potential effects of *Talinum triangulare Jacq. Willd.* crude ethanol extract was assessed. SGPT/ALT, SGOT/AST, Creatinine, Red Blood Cells (RBCs), PCV/Haematocrit and Weight in male and female experimental rats. All these parameters were shown and

determined before and after ten (10) days of oral administration.

3.3.1. Significant Difference and Effectiveness of *T. triangulare* in Various Concentration on the Weight of Male Experimental Rats

Table 2 shows the level of body weight before male Sprague Dawley (SD) rats are given orally, body weight after oral administration, mean weight difference and percentage reduction on experimental rats. The results showed that the highest body weight (129.50 g) was recorded before oral administration of T3 (200 mg/kg), followed closely by T2 (150 mg/kg) with 124.50 g, T0 male (standard diet) with 120.75 g, and T1 with 116.75 g showed the lowest body weight before oral administration.

After 10 days of oral administration, T1 (100 mg/kg) was found to have the largest increase in body weight from 116.75 g to 152.50 g with a 30.62 percent increase in

percentage. Following T0 male (standard diet) with a percentage increase of 28.78 per cent in body weight, T3 (200 mg/kg) from 129.50 g to 155.25 g with an increase of 19.88 per cent and T2 with the lowest increase of 16.06 per cent among other treatments.

Table 2: Effectiveness of Ceylon Spinach (*T. triangulare* Jacq. Willd) Crude Extract on the Weight of Male Experimental Rats after Ten (10) Days of Oral Administration

| Treatment | BWBOA (g) | BWAOA (g) | MDW (g) | Percentage change (%) |
|--------------------|-----------|-----------|---------|-----------------------|
| T0 (standard diet) | 120.75 | 155.50a | 34.75 | 28.78 |
| T1 (100 mg/kg) | 116.75 | 152.50b | 35.75 | 30.62 |
| T2 (150 mg/kg) | 124.50 | 144.50b | 20.00 | 16.06 |
| T3 (200 mg/kg) | 129.50 | 155.25b | 25.75 | 19.88 |

Legend: *Means followed by the same letter are not significant different at 0.05 level

BWBOA- Body Weight before Oral Administration; BWAOA – Body Weight after Oral Administration; MDW- Mean Difference in Weight; and Percentage Change

Table 3: Effectiveness of Ceylon Spinach (*T. triangulare* Jacq. Willd) Crude Extract in the Level of SGPT/ALT of Male Experimental Rats After Ten (10) Days of Oral Administration

| Treatment | NLSGPT | SLBOA (U/L) | SLAOA (U/L) | Mean difference in SGPT | Percentage change (%) |
|--------------------|-----------|-------------|-------------|-------------------------|-----------------------|
| T0 (standard diet) | 18-45 U/L | 89.78 | 116.45a | 26.68 | 29.71 |
| T1 (100 mg/kg) | 18-45 U/L | 106.33 | 83.23b | (23.10) | (21.73) |
| T2 (150 mg/kg) | 18-45 U/L | 67.90 | 85.80a | 18.20 | 26.80 |
| T3 (200 mg/kg) | 18-45 U/L | 56.93 | 105.63a | 48.70 | 85.54 |

Legend: *Means followed by the same letter are not significant different at 0.05 level NLGPT- Normal Level of Serum Glutamic-Pyruvic Transaminase; SLBOA- SGPT/ALT Level Before Oral Administration; SLAOA – SGPT/ALT After Oral Administration

Statistical analysis showed a significant difference in the treatments tested for decreasing levels of SGPT/ALT in male experimental rats before and after ten (10) days of oral administration in T1 (100 mg/kg) showing decreased levels of SGPT/ALT in male experimental doses. However, the two doses (T2 and T3) did not differ significantly from each other in terms of decreasing the SGPT/ALT level since the result implies that the use of Ceylon spinach crude extract in high dose elevates / increases the level of SGPT/ALT. It could be deduced that Ceylon Spinach (*T. triangulare*) crude extract increases the level of SGPT/ALT in male experimental rats. This means that Ceylon spinach is not effective in reducing the level of SGPT/ALT in male experimental rats.

An Ebuehi [24] study on the Neurochemical Impact of *Talinum triangulare* Aqueous Extract on Learning and Memory in Male Wistar Rats indicated that there was a high level of SGPT/ALT relative to control in the blood of treated rats, which could be due to possible metabolic stress. Thus, the crude extract of Ceylon Spinach (*Talinum triangulare* Jacq. Willd.) has an effect on the liver enzyme alanine transaminase (ALT) of male experimental rats after oral administration in T1 (100 mg/kg) for ten (10) days, which decreases in levels. But if ingested in high dosages, it

If the % is negative (-) number then this is a % decrease

This result is supported by a study conducted by [23], in which the rise in weight gain may be due to the fact that Ceylon Spinach/water leaf extract has appetizing and digestive stimulating properties verified in his work "New additives for broiler chicken." This digestion and stimulating properties must have aided in the utilization of feed thus resulting in enhanced growth.

3.3.2. Significant Difference and Effectiveness of *T. triangulare* in Various Concentrations in the SGPT/ALT (Alanine transaminase) of Male Experimental Rats

Table 3 shows the normal level of SGPT/ALT in male experimental rats, SGPT/ALT level before oral administration, SGPT/ALT level after oral administration, the mean difference and percentage reduction of experimental rats.

increases the level of SGPT/ALT in T0 (standard diet), T2 (150 mg/kg), and T3 (200 mg/kg). Nevertheless, although it shows that the level of SGPT/ALT decreases in T1 (100 mg/kg), if the level of SGPT/ALT in the control and experimental group is consumed minimally, it is too high compared with the normal level of SGPT/ALT before and after oral administration in experimental rats. This shows that the crude extract of Ceylon Spinach (*T. triangulare* Jacq. Willd.) is not recommended for people with liver disease because it elevates liver enzyme levels.

3.3.3. Significant Difference and Effectiveness of *T. triangulare* in Various Concentration in the SGOT/AST (Aspartate aminotransferase) of Male Sprague Dawley Rats

The data showed that the level of SGOT/AST based on the normal level of SD rats was too high. Wherein T1 (100 mg/kg) has the highest level of SGOT/AST with a level of 391.00 U/L, followed by T0 with a level of 311.35 U/L, T2 (150 mg/kg) with 276.00 U/L and T3 (200 mg/kg). After ten (10) days of oral administration the level of SGOT/AST in males, T0 (standard diet) has the highest level of SGOT/AST followed by T3 with 307.95 U/L, T2 with 232.18 U/L, and T1 (100 mg/kg) with the lowest level recorder as 197.85 U/L.

Table 4: Effectiveness of Ceylon Spinach (*T. triangulare* Jacq. Willd.) Crude Extract in the Level of SGOT/AST of Male Experimental Rats After Ten (10) Days of Oral Administration

| Treatment | NLSGOT | SLBOA (U/L) | SLAOA (U/L) | Mean difference in SGOT | Percentage change (%) |
|----------------|-------------|-------------|---------------------|-------------------------|-----------------------|
| T0 (control) | 74- 143 U/L | 311.35 | 375.58 ^a | 64.23 | 20.63 |
| T1 (100 mg/kg) | 74- 143 U/L | 391.00 | 197.85 ^b | (193.15) | (49.40) |
| T2 (150 mg/kg) | 74- 143 U/L | 276.00 | 232.18 ^b | (43.82) | (15.88) |
| T3 (200 mg/kg) | 74- 143 U/L | 242.80 | 307.95 ^a | 65.15 | 26.83 |

Legend: *Means followed by the same letter are not significant different at 0.05 level

NLSGOT- Normal Level of Serum Glutamic Oxaloacetic Transaminase; SLBOA– SGOT/AST Level Before Oral Administration; SLAOA– SGOT/AST Level After Oral Administration SGOT Level,

If the % is negative (-) number then this is a % decrease

The result shows that the decrease in SGOT/AST levels in male experimental rats with the exception of T3 (200 mg/kg) and the control group (T0) was found to have increased in males. T1 (100 mg/kg) had a mean difference of 193.15 U/L with a percentage reduction of 49.40 percent, followed by T2 (150 mg/kg) with a mean difference of 43.83 U/L and a reduction of 15.88 percent.

After ten (10) days of oral administration of Ceylon Spinach (*Talinum triangulare* Jacq. Willd.) crude extract (100, 150 and 200 mg/kg b.w.) on experimental rats, a significant difference was observed in the level of SGOT/AST (Aspartate aminotransferase) in oral administration by treatment, which indicates that SGOT/AST has an effect or relationship between oral administration and treatment. This result is in accordance with the work of [27] on the Studies on the biochemical effects of *Talinum triangulare* Jacq. Willd. in rats. That there was a significant decrease in SGOT/AST in T1 (100 mg/kg) and T2 (150 mg/kg) compared to T0 (standard diet) and T3 (200 mg/kg).

Statistical Analysis revealed significant differences in the effectiveness of various treatments used in reducing SGOT/AST level in male experimental rats before and after ten (10) days of oral administration. Further analysis using Tukey HSD showed insignificant difference between T1 (100 mg/kg), T2 (150 mg/kg) and T3 (200 mg/kg) and T0 (standard diet). This goes to show that at ten (10) days of oral administration, the use of Ceylon Spinach crude extract is not effective in reducing SGOT/AST in male experimental rats. This is confirmed by a study by Ezekwe, (2013), in which the experimental rats' AST levels were significantly increased at a dose of 100mg/kg of *T. triangulare* because AST is also found in other organs of the body such as the heart but at 200 and 400 mg/kg there was a decrease in AST when compared to the one at 100mg/kg.

3.3.4. Significant Difference and Effectiveness of *T. triangulare* in Various Concentrations in the Creatinine of Male Sprague Dawley Rats

The data shows that before the oral administration of the Ceylon Spinach (*T. triangulare* Jacq. Willd) in the males'

experimental group, T2 (150 mg/kg) has the highest creatinine level with 0.17 mg/dL, followed by T3 (200 mg/kg) with 0.15 mg/dL, T0 (standard diet) with the lowest level of creatinine level of 0.11 mg/dL. This indicates that the creatinine level before the oral administration is at average level based on the normal level of SD rats.

After ten (10) days of oral administration, the creatinine level has a significant increase wherein T3 (200 mg/kg) with 0.20 mg/dL followed by T1 (100 mg/kg) with 0.15 mg/dL, T2 (150 mg/kg) with 0.14 mg/dL compared to the T0 (control group) with 0.11 mg/dL with the lowest creatinine level. This means that the level of creatinine after ten (10) days of oral administration increases. The result shows that the increase in the creatinine level in males with the exception of T2 (100 mg/kg) and the control group (T0), were found to have been reduced. The highest increase was shown in T1 (100 mg/kg) with mean difference of 0.09 mg/dL and an increase percentage of 150.00 percent. T3 (200 mg/kg) has a mean difference of 0.05 mg/dL with an increase of 33.33 percent which yielded the lowest percentage increase in the experimental group.

It shows that the crude extract of Ceylon Spinach (*Talinum triangulare* Jacq. Willd.) increases the level of creatinine on male experimental rats in T1 (100 mg/kg) and T3 200 mg/kg after ten (10) days of oral administration. It indicates that the abundant presence of tannins in Ceylon Spinach (*Talinum triangulare* Jacq. Willd.) may have caused the increased creatinine level in SD rats.

Statistical analysis revealed no significant difference among the treatments studied in terms of decreasing Creatinine levels in male experimental rats before and after ten (10) days of oral administration. The result showed that the three treatments (T1, T2 and T3) differ significantly from each other as to effectiveness or as to decreasing creatinine level. It could be deduced that Ceylon Spinach (*T. triangulare*) crude extract can increase the level of creatinine in male experimental rats. This showed that the crude extract of Ceylon spinach is not effective in lowering/reducing the creatinine level of male experimental rats.

Table 5: Effectiveness of Ceylon Spinach (*T. triangulare* Jacq. Willd) Crude Extract in the Level of Creatinine of Male Experimental Rats After Ten (10) Days of Oral Administration

| Treatment | NCL | CLBOA (mg/dL) | CLAOA (mg/dL) | MDCL | Percentage change (%) |
|----------------|-----------------|---------------|---------------|--------|-----------------------|
| T0 (control) | 0.3 ± 0.1 mg/dL | 0.11 | 0.10a | (0.01) | (9.09) |
| T1 (100 mg/kg) | 0.3 ± 0.1 mg/dL | 0.06 | 0.15b | 0.09 | 150.00 |
| T2 (150 mg/kg) | 0.3 ± 0.1 mg/dL | 0.17 | 0.14a | (0.03) | (17.65) |
| T3 (200 mg/kg) | 0.3 ± 0.1 mg/dL | 0.15 | 0.20b | 0.05 | 33.33 |

Legend: *Means followed by the same letter are not significant different at 0.05 level

NCL- Normal Creatinine Level; CLBOA- Creatinine Level Before Oral Administration; CLAOA- Creatinine Level After Oral Administration; MDCL- Mean Difference in Creatinine Level

If the % is negative (-) number then this is a % decrease

Another reading contradicts the findings of this study wherein the extract of *T. triangulare* significantly reduced the serum level of creatinine. While control rats had a creatinine concentration of 133.44; the extract doses of 100, 250, 500, and 100 mg/kg decreased creatinine levels to 105.00, 94.20, 100.20, and 82.00 mmol/L. Creatinine changes are suggestive of kidney disease. Consequently, the results indicated that the extract did not affect the kidney harmfully [27].

3.3.5. Significant Difference and Effectiveness of *T. triangulare* in Various Concentrations in the RBC of Male Sprague Dawley Rats.

The data shows that before the oral administration of the Ceylon Spinach (*T. triangulare* Jacq. Willd) crude extract in male experimental group, T3 (200 mg/kg) has the highest RBC level of $7.97 \times 10^6/\text{mm}^3$, followed by T0 (standard diet) wherein the level of RBC is at $6.91 \times 10^6/\text{mm}^3$ and $6.65 \times 10^6/\text{mm}^3$, T2 (150 mg/kg) with $6.60 \times 10^6/\text{mm}^3$, T1 with $6.57 \times 10^6/\text{mm}^3$.

After ten (10) days of oral administration of the Ceylon Spinach (*T. triangulare* Jacq. Willd) crude extract in the experimental group, T3 (200 mg/kg) has the highest RBC level with $8.30 \times 10^6/\text{mm}^3$, followed by T2 (150 mg/kg) with a $7.47 \times 10^6/\text{mm}^3$ level of RBC, T0 (standard diet) with RBC level of $7.18 \times 10^6/\text{mm}^3$ in males and T1 (100 mg/kg) has the lowest RBC level in the experimental group. The result of the increase in RBC level in male experimental rats shows that T2 (150 mg/kg) has the highest mean difference of $0.87 \times 10^6/\text{mm}^3$ with a percentage increase of 13.18 percent. Followed by T1 (100 mg/kg) with a mean difference of $0.54 \times 10^6/\text{mm}^3$ and a percentage increase of 8.22 percent. T3 has the mean difference of $0.33 \times 10^6/\text{mm}^3$ with the lowest percentage increase of 4.17 compared to the control group (T0) with a mean difference of $0.27 \times 10^6/\text{mm}^3$ and $0.49 \times 10^6/\text{mm}^3$ with a percentage increase of 3.91 percent and 7.37 percent respectively.

Table 6: Effectiveness of Ceylon Spinach (*T. triangulare* Jacq. Willd) Crude Extract in the Level of Red Blood Cells of Male Experimental Rats after Ten (10) Days of Oral Administration

| Treatment | NRBCL | RBCLBOA $\times 10^6/\text{mm}^3$ | RBCLAOA $\times 10^6/\text{mm}^3$ | MDRBCL | Percentage change (%) |
|--------------------|---------------------------|--------------------------------------|--------------------------------------|--------|-----------------------|
| T0 (standard diet) | $6.9 \pm 0.3 \times 10^6$ | 6.91 | 7.18a | 0.27 | 3.91 |
| T1 (100 mg/kg) | $6.9 \pm 0.3 \times 10^6$ | 6.57 | 7.11b | 0.54 | 8.22 |
| T2 (150 mg/kg) | $6.9 \pm 0.3 \times 10^6$ | 6.60 | 7.47b | 0.87 | 13.18 |
| T3 (200 mg/kg) | $6.9 \pm 0.3 \times 10^6$ | 7.97 | 8.30b | 0.33 | 4.14 |

Legend: *Means followed by the same letter are not significant different at 0.05 level

NRBCL- Normal Red Blood Cell Level; RBCLBOA- Red Blood Cell Level Before Oral Administration; RBCLAOA- Red Blood Cell Level After Oral Administration; MDRBCL- Mean Difference in Red Blood Cell Level

If the % is negative (-) number then this is a % decrease

The data revealed that there is a significant increase in the level of RBC after ten (10) days of oral administration of crude extract of Ceylon Spinach (*Talinum triangulare* Jacq. Willd.) particularly in T2 (150 mg/kg). It can be deduced that if the RBC level is lowered because of such conditions, the possibility of Ceylon spinach when consumed minimally can raise the level of RBC in humans that can prevent anaemia. This is because of the abundant presence of saponins, tannins and other analyzed constituents of the plant extract.

Statistically, significant difference in the treatments used along effectiveness before and after ten (10) days of oral administration were obtained in T2 (150 mg/kg) showing the greatest increase in Red Blood cells level in male rats. The treatments of Ceylon spinach, however, did not differ significantly from each other as to the effectiveness in increasing the level of RBC in male experimental rats. This means that after ten (10) days of oral administration in male experimental rats, the results of the three (3) treatments: T1 (100 mg/kg), T2 (150 mg/kg), T3 (200 mg/kg) were comparable. T2 (150 mg/kg) has been shown to be the most effective treatment for Ceylon Spinach crude extract in increasing the level of RBC in male experimental rats. The

findings were supported by a study in which the RBC concentration was significantly increased compared to the control and the highest RBC level was achieved at 200 mg/kg. It denotes that *Talinum triangulare* leaf can be used to treat disease symptoms such as anaemia, and may also be used by pregnant women and rising children to increase blood pressure [10].

3.3.6. Significant Difference and Effectiveness of *T. triangulare* in Various Concentrations in the PCV/Haematocrit of Male Sprague Dawley Rats

After ten (10) days of oral administration of Ceylon spinach (*T. triangulare* Jacq. Willd.) in treatment groups, result showed that there was an increase of PCV level in T3 (200 mg/kg) with the highest level of 49.75 percent, followed by T2 (150 mg/kg) with 47.25 percent and T1 (100 mg/kg) with the lowest PCV level of 44.75 percent compared to the control group the T0 (standard diet) with 46.25 percent. The data revealed that after the oral administration of crude extract of Ceylon Spinach on experimental rats, the level of PCV/Haematocrit increases based on the normal level.

Table 7: Effectiveness of Ceylon spinach (*T. triangulare* Jacq. Willd) Crude extract in the Level of Packed Cell Volume (PCV)/Haematocrit of Male Experimental Rats After Ten (10) Days of Oral Administration

| Treatment | NPCVL | PCVLBOA (Haematocrit) % | PCVLAOA (Haematocrit) % | MDPCVL | Percentage change (%) |
|--------------------|--------------|-------------------------|-------------------------|--------|-----------------------|
| T0 (standard diet) | 48.3 ± 2.3 % | 42.25 | 46.25 ^a | 4.00 | 9.47 |
| T1 (100 mg/kg) | 48.3 ± 2.3 % | 41.00 | 44.75 ^b | 3.75 | 9.15 |
| T2 (150 mg/kg) | 48.3 ± 2.3 % | 42.25 | 47.25 ^b | 5.00 | 11.83 |
| T3 (200 mg/kg) | 48.3 ± 2.3 % | 48.00 | 49.75 ^b | 1.75 | 3.65 |

Legend: *Means followed by the same letter are not significant different at 0.05 level

NPCVL- Normal Packed Cell Volume Level; PCVLBOA- Packed Cell Volume Level Before Oral Administration; PCVLAOA- Packed Cell Volume Level After Oral Administration, MDPCVL- Mean Difference in the Packed Cell Volume Level

If the % is negative (-) number then this is a % decrease

Statistically, no significant difference in the PCV/Haematocrit along effectiveness before and after ten (10) days of oral administration wherein T2 (150 mg/kg) showed the greatest increase in PCV/Haematocrit level in male rats. The treatments of Ceylon Spinach, however, did not differ significantly from each other as to the effectiveness in increasing the level of PCV/Haematocrit in male experimental rats. This means that effects of the three (3) treatments: T1 (100 mg/kg), T2 (150 mg/kg), T3 (200 mg/kg) were compared to each other after ten (10) days of oral administration in male experimental rats wherein T2 (150 mg/kg) is the best treatment of Ceylon Spinach crude extract that could be effective in increasing the PCV/Hematocrit level in male experimental rats.

This means that Ceylon Spinach (*T. triangulare* Jacq. Willd) crude extract can normalize the level of PCV/Haematocrit in the blood when the haematocrit level is low. This finding can be attributed in particular to the T2 (150 mg/kg), with the highest percentage change in male and female experimental rats due to the biochemical constituents in *T. triangulare*.

3.3.7. Significant Difference and Effectiveness of *T. triangulare* in Various Concentrations in the Weight of Female Experimental Rats

Results showed that before oral administration T1 (100 mg/kg) recorded the highest body weight of 121.50 g in female experimental rats, followed closely by T0 (standard diet) with 116.50 g, T3 (200 mg/kg) with 114.25 g and T2 with 109.50 g showed the lowest body weight before oral administration. After ten (10) days of oral administration in female experimental rats, it was observed that T2 (150 mg/kg) has the highest increase in weight from 109.50g to 125.25 with a mean difference of 15.75g and has a calculated increase of 14.38 percent. Followed by T3 (200 mg/kg) from 114.25g to 127.25g with a mean difference of 13.00g and a calculated increase of 11.38 percent. T1 (100 mg/kg) from 121.50g to 128.25g with a mean difference of 6.75g and has the lowest increase of 5.56 percent among experimental group compared to the control group T0 (standard diet) from 116.50 to 133.25g with a mean difference of 16.75g and an increase of 14.38percent.

Analysis of variance revealed significant difference in the treatments studied in terms of increasing weight level in female experimental rats before and after ten (10) days of oral administration. The three (3) crude extract treatments,

however, did not differ significantly from each other as to the effectiveness in increasing weight in female experimental rats. This means that the different treatments of Ceylon Spinach crude extract could be effective in increasing body weight in female experimental rats.

Table 8: Effectiveness of Ceylon Spinach (*T. triangulare* Jacq. Willd) Crude Extract in the Weight of Female Experimental Rats After Ten (10) Days of Oral Administration

| Treatment | BWBOA (g) | BWAOA (g) | MDW (g) | Percentage change (%) |
|--------------------|-----------|----------------------|---------|-----------------------|
| T0 (standard diet) | 116.50 | 133.25 ^a | 16.75 | 14.38 |
| T1 (100 mg/kg) | 121.50 | 128.25 ^{ab} | 6.75 | 5.56 |
| T2 (150 mg/kg) | 109.50 | 125.25 ^b | 15.75 | 14.38 |
| T3 (200 mg/kg) | 114.25 | 127.25 ^b | 13.00 | 11.38 |

Legend: *Means followed by the same letter are not significant different at 0.05 level

BWBOA- Body Weight Before Oral Administration; BWAOA – Body Weight After Oral Administration; MDW- Mean Difference in Weight; and Percentage Reduction

• If the % is negative (-) number then this is a % decrease

This finding is confirmed by a study undertaken by Langhout, 2000, in which the rise in weight gain can be related to the fact that Ceylon Spinach/water leaf extract has appetizing and nutritionally stimulating properties recorded in his work "New Additives for Broiler Poultry." This digestion and relaxing properties may have led to feed usage, which may have contributed to increased production.

3.3.8. Significant Difference and Effectiveness of *T. triangulare* in Various Concentrations in the SGPT/ALT (Alanine transaminase) of Female Experimental Rats.

After ten (10) days of oral administration in female experimental rats, data showed that it was almost tripled the level based on the normal level of SGPT/AST. T3 (200 mg/kg) with 99.55 U/L followed by T2 (150 mg/kg) with 74.23 U/L and T1 (100 mg/kg) with 62.23 U/L as the lowest in the experimental groups but shows a very high level of SGPT/ALT compared to the normal level of SGPT/ALT in Sprague Dawley (SD) rats. The result shows that the increase in the level of SGPT/ALT with the exception of T1 (100 mg/kg) in females were found to have been reduced from 74.70 U/L to 62.23 U/L with a mean difference of 12.47 U/L and reduced to 16.69 percent. T3 (200 mg/kg) with a mean difference of 14.15 U/L and an increase of 16.57 percent. T2 (150 mg/kg) has a mean difference of 7.28

U/L and a calculated percentage of 10.87 percent compared to T0 (standard diet) has a mean difference of 92.73 U/L with an increase of 165.88 percent SGPT/ALT level.

After the oral administration of Ceylon Spinach (*Talinum triangulare Jacq. Willd.*) crude extract (100, 150 and 200 mg/kg b.w.) in female experimental rats, it was found out that there was a significant difference in the SGPT/ALT (Alanine transaminase) level, which implies that SGPT/ALT of female experimental rats has a relationship between the oral administration and treatment.

Statistical analysis revealed significant difference before and after ten (10) days of oral administration among the treatments studied in terms of decreasing SGPT/ALT levels in female experimental rats showing T1 (100 mg/kg) reduces the level of SGPT/ALT in female experimental rats. The three (3) Ceylon spinach crude extract treatments, did not differ significantly to each other as to reducing SGPT/ALT levels. The result implies that the use of Ceylon Spinach

crude extract elevates/increase the level of SGPT/ALT level. It could be deduced that Ceylon Spinach (*T. triangulare*) crude extract is ineffective in reducing SGPT/ALT in female experimental rats.

Thus, the Ceylon Spinach (*Talinum triangulare Jacq. Willd.*) crude extract has an effect to liver enzyme alanine transaminase (ALT) of the experimental rats after the oral administration of T1 (100 mg/kg) for ten (10) days subsequently the level decreases. But the level of the SGPT/ALT increases in T2 (150 mg/kg) and T3 (200 mg/kg). Nevertheless, even though it shows that the level of the SGPT/ALT decreases in T1 (100 mg/kg) if it is consumed minimally the level of SGPT/ALT in the control and experimental group is too high compared to the normal level of SGPT/ALT before and after the oral administration in SD rats. This shows that the Ceylon spinach (*T. triangulare Jacq. Willd.*) crude extract is not advisable to people with liver diseases because it elevates the level of liver enzymes.

Table 9: Effectiveness of Ceylon Spinach (*T. triangulare Jacq. Willd*) Crude Extract in the Level of SGPT/ALT of Female Experimental Rats after Ten (10) Days of Oral Administration

| Treatment | NLSGPT | SLBOA (U/L) | SLAOA (U/L) | Mean difference in SGPT | Percentage change (%) |
|----------------|-----------|-------------|----------------------|-------------------------|-----------------------|
| T0 (control) | 16-48 U/L | 55.90 | 148.63 ^{ab} | 92.73 | 165.88 |
| T1 (100 mg/kg) | 16-48 U/L | 74.70 | 62.23 ^{bc} | (12.47) | (16.69) |
| T2 (150 mg/kg) | 16-48 U/L | 66.95 | 74.23 ^{ab} | 7.28 | 10.87 |
| T3 (200 mg/kg) | 16-48 U/L | 85.40 | 99.55 ^{ab} | 14.15 | 16.57 |

Legend: *Means followed by the same letter are not significant different at 0.05 level

NLSGPT- Normal Level of Serum Glutamic-Pyruvic Transaminase; SLBOA- SGPT/ALT Level Before Oral Administration; SLAOA – SGPT/ALT After Oral Administration

• If the % is negative (-) number then this is a % decrease

3.3.9. Significant Difference and Effectiveness of *T. triangulare* in Various Concentration in the SGOT/AST (Aspartate aminotransferase) of Female Experimental Rats

The data showed that the level of SGOT/AST based on the normal level of female experimental rats was too high. Wherein T3 (200 mg/kg) has the highest level of SGOT/AST of 347.73 U/L, followed by T1 with a 320.40 U/L, T2 (150 mg/kg) with 249.55 U/L, T0 (standard diet) recorded to have the lowest SGOT/AST with 239.80 U/L in female experimental rats before the oral administration. The result shows that after ten (10) days of oral administration in female experimental rats, there was a decrease in the level of SGOT/AST with the exception of T0 (standard diet) were found to have been elevated compared to the experimental group wherein T1 (100 mg/kg) has the highest reduction in the level of SGOT/AST from 320.40 U/L to 242.43 U/L with a mean difference of 77.97 U/L and has percentage reduction of 29.34 percent followed by T3 (100 mg/kg) from 347.73 U/L to 311.05 U/L with a mean difference of 36.68 U/L and a reduction of 10.55 percent. T2 (150 mg/kg) from 249.55 U/L to 237.30 U/L has a mean difference of 12.25 U/L with the lowest reduction of 4.91 percent.

Table 10: Effectiveness of Ceylon Spinach (*T. triangulare Jacq. Willd*) Crude Extract in the Level of SGOT/AST of Female Experimental Rats After Ten (10) Days of Oral Administration

| Treatment | NLSGOT | SLBOA (U/L) | SLAOA (U/L) | Mean difference in SGOT | Percentage change (%) |
|--------------------|------------|-------------|---------------------|-------------------------|-----------------------|
| T0 (standard diet) | 65-203 U/L | 239.80 | 645.95 ^a | 406.15 | 169.37 |
| T1 (100 mg/kg) | 65-203 U/L | 320.40 | 242.43 ^b | (77.97) | (24.34) |
| T2 (150 mg/kg) | 65-203 U/L | 249.55 | 237.30 ^b | (12.25) | (4.91) |
| T3 (200 mg/kg) | 65-203 U/L | 347.73 | 311.05 ^b | (36.68) | (10.55) |

Legend: *Means followed by the same letter are not significant different at 0.05 level

NLSGOT- Normal Level of Serum Glutamic Oxaloacetic Transaminase; SLBOA– SGOT/AST Level Before Oral Administration; SLAOA– SGOT/AST Level After Oral Administration SGOT Level,

• If the % is negative (-) number then this is a % decrease

Statistical Analysis revealed no significant differences in the effectiveness of various treatments used in reducing SGOT/AST level in female experimental rats before and after ten (10) days of oral administration. Further analysis using Tukey HSD showed no significant different between the treatment group and the control group. This means that

after ten (10) days of oral administration of the three (3) treatments: T1 (100 mg/kg), T2 (150 mg/kg), T3 (200 mg/kg) is an effective in reducing SGOT/AST in female experimental rats. This implies that the Ceylon spinach crude extract is effective in lowering or reducing the SGOT/AST of female experimental rats.

This result is in accordance with the work of Arit, 2007 on the Studies on the biochemical effects of *Talinum triangulare Jacq. Willd.* in rats. That there was a significant decrease in the level of SGOT/AST in female SD rats in T1 (100 mg/kg), T2 (150 mg/kg), T3 (200 mg/kg) compared to T0 (control diet).

3.3.10. Significant Difference and Effectiveness of *T. triangulare* in Various Concentrations in the Creatinine of Female Experimental Rats.

The data showed that before the oral administration of the Ceylon Spinach (*T. triangulare Jacq. Willd.*) in the female experimental rats, T0 (standard diet) has the highest creatinine level with 0.19 mg/dL, followed by T2 (150 mg/kg) with 0.17 mg/dL, T1 (100 mg/kg) with 0.13 mg/dL, and T3 with 0.09 mg/dL. This indicates that the creatinine level before the oral administration is at average level based on the normal level of SD rats.

Statistical analysis revealed no significant difference among the treatments studied in terms of decreasing Creatinine levels in female experimental rats before and after ten (10) days of oral administration. The result implies that the use of Ceylon Spinach crude extract elevates/increase the level of creatinine level. It could be deduced that Ceylon Spinach (*T. triangulare*) crude extract is not effective in lowering the level of creatinine in female experimental rats.

It shows that the crude extract of Ceylon Spinach (*Talinum triangulare Jacq. Willd.*) increases the level of creatinine on SD rats after ten (10) days of oral administration. It indicates that the abundant presence of tannins in Ceylon Spinach (*Talinum triangulare Jacq. Willd.*) may have caused the increased creatinine level in SD rats. The rates of creatinine are also associated with muscle mass or the volume of muscle in the body that may decline with age or illness. High levels may mean less efficient or weakened muscles, for example in a disorder such as muscular dystrophy (MD) [28]. But high levels may also indicate a kidney infection, as these organs get rid of the body's waste products and maintain the blood healthy. This is confirmed by the 1979 research undertaken [29] which showed that some of the studied components of the vegetable species may be completely toxic to both humans and farm animals, and that some are tannin-specific organisms.

Table 11: Effectiveness of Ceylon Spinach (*T. triangulare Jacq. Willd.*) Crude Extract in the Level of Creatinine of Female Experimental Rats After Ten (10) Days of Oral Administration

| Treatment | NCL | CLBOA (mg/dL) | CLAOA (mg/dL) | MDCL | Percentage change (%) |
|--------------------|-----------------|---------------|-------------------|--------|-----------------------|
| T0 (Standard diet) | 0.3 ± 0.1 mg/dL | 0.19 | 0.12 ^a | (0.07) | (36.84) |
| T1 (100 mg/kg) | 0.3 ± 0.1 mg/dL | 0.13 | 0.17 ^b | 0.04 | 30.77 |
| T2 (150 mg/kg) | 0.3 ± 0.1 mg/dL | 0.17 | 0.20 ^b | 0.03 | 17.65 |
| T3 (200 mg/kg) | 0.3 ± 0.1 mg/dL | 0.09 | 0.21 ^b | 0.12 | 133.33 |

Legend: *Means followed by the same letter are not significant different at 0.05 level

NCL- Normal Creatinine Level; CLBOA- Creatinine Level Before Oral Administration; CLAOA- Creatinine Level After Oral Administration; MDCL- Mean Difference in Creatinine Level

If the % is negative (-) number then this is a % decrease

3.3.11. Significant Difference and Effectiveness of *T. triangulare* in Various Concentrations in the RBC of Female Sprague Dawley Rats

The data shows that before the oral administration of the Ceylon Spinach (*T. triangulare Jacq. Willd.*) crude extract in female experimental group, T1 (100 mg/kg) has the highest RBC level of $6.95 \times 10^6/\text{mm}^3$, followed by T3 (200

mg/kg) with $6.75 \times 10^6/\text{mm}^3$, T0 (standard diet) with $6.65 \times 10^6/\text{mm}^3$ and T2 (150 mg/kg) with $6.32 \times 10^6/\text{mm}^3$.

The data showed a significant increase in the RBC level after ten (10) days of oral administration of Ceylon Spinach crude extract (*Talinum triangulare Jacq. Willd.*) and T2 (150 mg / kg) was the effective treatment to increase the RBC levels in SD rats.

Table 12: Effectiveness of Ceylon Spinach (*T. triangulare Jacq. Willd.*) Crude Extract in the Level of Red Blood Cells of Female Experimental Rats after Ten (10) Days of Oral Administration

| Treatment | NRBCL | RBCLBOA $\times 10^6/\text{mm}^3$ | RBCLAOA $\times 10^6/\text{mm}^3$ | MDRBCL | Percentage change (%) |
|----------------|---------------------------|-----------------------------------|-----------------------------------|--------|-----------------------|
| T0 (control) | $7.7 \pm 0.3 \times 10^6$ | 6.65 | 7.14 ^{ab} | 0.49 | 7.37 |
| T1 (100 mg/kg) | $7.7 \pm 0.3 \times 10^6$ | 6.95 | 7.27 ^{ab} | 0.32 | 4.60 |
| T2 (150 mg/kg) | $7.7 \pm 0.3 \times 10^6$ | 6.32 | 7.08 ^{ab} | 0.76 | 12.02 |
| T3 (200 mg/kg) | $7.7 \pm 0.3 \times 10^6$ | 6.75 | 7.41 ^{ab} | 0.66 | 9.77 |

Legend: *Means followed by the same letter are not significant different at 0.05 level

NRBCL- Normal Red Blood Cell Level; RBCLBOA- Red Blood Cell Level Before Oral Administration; RBCLAOA- Red Blood Cell Level After Oral Administration; MDRBCL- Mean Difference in Red Blood Cell Level

• If the % is negative (-) number then this is a % decrease

Statistically, no significant difference in the treatments used along effectiveness before and after ten (10) days of oral administration wherein T2 (150 mg/kg) showed the greatest increase in Red Blood cells level in female rats. The three (3) treatments of Ceylon spinach, however, did not differ significantly from each other as to the effectiveness in increasing the level of RBC in female experimental rats. This implies that the Ceylon spinach crude extract is effective in increasing the RBC level in female experimental rats. This is confirmed by the [10] study in which the RBC concentration is significantly increased as compared with the control and the maximum RBC level at 200 mg/kg was obtained. This indicates *T. triangulae* leaves may be used to treat disease conditions such as anaemia, and may be used by pregnant women and growing children to increase their blood levels.

3.3.12. Significant Difference and Effectiveness of *T. triangulare* in Various Concentrations in the PCV/Hematocrit of Female Experimental Rats

The result shows that the PCV level in experimental group, with the exception of T1 (100 mg/kg), were found to have

been reduced from 46.75 percent to 45 percent with a mean difference of 1.75 percent and reduced by 3.73 percent. The highest increase (being the difference of PLBOA and PLAOA) was observed in T2 (150 mg/kg) at 7.75 percent PCV level for a percentage increase of 19.62 percent followed by T3 (200 mg/kg) with 4.00 percent haematocrit level with a calculated percentage increase of 9.58 compared to the control group T0 (standard diet with 2.25 percent mean difference and an increase of 5.23 percent.

Statistically, there was no significant difference in efficacy in PCV/Haematocrit after ten (10) days of oral administration in which T2 (150 mg/kg) showed the highest increase in PCV/Haematocrit levels in female rats. Treatment of Ceylon spinach has no significant difference in efficacy in increasing the level of PCV/Haematocrit in male experimental rats. It implies that the Ceylon Spinach is effective in increasing the PCV/Haematocrit level in female experimental rats particularly in T2 (150 mg/kg) with an increase of 19.62 percent.

Table 13: Effectiveness of Ceylon spinach (*T. triangulare* Jacq. Willd) crude extract in the Level of Packed Cell Volume (PCV)/Haematocrit of Female Experimental Rats After Ten (10) Days of Oral Administration

| Treatment | NPCVL | PCVLBOA (Haematocrit) % | PCVLAOA (Haematocrit) % | MDPCVL | Percentage change (%) |
|--------------------|--------------|-------------------------|-------------------------|--------|-----------------------|
| T0 (standard diet) | 51.2 ± 2.6 % | 43.00 | 45.25 ^a | 2.25 | 5.23 |
| T1 (100 mg/kg) | 51.2 ± 2.6 % | 46.75 | 45.00 ^{ab} | (1.75) | (3.74) |
| T2 (150 mg/kg) | 51.2 ± 2.6 % | 39.50 | 47.25 ^b | 7.75 | 19.62 |
| T3 (200 mg/kg) | 51.2 ± 2.6 % | 41.75 | 45.75 ^b | 4.00 | 9.58 |

Legend: *Means followed by the same letter are not significant different at 0.05 level

NPCVL- Normal Packed Cell Volume Level; PCVLBOA- Packed Cell Volume Level Before Oral Administration; PCVLAOA- Packed Cell Volume Level After Oral Administration, MDPCVL- Mean Difference in the Packed Cell Volume Level

• If the % is negative (-) number then this is a % decrease

In phytochemical analysis, alkaloids, flavonoids, glycosides, saponins, enzymes, tannins, hormones, resins, and terpenoids were produced. Results show potential benefits in fighting blood disorders including anaemia [30].

3.4 Acute Oral Toxicity Test (LD50)

The sample that is marked as Ceylon Spinach (*Talinum triangulare* Jacq. Willd.) was submitted to Department of Science and Technology- ITDI (Industrial Technology Development Institute), Standards and Testing Division, Gen. Santos Ave., Bicutan, Taguig City, Metro Manila for LD50 Oral Toxicity Test screening employing the standard methods. The median lethal dose (LD50) of the sample, administered orally to ICR mice is 16.362 ± 1.9607 g/kg. Toxidrome ranged from decreased motor activity and respiratory rate, grooming, hyperemia, ptosis, piloerection, loss of grip strength, pinna and righting reflexes, defecation, urination, passivity, ataxia, tremors, convulsion and death of mice.

Table 14 shows the behavioral observation/toxidrome after oral administration of sample to ICR Mice for fourteen (14) days. Mice received oral limit doses of 10 g/kg, 15 g/kg and 22 g/kg respectively. Treated mice were monitored and observed for signs of toxicity and mortality for two (2)

weeks for signs of treatment-related effects, followed by necropsy.

Table 14: Behavioral Observation/Toxidrome after Oral Administration of Sample to ICR Mice

| Dose g/kg | No. of animals | Observation |
|-----------|----------------|---|
| 10 | 10 | Fifteen (15) minutes after dosing, the following manifestations were observed: decreased motor activity and respiratory rate, grooming, hyperemia, ptosis, piloerection and death of one (1) mouse after 1 hour. The remaining nine (9) mice recovered after 24 hours and until the 14 days period of observation. |
| 15 | 10 | Five (5) to fifteen (15) minutes after dosing, the following manifestations were observed: decreased motor activity and respiratory rate, grooming, hyperemia, ptosis, piloerection, loss of grip strength, pinna and righting reflexes, urination, passivity (+6), ataxia, tremors, convulsion and death of two (2) mice after 30 minutes followed by two (2) mice after 1.5 hours. The remaining six (6) mice recovered after 24 hours and until the 14 days period of observation. |
| 22.5 | 10 | Five (5) to fifteen (15) minutes after dosing, the following manifestations were observed: decreased motor activity and respiratory rate, grooming, hyperemia, ptosis, piloerection, loss of grip strength, pinna and righting reflexes, defecation, urination, passivity (+6), ataxia, tremors, convulsion and death |

| | | |
|--|--|--|
| | | of six (6) mice after 25-48 minutes followed by two (2) mice after 1.5 hours. The remaining two (2) mice recovered after 24 hours and until the 14 days period of observation. |
|--|--|--|

It is evident in the Table 15 the results of the summary of mortality ratio of animals administered orally on the LD50 oral toxicity test revealed that after two weeks oral administration of 10 g/kg of ethanol crude extract of the Ceylon Spinach (*Talinum triangulare Jacq. Willd.*) in Group I, 1 deceased out of 10 ICR mice samples from day 1 to day

14 with a reduction of 10%. Group II with 15 g/kg of oral administration of the ethanol crude extract of the Ceylon Spinach (*Talinum triangulare Jacq. Willd.*) has reduced by 40 percent out of 10 mice samples from day 1 to day 14. Compared to Group III with 80% reduction in 22.5 g/kg oral administration of the Ceylon Spinach (*Talinum triangulare Jacq. Willd.*) ethanol crude extract from day 1 to day 14 to 10 ICR mice samples. As to the toxicological effect of *T. triangulare* ethanol crude extract on the different groups,

Table 15: Summary of Mortality Ratio of Animals Administered Orally with Ceylon Spinach (*T. triangulare Jacq. Willd.*) Ethanol Crude Extract

| Group No. | Dose g/kg | no. of animals | No. of animals mortality | no. of animals alive | Mean difference | Percentage reduction % |
|-----------|-----------|----------------|--------------------------|----------------------|-----------------|------------------------|
| 1 | 10 | 10 | 1 | 9 | 1 | 10 |
| 2 | 15 | 10 | 4 | 6 | 4 | 40 |
| 3 | 22.5 | 10 | 8 | 2 | 8 | 80 |

Scale: 0-10% (non-toxic), 10-40% (low-toxic), 40-80% (medium toxic), 80-100% (highly toxic)

No prominent lesions were found on the vital organs of all checked animals during the gross inspection. Weight gain was found in all remaining species. They were euthanized after 14 days of observation, after the experimental animals' necropsy findings.

3.5. Developed IEC Material of Ceylon spinach (*Talinum triangulare Jacq. Willd.*)

Information Education and Communication is a material that was developed by the researcher to create awareness and increase knowledge of the community about the plant material used in the study. The Information and Education Communication (IEC) material which was the output of this study was the Ceylon Spinach (*Talinum triangulare Jacq. Willd.*) brochure. This material was developed as a tool for realizing the vision, Health for All, and Health in People's Hands, hoping to provide an alternative means to reach people with quality health care services. This is also to empower people to manage their own health by using available resources (including medicinal plants) from the community in terms of the benefits of Ceylon Spinach as an indigenous vegetable.

The IEC material informs the public about the importance of this species in the medical world and it is hoped that the readers or users of this brochure will realize and experience the value and healing benefits of the Ceylon Spinach plant, particularly those contained herein, and will be inspired to study more about it and share their knowledge and experiences with others. Also, to encourage people in their backyards to grow the Ceylon Spinach. This gives people the power to manage their own health.

3.6. Validity of the Ceylon spinach (*Talinum triangulare Jacq. Willd.*) Brochure

Table 16 shows the level of validity of the developed IEC material. Indicators involve appearance and structure, complexity, methodology of writing, graphics/pictures, and consistency of study.

that the IEC material could be used through this study to disseminate information or advocacy campaign to the community to inform them about the health benefits that can be obtained from Ceylon Spinach (*Talinum triangulare Jacq. Willd.*) so that they will be aware of and encourage the plant to be cultivated and used. However, further reviews are still needed for its improvement.

Table 16: The Level of Validity of the Developed Information Communication (IEC) Material

| Criteria | Validators' rating | | | | | | | |
|---|--------------------|---|---|---|---|-------|------|-----|
| | 1 | 2 | 3 | 4 | 5 | Total | Mean | DR |
| 1) Attractiveness and Organization (Organization). The brochure has exceptionally attractive formatting and well-organized information. | 4 | 4 | 4 | 4 | 4 | 20 | 4.00 | VHV |
| 2) Content – Accuracy (Ideas). The brochure has all of the required information. | 4 | 4 | 4 | 4 | 4 | 20 | 4.00 | VHV |
| 3) Writing-Mechanics (Conventions). All of the writing is done in complete sentences. Capitalization and punctuation are correct throughout the brochure. | 3 | 4 | 4 | 4 | 4 | 19 | 3.80 | VHV |
| 4) Graphics/Picture. The graphics go well with the text and there is a good mix of text and graphics. | 3 | 4 | 4 | 4 | 4 | 19 | 3.80 | VHV |
| 5) Research Quality. There are many citation from a variety of sources accurately listed on the brochure | 4 | 4 | 4 | 4 | 4 | 20 | 4.00 | VHV |
| Grand Mean | 3.6 | 4 | 4 | 4 | 4 | 19.6 | 3.92 | VHV |

Legend: Descriptive Rating (DR), VHV- Very High Valid

Equally, data revealed that the five (5) validators agreed on the brochure's validity as Very High Valid (VHV). It implies



Plate 2 (a): Developed Information and Education Communication Brochure of Ceylon Spinach



Plate 2 (b) Developed Information and Education Communication Brochure of Ceylon Spinach

4. Discussion

The study aimed to determine the potential of Ceylon Spinach (*Talinum triangulare Jacq. Willd.*) on selected haematological, liver and kidney parameters. Specifically, this study aims to (1) determine the phytochemicals of Ceylon Spinach (*Talinum triangulare Jacq. Willd.*) leaf extract using ethanol as a solvent; (2) determine the significant difference of the effectiveness of Ceylon Spinach (*Talinum triangulare Jacq. Willd.*) in various concentrations; determine the level of PCV (Packed Cell Volume), RBCs, experimental rats; (4) determine the toxicity level of Ceylon Spinach (*Talinum triangulare Jacq. Willd.*) on ICR mice; (5) develop Information and Education Communication (IEC) Materials on Ceylon Spinach (*Talinum triangulare Jacq. Willd.*); and (6) Level of validity of the IEC Material.

The researcher came up with the following salient findings wherein the qualitative phytochemical analysis showed that the leaves of *T. triangulare Jacq. Willd.* contained saponins, tannins, sterols, triterpenes, glycosides, flavonoids and alkaloids.

The administration of 150 mg/kg (T2) crude extract can increase the level of RBC and PCV/Haematocrit in male and female experimental rats after ten (10) days of oral administration.

There is a significant difference in the efficacy of the different concentrations used to either increase or decrease the levels of SGPT/ALT, SGOT/AST, RBC, PCV/Hematocrit and Creatinine.

The findings of the acute oral toxicity (LD50) test showed that after two weeks of oral administration of the Ceylon Spinach ethanol crude extract (*Talinum triangulare Jacq. Willd.*), Group I (10g/kg) had a percentage reduction of 10 percent; Group II (15 g/kg) had a reduction of 40 percent and Group III (22.5 g/kg) had a reduction of 80 percent.

The Information and Education Communication (IEC) material which was the output of this study was the Ceylon spinach (*Talinum triangulare Jacq. Willd.*) brochure that composed the plant profile, taxonomic classification, propagation and planting, phytochemical analysis, medicinal/health benefits and known hazards.

The data revealed that based on the attractiveness and organization (4.00), content-accuracy (4.00), writing mechanics (4.00), graphic picture (3.80), and writing mechanics (3.80) with a grand mean of 3.92.

5. Conclusion

The following conclusions were drawn based on the findings and observations gathered by the researcher:

The results of the phytochemical analysis indicate that Ceylon Spinach leaves (*Talinum triangulare Jacq. Willd.*) contain an appropriate number of bioactive components such as tannins, saponins, sterols, triterpenes, glycosides, among others, indicating that Ceylon Spinach leaves could significantly contribute to the management of blood-related

Among the three (3) treatments of ethanol crude extract of *Talinum triangulare Jacq. Willd.* (T1- 100 mg/kg, T2- 150 mg/kg, T3-200 mg/kg), T2 has been consistent and successful in achieving the highest improvement in terms of increasing the level of RBC and PCV/Haematocrit, which can significantly contribute to the treatment of anaemia compared to creatinine, SGPT/ALT and SGOT/AST, in which the level is also increased but may not be safe for people with liver disorders without medical supervision.

There was a significant difference in the efficacy of different concentrations in increasing RBC and PCV/Hematocrit levels in experimental rats, both male and female, in the 150 mg / kg ethanol crude extract of Ceylon Spinach, where the leaves of the plant material have a vital effect in the treatment of haematological diseases but not in kidney and liver diseases.

Ceylon Spinach (*T. triangulare Jacq. Willd.*) Ethanol crude extract showed a high toxic effect when consumed in high doses (150 mg/kg to 200 mg/kg).

The IEC material was created in the form of a brochure for a more successful awareness drive for the community, academy and other institutions to understand and experience the importance and healing benefits of Ceylon Spinach, and to be encouraged to learn more for them and share their knowledge and experiences with others. This will also encourage the people in their backyards to grow the Ceylon Spinach. It will result in people being empowered to manage their own health.

Data revealed that the five (5) experts agreed on the validity of the brochure as Very High Valid (VHV) and therefore the IEC material was an effective tool for disseminating information to the community.

6. Acknowledgements

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