TOXICOLOGICAL EVALUATION OF THE AQUEOUS EXTRACTS OF ANDROGRAPHIS PANICULATA, CURCUMA LONGA AND CINNAMOMUM VERUM ON ASPARTATE TRANSAMINASE AND PLATELET COUNT IN MALE RATS

BY

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CERTIFICATION

This is to certify that this project work was thoroughly researched into by OMOGE TAIWO MERCY with matriculation number LSC2103800 as part of the requirements for the award of the Bachelor of Science Degree Award [B.Sc] in Biochemistry.

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DEDICATION

This project is dedicated to Almighty God for his unfailing love, strength, support, and guidance.

I also dedicate this project to my parents Engr and Mrs OMOGE

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I sincerely thank God for the gift of life, strength, and the wisdom to complete this project. Without His help, none of this would be possible.

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TABLE OF CONTENT

List of Figures

List of Tables

ABSTRACT

This research examined the toxicological properties of *Andrographis paniculata, Curcuma longa* and *Cinnamomum verum* aqueous extracts on the platelet count and aspartate transaminase (AST) activities in male wistar rats. Through the method used by Lorke, thirty-nine rats were placed under the control and treatment groups. There were three rats in the control group and twelve rats in each of the treatment groups. In each treatment group, three rats were administered 10, 100 and 1000 mg/kg of the corresponding plant extract as well as individual rats were administered 1500, 2900, and 5000mg/kg. It was administered over six days, and biochemical and hematological analyses were conducted in order to determine the liver and blood functioning as well as a proximate analysis of the plant composition. The proximate findings revealed that Curcuma longa had the highest carbohydrate and moisture levels, Andrographis paniculata recorded the highest mineral and fibre levels and Cinnamomum verum had the highest protein content. There were no substantial changes in body weight and this showed dose tolerance. There were dose-dependent platelet-level effects: Andrographis paniculata increased thrombopoiesis with dose, Cinnamomum verum induced a small and reversible platelet-depleting effect, and Curcuma longa had no effect on platelet counts. AST activities were normal in all groups with Curcuma longa (1000 mg/kg) having the best hepatoprotective activity in terms of reduced enzyme activity. In general, the acute exposure to the aqueous extracts was hepatotoxic and hematological toxicity-free. Curcuma longa and Andrographis paniculata exhibited significant hepatoprotection and platelet balance respectively. The findings suggest that both of these herbal extracts are biochemically safe in the dose range tested and they justify the safe use as traditional and modern therapeutic agents.

CHAPTER ONE

1.0 Background of the Study

For years herbs have been the most trusted means of medicinal care for mankind. According to the World Health Organization (WHO, 2022), almost 80 percent of the world population continues to rely on herbal preparations as one of the key elements of primary health care. Affordability, accessibility, and a strong cultural belief in traditional medicine enhance this dependence in developing regions (Nagajothi *et al*., 2018). The world herbal market has grown to a multibillion-dollar market in the past few decades as the preference of consumers toward natural remedies grows (Ahmed and Ibrahim, 2024).

Nonetheless, the notion that herbal medicine is safe and sound due to its naturalness is a scientific fallacy. Any biological active substance may have toxic effects when taken in the wrong amounts or during a long period of time (Lorke, 1983; Zbinden, 1981). The natural bias, which is also known as the assumption of universal safety, has resulted in uncontrolled consumption of plant-based preparations that have not been thoroughly toxicologically tested (Ahmed and Ibrahim, 2024). Thus, the systematic analysis of the safety and physiological effects of popular herbal extracts is an essential need of the public-health.

The research involves the toxicology study of aqueous extracts of three commonly used medicinal plants *Andrographis paniculata*, *Curcuma longa*, and *Cinnamomum verum* in Aspartate Transaminase (AST) and Platelet Count in male Wistar rats. The reasons behind these parameters are that AST is a sensitive biochemical index of hepatocellular integrity, whereas platelet count is an indicator of vascular and bone-marrow health (OECD, 2018). Notably, the research utilizes aqueous extraction, which is a close replica of the conventional preparation

technique (decoction and infusion) as opposed to the use of organic solvents, which changes the phytochemical profiles (Solawati *et al*., 2022). The research will determine the safe dosage ranges, No Observed Adverse Effect Levels (NOAEL), as well as determine what pitfalls may be linked to chronic herbal usage by comparing the sub-acute (28-day) effects of the three extracts.

1.1 Literature Review

1.1.1 *Andrographis paniculata* (King of Bitters)

A. Botanical Classification and Traditional Significance.

Andrographis paniculata (Burm.f.) Nees is a plant of the family. It is an erect annual herb that is very common in India, Sri Lanka, Southeast Asia and some parts of Africa. It is a traditional medicine that is considered valuable in the treatment of fever, liver diseases, respiratory infections, and digestive disruptions (Jayakumar *et al*., 2013). It is also referred to as Kalmegh in Ayurveda due to its bitter taste, as well as commonly used in herbal therapy in Africa as a liver tonic, immune booster (Nagajothi *et al*., 2018).

B. Major Phytochemistry

The medicinal effect of the plant is mainly based on diterpenoid lactones like andrographolide, neoandrographolide, and deoxyandrographolide (Chen *et al*., 2021). These substances have a high antioxidant and anti-inflammatory effect through the regulation of NF-kB and cytokine pathways. Other radical-scavenging capacity is provided by flavonoids, xanthones, and polyphenols (Adegboyega *et al*., 2024).

C. Pharmacological Potency

Studies through experimentation demonstrate widespread pharmacological impacts such as hepatoprotective, antipyretic, antiviral and immunomodulatory activities. *Andrographis paniculata* extract improves the hepatic antioxidant enzyme and protects against the injury caused by xenobiotics in rats (Nagajothi *et al*., 2018). TNF-a and IL-1b are other inflammatory mediators that Andrographolide suppresses (Chen et al., 2021).

D. Pre-Current Hepatological and Hematological Data in Toxicology.

OECD 407 toxicological tests demonstrate that no deaths occur at the limit of 2000 mg /kg body weight; there were mild reversible enzyme effects at higher levels (Solawati *et al*., 2022). Sub-acute research shows decrease in AST and ALT activities, which are evidence of hepatoprotective activity (Fan *et al*., 2020). During long-term exposure, minor gastrointestinal disturbances and temporary platelet count have been observed (Ahmed & Ibrahim, 2024).

Andrographis paniculata

1.1.2 *Curcuma longa* (Turmeric)

A. Botanical Classification and Traditional Significance.

Curcuma longa. is a rhizomatous perennial herb of the family Zingiberaceae, which is widely grown all over tropical Asia. It is called Turmeric and has been exploited in Ayurvedic and Unani systems to cure liver diseases, inflammation and gastrointestinal issues over a period of four thousand years (Prasad and Aggarwal, 2011; Sanusi, 2019).

B. Major Phytochemistry

The major active constituents include curcuminoids, that is, curcumin, demethoxycurcumin, and bis-demethoxycurcumin, and volatile oils, including turmerone and zingiberene (Murugan et al., 2021). Curcumin has strong antioxidant properties because it scavenges reactive oxygen species and activates antioxidant enzymes through the Nrf2 pathway (Sengupta *et al*., 2023).

C. Pharmacological Potency

Curcuma longa has hepatoprotective, anti-inflammatory, antimicrobial, and anticancer activity pharmacologically. It improves the secretion of bile, regulates the metabolism of lipids, and stabilizes the cell membranes (Murugan *et al*., 2021). Its safety and multifunctional activity is why it is still used in traditional and modern preparations.

D. Hepatological and Hematological Existing Data in Toxicology.

Sub-chronic toxicity research indicates that dosages of 500 mg kg-1 day-1 have no major effect on serum AST, ALT, and platelet count (Mulyani et al., 2022; Wakil et al., 2024). Despite the fact that curcumin has weak anti-platelet properties, they are within the physiological range at therapeutic levels (Ahmed & Ibrahim, 2024). Long-lasting therapy has not demonstrated any cases of hepatic necrosis or hematologic stagnation, which proves that it has a high safety margin.

Curcuma longa

1.1.3 *Cinnamomum verum* (True Cinnamon)

A. Botanical Classification and Traditional Significance.

Cinnamomum verum J. Presl (family Lauraceae) is a tree, evergreen and native to Sri Lanka and south India. True cinnamon is the inner bark and was considered a spice and a medicine during centuries. It is also common in conventional medicine to treat diabetes, digestive discomfort and respiratory diseases (Wang *et al*., 2023).

B. Major Phytochemistry

This essential oil is characterized by cinnamaldehyde, eugenol, and cinnamic acid that have their back up by minor ones, linalool, and coumarin (Guo *et al*., 2024). These are very potent antioxidants and antimicrobial compounds. *Cinnamomum* verum and *Cinnamomum cassia* are not similar due to the lower coumarin content, which is why the first one is safer (IU *et al*., 2022).

C. Pharmacological Potency

Research ascribes many pharmacologic activities to Cinnamomum verum: antidiabetic, lipid-modulating, anti-inflammatory and antimicrobial. Cinnamaldehyde increases the sensitivity of insulin receptor and promotes the use of glucose in the hepatic region (Wang *et al*., 2023)).

D. A. Current Hepatological and Hematological Data in Toxicology.

The results of acute and sub-chronic toxicity tests show no negative effects at 2000 mg kg -1 day -1 (IU *et al*., 2022). Hepatoprotective-like aqueous extracts substantially decrease increased

hepatotoxic model levels of AST and ALT (Bellassoued *et al*., 2019). The hematological indices are also stable, which proves the safety of moderate drinking.

Cinnamomum vernum

1.2 Justification of Toxicological Endpoints.

The selection of aspartate transaminase (AST) and platelet count as the parameters of evaluation gives a complementary information about the hepatic and hematologic integrity.

AST or serum glutamate oxaloacetate transaminase (SGOT) is responsible of transferring the amino-group between aspartate and a-ketoglutarate. The presence of AST in blood speaks of hepatocellular damage (OECD, 2018). High AST levels are hence an exquisite indicator of hepatic toxicity or protection (Murugan et al., 2021).

Platelets, however, play a crucial role in coagulation and repairing of vascularity. Any change in the number of platelets can indicate bone-marrow inhibition or peripheral destruction (Ahmed & Ibrahim, 2024). Certain phytochemicals, in particular, curcumin and cinnamaldehyde might have the effects on platelet aggregation, so this parameter is significant in evaluating the sub-acute safety (Sengupta et al., 2023). Therefore, the AST and platelet count should be simultaneously evaluated to create a toxicological portrait of the chosen plant extracts, incorporating the hepatic, hematologic, and systemic requirements.

1.2.1 Aspartate Transaminase (AST); As the cornerstone of hepatotoxicity marker activities.

AST (serum glutamate oxaloacetate transaminase (SGOT)) is among the most valid biochemical indicators to measure liver functioning as well as identify hepatocellular damage. AST is a reversible enzyme that promotes the exchange of an amino group between aspartate and a-

ketoglutarate to oxaloacetate and glutamate (Abdulrahman *et al*., 2021). It is also spread across different tissues such as liver, heart, kidney, skeletal muscles and brain although the hepatic cells contain the largest amount of the same. Thus, AST is released into the bloodstream with the destruction of the integrity of the hepatocellular membrane, which occurs due to the toxicity, oxidative stress, or excessive metabolic load (OECD, 2018).

The AST activity is used as a cornerstone marker in toxicological and pharmacological experiments due to the fact that it gives a quantitative measure of the damage to the hepatocytes. The higher the AST activity, the higher the degree of necrosis of liver cells, especially in acute or sub-acute hepatotoxicity (Sengupta *et al*., 2023). Nonetheless, AST is found in other non-hepatic tissues and therefore, its application is best combined with alanine transaminase (ALT), which is more sensitive to liver parenchymal damage (Murugan *et al*., 2021).

AST levels in serum are an important endpoint that is used to measure toxicity and hepatoprotection. In herbal research, the decrease in AST after plant extracts intake reveals that the research may have hepatoprotective properties due to antioxidant and membrane-stabilizing production (Solawati *et al*., 2022). On the other hand, elevated levels of a substantial magnitude are indicative of hepatocellular leakage due to metabolic stress or phytochemical toxicity.

The effects of plant-based bioactives on the levels of AST have been reported in several studies. As an example, *Andrographis paniculata* extract has been demonstrated to normalize the AST activity in rats subjected to hepatic injury caused by carbon tetrachloride (Fan et al., 2020). On the same note, *Curcuma longa and Cinnamomum verum* carry curcuminoids and cinnamaldehyde, respectively, that eliminate free radicals and enzyme-modulating in the liver, respectively, which prevents oxidative damage (Wang et al., 2023; Guo et al., 2024).

Therefore, inclusion of AST in this toxicological assessment gives a biochemical backbone towards hepatic response of aquatic extracts of the chosen plants. AST change degree provides a first-hand confirmation of cell integrity or cell damage indicating safe dosage and defining the possible therapeutic range of these historically utilized botanicals (Ahmed and Ibrahim, 2024).

1.2.2. Hematological Indicator Platelet Count.

Platelets or thrombocytes are minute, non-nucleated fragments of cytoplasm of megakaryocytes in the bone marrow. They are critical to hemostasis, which is the process that inhibits excessive loss of blood after the vascular injury. In addition to platelets in the formation of clots, they are also involved in inflammation, immune reaction, tissue repair, and vascular integrity (Harrison et al., 2022).The platelet count is an important hematological indicator in toxicology and pharmacology since it indicates the functional ability of the bone marrow and the health of the circulatory system (OECD, 2018). Normal mammalian platelet count is 150,000 to 450,000/mL in the blood, and variations outside this range might signify many physiological abnormalities (Murugan *et al*., 2021). Bone marrow suppression, an augmented peripheral destruction, or even poisonous assault on the hematopoietic tissues may lead to a reduction in the number of platelets (thrombocytes). On the other hand, there are high levels of platelets (thrombocytosis) which are a compensatory reaction to inflammation, tissue injury, or dehydration (Sengupta *et al*., 2023). Platelet count is a key hematological parameter in toxicological tests of medicinal plants since most of the phytochemicals may also act on hematopoiesis. As an example, alkaloid and phenolic compounds could either stimulate or suppress bone marrow. A response that is concentration, time and exposure dependent (Ahmed and Ibrahim, 2024). The platelet count will be monitored to assess the negative or positive outcome of the plant extracts on the formation of

blood and stability of vessels. This parameter is relevant according to research evidence. The anti-thrombotic effect of curcuminoids has been reported to have a mild effect of reducing platelet aggregation of curcuma longa though still with a physiological normal level of therapeutic dose (Prasad & Aggarwal, 2011). *Cinnamomum verum* is a source of cinnamaldehyde, which is capable of controlling platelet activation, preventing the production of thromboxane A2 to help improve circulation without leading to bleeding (Bellassoued *et al*., 2019). Concurrently, *Andrographis paniculata* extract exhibited hematopoietic balance in keeping the rodent toxicity model of platelets and red cells intact (Fan *et al*., 2020). As such, the assessment of platelets counts and AST gives a holistic assessment of hepatic and hematological safety. AST indicates the integrity of liver cells, but the number of platelets indicates potential toxicity of the system or of marrow. All these parameters obtain a comprehensive evaluation of the possible impact of aqueous plant extracts in the experimental toxicology that contributes to the safe incorporation of these botanicals into the sphere of evidence-based medicine (Wang *et al.*, 2023).

1.3 Scope and Limitation of the Study

The present study is designed to evaluate the toxicological effects of aqueous extracts of *Andrographis paniculata*, *Curcuma longa*, and *Cinnamomum verum* on specific biochemical and hematological parameters in male Wistar rats. The study's scope encompasses all activities from extract preparation and dosage standardization to animal experimentation, data collection, and analysis, focusing on establishing a comparative toxicological profile for the selected plant species.

1.3.1Scope of the Study

1. **Plant Material and Extraction Method:**

   The experiment uses aqueous extracts that have been made using certified plant samples, that is, as leaves of *Andrographis paniculata*, rhizomes of *Curcuma longa*, and bark of *Cinnamomum verum*. The extraction technique was selected, as it was adopted to be similar to the traditional approach of using herbs in decoction and infusion adopted by the local people (Elfahmi et al., 2006; Rahman *et al.*, 2021). This method is ecologically and culturally relevant, as it guarantees the possibility of direct comparison of laboratory data and actual herbal use traditions in the world.

2. **Experimental Animal Model:**

   The physiological and metabolic similarity of healthy adult male Wistar rats to humans makes them an experimental model in the toxicological study (OECD, 2018). The given model enables the same and reproducible evaluation of biochemical and hematological indices after controlled exposure to the plant extracts.

3. **Toxicological Evaluation Parameters:**

   Two biomarkers were selected to depict different physiological systems: Aspartate Transaminase (AST); this is a type of enzyme found in the liver that represents the functioning of hepatocytes and hepatocellular integrity. Platelet Count; a hematological index that shows the condition of bone marrow and circulatory. They were chosen as these endpoints to offer a dual-system evaluation of the toxicity and offer the

understanding on the hepatic and hematopoietic effects (Murugan *et al*., 2021; Bellassoued et al., 2019).

4. **Duration and Dosing Regimen:**

The experiment examines the acute toxicity after an exposure period of 6 days on the basis of OECD guidelines (Test Guideline 407). Doses were chosen according to a previous acute toxicity study (determination of an LD50) in order to define a safe yet pharmacologically active range. This study can identify small toxic effects which otherwise might not be detected in short-term studies (Solawati *et al*., 2022).

5. **Analytical and Statistical Procedures:**

The biochemical and hematological data obtained were analyzed using standardized statistical tools such as Analysis of Variance (ANOVA) to compare results between control and treatment groups. This ensures statistical validity and facilitates accurate interpretation of the toxicological responses (Wang *et al*., 2023).

6. **Comparative Focus:**

The study's comparative framework allows for direct cross-evaluation of the three plant extracts under identical experimental conditions. This not only enhances scientific robustness but also identifies which plant exhibits the most favorable safety profile, serving as a foundation for prioritizing future mechanistic or clinical studies (Ahmed & Ibrahim, 2024).

1.5.2 Limitations of the Study

Although it is scientifically rigorous, the research paper recognizes some methodology and logistical limitations that could affect the interpretation of the results or its extrapolation:

1. **Species-Specific Differences:**

Although Wistar rats are the standard model of toxicity testing, there is a possibility that interspecies metabolism can restrict the extrapolation of results to humans. To confirm the findings in human populations, they should be subjected to further clinical studies (OECD, 2018).

2. **Limited Biomarker Scope:**

The paper mainly concentrates on AST and platelet count as the indicative biochemical and hematological parameters. These are vital signs of toxicity, but they do not include all the potential systemic or molecular changes that can be experienced, including oxidative stress or histopathological changes (Murugan *et al*., 2021).

3. **Duration of Exposure in Short-term:**

Only acute (6-day) toxicity is evaluated in the research. There was no evaluation of long-term (chronic) toxicity, or reproductive or genetic toxicity, so it might not provide insight into long-term effects or hereditary effects (WHO, 2022).

4. Phytochemical Variability:

The various chemical composition of the plant extracts may differ with geographical origin, season of harvest and environmental factors. Even though authentication and standardization have been carried out, minor differences in concentrations of phytochemicals may interfere with the reproducibility (Rahman *et al*., 2021).

5. Single Extraction Solvent:

Although aqueous extraction has been shown to be more culturally relevant, it rejects other types of solvents, which could possibly have varying toxicity profiles. The results of the present study, therefore, cannot be generalized to water-based preparations to ethanol or methanol preparations (Elfahmi *et al*., 2006).

6. Uncontrollable External Factors:

Complex environmental or dietary interactions which are in human herbal consumption cannot be fully recreated in laboratory-based research. The variables can alter absorption or metabolism and change the effect of toxicity (Ahmed and Ibrahim, 2024).

CHAPTER TWO

2.0 MATERIALS AND METHOD

2.1. Materials

The materials used for this study are the samples *Andrographis paniculata, curcuma longa* and *Cinnamomum verum*, male Wistar rat and laboratory apparatus.

2.1.1 Apparatus

Gavage, Test tube, Conical flask, Measuring cylinder, Beaker, Micro pipette, Foil paper, Extraction jar, Pot, Cheese cloth, Rubber bucket, Glass rod, Soft tissue paper, Hand gloves, Muslin cloth, Kjeldahl flask, Gas cylinder

2.1.2 Chemicals and Reagent

Andrographis paniculata , Cinnamomum verum, Curcuma longa, Chloroform, Distilled water, Picric Acid, Ethanol, Nitric acid, Nessler's reagent, Concentrated sulphuric acid, Potassium hydroxide

2.1.3 Equipment

Weighing balance, Water bath, Centrifuge, Spectrophotometer, Hot plate, Muffle furnace, Drying oven, Stop watch

2.1.4 Other materials

Latex gloves Cotton wool, Syringe Masking tape Detergent

2.2 Study area

This research was carried out in Advance laboratory of the Department of Biochemistry, Faculty of Life science, University of Benin, Benin city, Edo state, Nigeria.

2.3 Study selection

We only concluded original research studies conducted on healthy male albino Wistar rats having an initial weight ranging from 90 g- 120 g. Overall, thirty-nine (39) animals were provided and fed for a period of 2 weeks. The animals were randomly placed in groups irrespective of their weight.

2.4 Experimental animal

A total of thirty-nine (39) wistar rats were obtained from the animal house of the Department of Biochemistry. The animals were distributed into different cages in groups

2.5 Collection of plant materials

Pure samples of *Andrographis paniculata, Cinnamomum verum* and *Curcuma longa* were obtained from the market and were dried and milled. The plant samples were identified and authenticated by a botanist from the Department of Plant Biology and Biotechnology, University of Benin. Voucher specimens were prepared and deposited in the departmental herbarium with the following identification numbers:

Andrographis paniculata (Voucher No. UBH–AP599)

Curcuma longa (Voucher No. UBH–CL–002)

Cinnamomum verum (Voucher No. UBH–CV–395)

2.6 Extraction Method

2.6.1 Aqueous Extraction of *Andrographis Paniculata* Using Decoction Method

Three hundred (300) grams of *Andrographis paniculata* plant was weighed accurately using a calibrated analytical balance. The weighed sample was poured in a pot containing three (3) litres of boiling distilled water and allowed to boil for fifteen (15) minutes. After boiling, it was allowed to cool then filtered. The filtrate was poured back into the pot and evaporated to dryness under low heat. The extract was scooped and transferred to an already weighed stainless steel plate and the total weight was taken.

2.6.2 Ethanol Extraction of *Andrographis Paniculata* Using Decoction Method

Three hundred (300) grams of *Andrographis paniculata* plant was weighed accurately using a calibrated analytical balance. The weighed sample was poured into an extraction jar and three (3) liters of ethanol was added with occasional stirring and was covered for two (2) hours. The mixture was stirred again after two (2) hours, covered and left for forty-eight (48) hours. After forty-eight (48) hours, it was stirred, filtered and concentrated by evaporating to dryness under low heat. The extract gotten was transferred to an already weighed stainless steel plate and the final weight was taken.

2.6.3 Aqueous Extraction of *Cinnamomum verum* Using Cold Maceration Method

One thousand two hundred (1200) grams of *Cinnamomum verum* was accurately weighed using a calibrated analytical balance. The weighed sample was poured into a rubber bucket and six (6) litres of distilled water was added, the mixture was stirred and covered for twenty-four (24) hours. After twenty-four (24) hours, the filtrate was separated from the residue by sieving with a cheese cloth. The filtrate was freeze dried for seven (7) days after which the final weight was taken.

2.6.4 Aqueous Extraction of Curcuma longa Using Cold Maceration Method

One thousand two hundred (1200) grams of Curcuma longa was accurately weighed using a calibrated analytical balance. The weighed sample was poured into a rubber bucket and six (6) litres of distilled water was added, the mixture was stirred and covered for twenty-four (24) hours. After twenty-four (24) hours, the filtrate was separated from the residue by sieving with a cheese cloth. The filtrate was freeze dried for seven (7) days after which the final weight was taken.

2.7 Proximate Analysis

Proximate analysis is carried out to determine the estimated nutrient value of a sample.

2.7.1 Proximate analysis procedure: The samples were oven dried at 60°C for 24 hours using an oven and allowed to cool, and was processed for proximate content determination by passing the sample through a grinder a sufficient number of times to obtain a uniform mixture.

2.7.2 Determination of moisture content

The moisture content of food samples was determined using A.O.A.C (2000) method (The gravimetric method).

Principle:

The moisture content is determined from the difference in weight after complete evaporation of moisture.

Procedure:

One gram of food samples was weighed in crucibles and oven dried at 105^{o}C to a constant weight. The samples were cooled in a desiccator and weighed.

Moisture loss = initial weight– final weight (weight after drying)

$$\% \text{ moisture} = \frac{\text{Loss in weight}}{\text{Weight of sample}} \times 100$$

2.7.3 Determination of crude protein content

Nitrogen was been determined using the micro-Kjeldahl method (A.O.A.C 2000) and crude protein content will be subsequently calculated by multiplying the nitrogen content by a factor of 6.25

Principle:

Proteins and other food components are digested with sulphuric acid in the presence of catalysts. Ammonium sulphate is produced from the total organic nitrogen in the food. Alkali is then used to neutralize the acid digest to produce ammonium which is steam distilled directly

into a hydrochloric acid containing the indicator methylene red. Hydrochloric acid and ammonium react to produce ammonium chloride which is then titrated with sodium hydroxide. A blank determination is run to determine the nitrogen content of the reagents. The percentage nitrogen is calculated. The percent nitrogen multiplied by the conversion factor for that particular food produces the percent protein in a food.

Procedure:

One milliliter of 4% $CuSO_4$, H_2SO_4 and 0.8g of K_2SO_4 were placed in the micro Kjeldahl flask. Varying quantity of food samples depending on nitrogen content was added to the reagents in micro Kjeldahl flask. It was digested first at low temperature until frosting ceases, then at a high temperature, until the solution was clear, pale yellow or light blue. The flask was left to cool, and 4ml of distilled water was gradually added and content were distilled using a Kjeldahl distillation apparatus. 10 mL of 30% NaOH was used to liberate during distillation ammonium. Ammonium was collected in 0.01 M HCl (a drop of methylene red was added to the HCl.). The distillate was titrated with 0.01M NaOH. Nitrogen content of samples was calculated from the volume of HCl neutralized by NaOH.

Calculations

$$\%\text{nitrogen} = \frac{\text{titer value (blank)} - \text{titer value (distillate)} \times 0.14}{\text{Weight of sample}}$$

Protein = % nitrogen x 6.25

100

% protein = protein x ----------

Initial weight

2.7.4 **Determination of lipid content**

Crude fat was be determined using Soxhlet extraction A.O.A.C (2000)

Principle:

The free lipid content consists of neutral fats (triglycerides) and free fatty acid was determined by extracting the dried and ground material with diethyl ether in a continuous extraction apparatus (Soxhlet extractor).

Procedure:

The weight of an empty flask was determined. One gram of sample was wrapped and placed in an extraction thimble. The thimble was plugged with cotton wool to avoid loss of sample. The thimble was placed in the extractor. An already weighed, clean and dry soxhlet extractor flask was attached to bottom of the extractor. Petroleum ether (500mls) was poured into dry soxhlet flask and the heating mantle switched on so that the petroleum ether boiled. Heating continued for eight hours after which the solvent was siphoned completely into flask and taken to dryness by distillation. The flask was removed dried to a constant weight. The cooled, weighed and the amount of extracted lipids was calculated from the difference between the weight before and after extraction.

Calculation:

Weight of empty porous thimble = w_o

Weight of thimble +ground sample = w1

Weight of ground sample = w1- w_o

Weight of empty extraction flask = w2

Weight of extraction flask + ether = w3

$$\%\text{lipid} = \frac{W3-W2}{W1-W_o} \times 100$$

2.7.5 **Determination of ash content**

Ash content was determined using the method of AOAC (2000).

Principle:

The total ash complete is estimated by complete removal of organic material using ignition.

Procedure:

One gram of samples was weighed in crucibles and ignited in a furnace at 500-600^{o}C for 3 hours until it turns to ashes completely. It was then cooled in a desiccator and weighed immediately at room temperature.

Calculation:

$$\% \text{ Ash} = \frac{W_2-W_0}{} \times 100$$

W_1-W_0

2.7.6 **Determination of dietary fiber**

This was determined by enzymatic-gravimetric method as described by A.O.A.C (2000)

Procedure:

One gram of each samples(w_0) was boiled in 200ml 0f sulphuric acid and boiled ently for thirty minutes the boiled sample were filtered through a muslin cloth which was rinsed with hot distilled water. 200ml 0f sodium hydroxide was added to the residue and allowed to boil for 30min, it was then rinse with hot distilled water. it was also rinsed with hydrochloric acid. it was finally rinsed three times with petroleum ether. it was allowed to drain, then dried in the oven and eventually allowed to cool in a dessicator and weighed(w_1). The samples were ashed at 500 ^{o}c, for 90minutes in a muffle furnace coole in a dessicator and weighed(w_2).c

calculation:

$$\% \text{ crude fibre} = \frac{w_1 - w_2}{w_0} \times 100$$

2.7.7 **Determination of carbohydrate content**

carbohydrate content was determined by obtaining the difference after adding up the % protein content, % fiber content and % lipid then subtracting from one hundred.

% carbohydrate = 100 - % lipid + % protein + % ash + % fiber

2.8 Study Design:

Thirty-nine (39) rats were weighed and nine (9) were randomly placed in each group for 10mg, 100mg and 1000mg of each sample, three (3) for 1500mg, 2900mg and 5000mg of each sample and three (3) for control irrespective of their weight and were labelled according to their pattern of acclimatization and were administered appropriately. This was done for all the animals and the cages were labelled.

Group 1 – Normal control

Group 2 – 10mg, 100mg, 1000mg *Andrographis paniculata*

Group 3 – 10mg, 100mg, 1000mg *Cinnamomum verum*

Group 4 – 10mg, 100mg, 1000mg *Curcuma longa*

Group 5 – 1500mg, 2900mg, 5000mg *Andrographis paniculata*

Group 6 – 1500mg, 2900mg, 5000mg *Cinnamomum verum*

Group 7 – 1500mg, 2900mg, 5000mg *Curcuma longa*

2.9 Determination of Body Weight

The weight of the animals was checked before and after administration of the sample extracts using a weighing balance and results were recorded as;

1. Body weight before treatment
2. Body weight after treatment

2.10 Experiment Termination and Sample Collection

Fourteen (14) days after administration, the rats were fasted overnight, anesthetized with chloroform in a closed chamber, sacrificed and the blood was extracted from the heart and liver using a syringe. The extracted blood was poured in an EDTA container and a plain container for each sample collected for hematology, kidney and liver function test.

2.11 Estimation of Aspartate Transaminase concentration

2.11.1Principle

The entire experiment hinges on linking the invisible action of the Aspartate Aminotransferase (AST) enzyme to a measurable change in color. First, the AST enzyme, present in the serum sample, catalyzes a transamination reaction. It transfers an amino group from L-Aspartate to 2-Oxoglutarate, producing two new molecules: L-Glutamate and Oxaloacetate. The amount of Oxaloacetate generated is directly proportional to how active the AST enzyme was in the patient's sample. Second, the solution is treated with the R2 Dye Reagent (2,4-Dinitrophenylhydrazine or 2,4-DNPH). This reagent reacts specifically with the newly formed Oxaloacetate, creating an intermediate compound. Finally, the strong alkaline solution, R4 Sodium Hydroxide (NaOH), is added. This stops the enzymatic reaction, shifts the pH dramatically, and converts the intermediate compound into a stable, intense, yellow-brown hydrazone color. This final color's intensity is what we measure. The darker the color, the more Oxaloacetate was produced, and therefore, the higher the AST enzyme activity.

2.11.2 Procedure for AST Activity Determination

The determination of aspartate aminotransferase (AST) activity was performed using a carefully timed and standardized protocol to obtain accurate results. The procedure relied on a reagent blank to correct for any background absorbance arising from the chemical reagents.

The assay began by adding 50 µL of R1 AST buffer, containing the key substrates L-aspartate and 2-oxoglutarate, into the sample tube. To this mixture, 10 µL of the patient's serum sample was added. The reaction mixture was then incubated at 37 °C for 30 minutes to allow the AST enzyme in the serum to catalyze the conversion of L-aspartate and 2-oxoglutarate to oxaloacetate and glutamate. In parallel, a reagent blank was prepared using the same buffer volume (500 µL) but replacing the serum with 10 µL of distilled water. This served as a control to identify any background color changes caused by the reagents themselves.

Following the incubation period, 50 µL of R2 dye reagent (DNPH) was added to both the sample and blank tubes. The mixture was allowed to stand at room temperature for 10 minutes to permit complete reaction between DNPH and the oxaloacetate produced, forming a hydrazone derivative responsible for the color development. Subsequently, 1000 µL of R4 sodium hydroxide (NaOH) was added to each tube to stabilize the developed color. The tubes were allowed to stand for an additional 5 minutes to achieve uniform color stabilization.

After stabilization, the spectrophotometer was set to a wavelength of 546 nm. The instrument was first zeroed using the reagent blank to eliminate background interference, and then the absorbance of the sample was measured. The intensity of the developed color was directly proportional to the AST activity in the patient's serum.

2.11.3 Calculation of AST Activity

The absorbance obtained from the sample was compared with that of a pyruvate standard of known concentration treated under identical conditions. The activity of AST in the serum sample was calculated using the formula:

AST Activity (U/L) = (Absorbance of Sample / Absorbance of Standard) × Concentration of Standard

In this expression, the absorbance of the sample represents the optical density of the test serum, while the absorbance of the standard corresponds to that of the reference pyruvate solution. The concentration of the standard is a known constant, and the factor is provided by the manufacturer to account for specific assay conditions such as temperature, incubation time, and reagent volumes. This calculation translates the color intensity measured at 546 nm into enzymatic activity, expressed as Units per Liter (U/L), which reflects the level of AST present in the patient's serum.

3.0 RESULT

3.1 Proximate analysis of *Andrographis paniculata, Cinnamomum verum* and *Curcuma longa*

Table 3.1

Parameter (%)	*Andrographis paniculata*	*Cinnamomum verum*	*Curcuma longa*
Moisture content	10.63 ± 0.47^{a}	13.80 ± 0.49^{b}	15.93 ± 1.08^{b}
Ash content	11.70 ± 0.23^{c}	5.23 ± 0.49^{a}	9.23 ± 0.58^{b}
Crude fibre	15.53 ± 0.34^{c}	14.30 ± 0.26^{b}	4.20 ± 0.11^{a}
Crude fat	1.30 ± 0.03^{a}	1.25 ± 0.03^{a}	1.20 ± 0.03^{a}
Crude protein	7.97 ± 0.44^{b}	8.86 ± 0.02^{b}	6.86 ± 0.23^{a}
Carbohydrate	52.85 ± 0.39^{a}	56.54 ± 0.65^{b}	62.56 ± 1.00^{c}

3.2 The effect of *Andrographis paniculata, Cinnamomum verum* and *curcuma longa* on body weight

Table 3.2

Group	**Body weight Week 0 (g) (initial body weight)**	**Body weight (g) (before administration)**	**Body weight (g) (after administration)**	**Mean difference %**
Group 1 (control)	137.7333±5.95475[b]	140.2033±6.5358[b, c]	2.4700±.59025[a, b]	
Group2 (A.P 10mg/kg)	115.3600±6.06103[a]	130.8767±3.72680[b]	15.5167±7.00203[c]	
Group 3 (A.P 100mg/kg)	138.5533±2.94568[b]	141.2167±2.40234[c]	2.6633±1.28650[a, b]	
Group 4 (A.P 1000mg/kg)	148.0633±2.27608[b]	152.0467±2.47469[d]	3.9833±3.21844[a, b]	
Group 5 (A.P	175.4700±.56199[c]	.0000±.00000[a]	.0000±.00000[a]	

1500mg/kg)			
Group 6 (A.P 2900mg/kg)	186.3767±.53042[d]	175.3000±.59808[e]	11.0767±.09262[b]
Group 7 (A.P 5000mg/kg	186.7667±.65282[d]	187.3100±.49095[f]	1.0167±.66167[a]
Group 8 (Cin 10mg/kg)	158.6667±.28672[b]	166.6167±1.7309[b, c]	8.1300±1.41288[b, c]
Group 9 (Cin 100mg/kg)	159.6367±.11609[b]	162.6167±6.55834[b]	9.8400±.13000[c, d]
Group 10 (Cin 1000mg/kg)	162.0267±4.36904[b]	174.8833±3.13846[c]	12.8567±2.1795[d, e]
Group 11 (Cin 1500mg/kg)	198.1600±.24987[c]	210.3833±.63304[d]	12.2233±.55682[d]
Group 12 (Cin 2900mg/kg)	203.3533±.3497[c, d]	219.3433±.45241[d]	15.9900±.59408[e]
Group 13 (Cin 5000mg/kg)	207.4667±.57828[d]	213.4633±.35974[d]	5.9967±.90554[b]
Group 14 (Turm 10mg/kg)	170.0300±.30665[c]	178.0167±2.88482[c]	7.9867±2.75469[a, b]
Group 15 (Turm 100mg/kg)	174.3400±1.98258[c]	183.8467±1.65532[c,d]	9.5067±.37676[b]

Group (Turm 1000mg/kg)	16	177.6933±.3053[c, d]	183.9333±2.6300[c, d]	6.2400±2.85553[a, b]
Group (Turm 1500mg/kg)	17	162.4500±.39887[b]	167.4700±.44306[b]	5.0200±.49000[a, b]
Group (Turm 2900mg/kg)	18	189.1000±.92147[e]	195.6167±.62229[e]	6.5167±1.53851[a, b]
Group (Turm 5000mg/kg)	19	184.2233±.5318[d, e]	189.2067±.57476[d, e]	4.9833±1.10197[a, b]

KEY: Body weight values recorded after every seven (7) days of oral administration starting from week one (1).

Body weight for week 0,1, 2: values are expressed as mean ± standard error

3.3 THE EFFECT OF *ANDROGRAPHIS PANICULATA, CINNAMOMUM LONGA* AND *CURCUMA VERNUM* ON PLATELET OF MALE RATS.

Group	PLT *Andrographis paniculata*	PLT *Curcuma longa*	PLT *Cinamomum vernum*
Control	562.67 ± 20.83	562.67 ± 20.83	565.67 ± 20.85
10 mg/kg	213.00 ± 51.19	573.67 ± 50.07	471.67 ± 40.99
100 mg/kg	428.00 ± 66.05	497.00 ± 82.64	308.33 ± 101.17
1000 mg/kg	608.00 ± 57.64	392.67 ± 102.13	562.33 ± 24.69
1500 mg/kg	—	625.00 ± 15.01	345.00 ± 8.66
2900 mg/kg	800.00 ± 24.58	488.00 ± 1.15	543.00 ± 8.08
5000 mg/kg	567.00 ± 9.81	450.00 ± 11.55	416.00 ± 6.93

3.4 The effect of *Andrographis paniculata, Cinnamomum longa* and *Curcuma verum* on Aspartate transaminase of male rats.

Group	AST.AP (U/L)	AST.TUM (U/L)	AST.CIN (U/L)
Control	101.33 ± 2.96[a]	101.33 ± 2.96[a]	101.33 ± 2.96[a]
10 mg/kg	88.57 ± 11.18[a]	98.50 ± 2.29[a]	63.87 ± 2.20[a]
100 mg/kg	93.33 ± 1.99[a]	96.57 ± 1.31[a]	88.73 ± 12.02[a]
1000 mg/kg	100.30 ± 8.08[a]	72.80 ± 7.73[b]	76.80 ± 11.89[a]
1500 mg/kg	—	95.80 ± 1.18[a]	103.0 ± 3.04[a]
2900 mg/kg	94.60 ± 2.99[a]	101.20 ± 1.16[a]	94.20 ± 1.24[a]
5000 mg/kg	99.30 ± 0.84[a]	97.50 ± 1.21[a]	98.00 ± 1.65[a]

CHAPTER FOUR

DISCUSSION OF RESULTS

Table 3.1 shows the variation of moisture content, ash content, crude fibre, crude fat, crude protein and carbohydrate across *Andrographis paniculata, Cinnamomum verum* and *Curcuma longa*. Values are expressed as mean ± standard error of mean (SEM) for each plant, values with different subscripts are significantly different at $p<0.05$. The proximate analysis of *Andrographis paniculata, Cinnamomum verum,* and *Curcuma longa* revealed distinct differences in their nutritional composition. Moisture content was highest in Curcuma longa (15.93 ± 1.08%), followed by *Cinnamomum verum* (13.80 ± 0.49%) and *Andrographis paniculata* (10.63 ± 0.47%). The higher moisture value in Curcuma longa suggests greater susceptibility to microbial spoilage, whereas the lower value observed in *Andrographis paniculata* indicates better storage stability and reduced moisture-related degradation (Ganogpichayagrai, 2020).

Ash content, which represents the total mineral composition, was highest in *Andrographis paniculata* (11.70 ± 0.23%), followed by Curcuma longa (9.23 ± 0.58%) and the least in *Cinnamomum verum* (5.23 ± 0.49%). This finding indicates that *Andrographis paniculata* may contain higher concentrations of essential minerals such as calcium, potassium, and magnesium that are vital for enzymatic activity and electrolyte balance (Ganogpichayagrai, 2020). Crude fibre content was also greatest in Andrographis paniculata (15.53 ± 0.34%), slightly higher than

Cinnamomum verum (14.30 ± 0.26%) and markedly higher than Curcuma longa (4.20 ± 0.11%). High fibre values in *Andrographis* and *Cinnamomum* suggest that these herbs may enhance gastrointestinal motility, promote bowel health, and aid detoxification processes (Buonomo *et al*., 2018).

The crude fat contents of the three extracts were relatively low and statistically similar, ranging from 1.20 ± 0.03% to 1.30 ± 0.03%. This low lipid level implies that the extracts are unlikely to contribute excessive fat to the diet, which supports their use in health management and weight control (Ganogpichayagrai, 2020). The protein content was highest in *Cinnamomum verum* (8.86 ± 0.02%), followed by *Andrographis paniculata* (7.97 ± 0.44%) and *Curcuma longa* (6.86 ± 0.23%). This suggests that *Cinnamomum verum* may contain a higher concentration of nitrogenous compounds, which play roles in tissue repair, immune function, and metabolic enzyme synthesis (Oluwole et al., 2021). In contrast, carbohydrate content was highest in Curcuma longa (62.56 ± 1.00%), followed by *Cinnamomum verum* (56.54 ± 0.65%) and Andrographis paniculata (52.85 ± 0.39%). This implies that *Curcuma longa* could serve as a rich source of energy, complementing its phytochemical benefits (Singh and Sharma, 2020). The body weight of experimental rats is an important indicator of overall health, reflecting the physiological impact of administered substances. During the seven-day treatment period, the control group showed a mean weight increase from 137.73 ± 5.95 g to 140.20 ± 6.53 g. Rats treated with *Andrographis paniculata* at 10 mg/kg exhibited a greater mean weight gain (15.52 ± 7.00 g) compared with the control, indicating potential improvement in appetite and metabolic activity at low doses. However, at 100 mg/kg and 1000 mg/kg, the mean differences were moderate (2.66 ± 1.29 g and 3.98 ± 3.22 g, respectively), showing a stabilizing trend. The 2900 mg/kg group demonstrated a higher mean gain (11.08 ± 0.09 g), while the highest dose (5000

mg/kg) produced a minimal change (1.02 ± 0.66 g), suggesting that the extract may stimulate weight gain only within an optimal dose range (Rahman et al., 2019). For *Cinnamomum verum*, body weight changes followed a positive trend across increasing doses. Rats treated at 10 mg/kg recorded an increase of 8.13 ± 1.41 g, while 100 mg/kg and 1000 mg/kg doses produced mean gains of 9.84 ± 0.13 g and 12.86 ± 2.18 g, respectively. The highest increase was seen at 2900 mg/kg (15.99 ± 0.59 g), indicating that *Cinnamomum verum* may enhance energy metabolism and feed efficiency at higher concentrations (Buonomo et al., 2018). Similar observations were reported by previous researchers who attributed weight increases in cinnamon-treated animals to improved glucose utilization and digestive efficiency (Mehrpouri and Hamidpour, 2020).

Curcuma longa demonstrated moderate increases in body weight across all groups, ranging from 5.02 ± 0.49 g to 9.51 ± 0.38 g, with the highest mean difference occurring at 100 mg/kg (9.51 ± 0.38 g). This indicates that the extract supported normal growth and did not exert appetite-suppressing or toxic effects. The general pattern across all treatments shows that none of the extracts caused a reduction in body weight, suggesting their overall safety and absence of toxic metabolic interference during the seven-day period (Farzaei *et al*., 2018).

Platelet count is a sensitive marker of bone marrow activity and thrombopoietic function. In the present study, platelet counts exhibited a dose-dependent variation among the treatment groups. The *Andrographis paniculata* group showed a marked reduction in platelet count at the 10 mg/kg dose, followed by a pronounced increase at higher doses (up to 2900 mg/kg). This biphasic response indicates an initial mild suppression of thrombopoiesis possibly due to adaptive physiological response, which was subsequently followed by stimulation of platelet production at higher concentrations. The elevation observed at 2900 mg/kg suggests that *Andrographis*

paniculata may enhance haematopoietic recovery and thrombopoiesis at optimal doses (Onderoglu, 1999). *Curcuma longa* administration resulted in relatively stable platelet levels throughout the treatment period, with values remaining within normal physiological ranges. This suggests that short-term administration of *Curcuma longa* neither suppressed nor exaggerated thrombopoiesis, implying safety on haematological function. Conversely, *Cinnamomum verum* produced a moderate decline in platelet count at lower and mid-dose levels, which could be associated with the anti-aggregatory properties of cinnamaldehyde and polyphenolic compounds present in cinnamon. These bioactive constituents have been shown to modulate platelet aggregation and reduce thrombosis risk (Mehrpouri and Hamidpour, 2020). However, at higher doses, platelet counts normalised, indicating a transient, non-toxic modulation of platelet function rather than suppression of platelet synthesis.

Aspartate Aminotransferase (AST) serves as a biochemical marker for hepatocellular integrity and liver function. The results indicated no significant increase in AST activity across treatment groups for *Andrographis paniculata, Curcuma longa,* and *Cinnamomum verum* when compared to the control. The control group recorded 101.33 ± 2.96 U/L, while *Andrographis paniculata* ranged between 88.57 ± 11.18 U/L and 100.30 ± 8.08 U/L, demonstrating stability and lack of enzyme leakage. *Curcuma longa* showed the lowest AST activity (72.80 ± 7.73 U/L at 1000 mg/kg), representing a significant decrease that may indicate hepatoprotection. In contrast, *Cinnamomum verum* values fluctuated between 63.87 ± 2.20 U/L and 108.50 ± — U/L but remained within physiological limits (Farzaei *et al*., 2018). The significant reduction in AST at 1000 mg/kg of Curcuma longa is consistent with its documented hepatoprotective and antioxidant properties. Curcumin, the active constituent, may enhance membrane stability and reduce oxidative damage to hepatocytes (Khan et al., 2019). The consistent results for

Andrographis paniculata and *Cinnamomum verum* further suggest that short-term exposure to these extracts does not elicit hepatic toxicity or interfere with metabolic enzyme function.

Taken together, the findings from this study demonstrate that the three herbal extracts – *Andrographis paniculata, Cinnamomum verum,* and *Curcuma longa* – exhibit beneficial nutritional and physiological properties without evidence of toxicity following seven days of oral administration. The proximate composition showed that Curcuma longa had the highest carbohydrate and moisture content, making it an excellent energy source but potentially less stable in storage, while *Andrographis paniculata* contained the highest mineral and fibre content, suggesting strong detoxification and digestive benefits. *Cinnamomum verum* presented the greatest protein concentration, implying its role in tissue repair and metabolic regulation. Body weight assessment revealed that all three extracts supported growth and did not impair metabolism, with notable increases observed in rats treated with higher doses of Cinnamomum *verum and Curcuma longa*. The platelet analysis further indicated that Andrographis paniculata may enhance thrombopoiesis at higher doses, while *Cinnamomum verum* exhibited mild anti-platelet activity consistent with its known pharmacological properties. *Curcuma longa* maintained stable platelet levels, highlighting its haematological safety. The AST results provided biochemical evidence of safety and hepatoprotection, particularly for *Curcuma longa*, which showed a significant reduction in enzyme activity at 1000 mg/kg (Farzaei *et al*., 2018). Overall, these findings confirm that the three medicinal plants are biochemically safe, possess diverse nutritional benefits, and hold potential as hepatoprotective and haematopoietic agents. Further studies involving longer exposure durations and additional biochemical markers are recommended to substantiate their long-term safety and therapeutic efficacy (Buonomo *et al*., 2018; Rahman *et al*., 2019).

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APPENDIX

Proximate analysis

Descriptives						
	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean	
					Lower Bound	Upper Bound
A1	3	10.6333	.81445	.47022	8.6101	12.6565
A2	3	13.8000	.85440	.49329	11.6776	15.9224
A3	3	15.9333	1.88237	1.08679	11.2573	20.6094
Total	9	13.4556	2.56277	.85426	11.4856	15.4255

A1	3	11.7000	.40000	.23094	10.7063	12.6937
A2	3	5.2333	.85049	.49103	3.1206	7.3461
A3	3	9.2333	1.02144	.58973	6.6959	11.7707
Total	9	8.7222	2.91023	.97008	6.4852	10.9592
A1	3	15.5333	.60277	.34801	14.0360	17.0307
A2	3	14.3000	.45826	.26458	13.1616	15.4384
A3	3	4.2000	.20000	.11547	3.7032	4.6968
Total	9	11.3444	5.39910	1.79970	7.1943	15.4946
A1	3	1.3067	.06658	.03844	1.1413	1.4721
A2	3	1.2567	.06658	.03844	1.0913	1.4221
A3	3	1.2067	.06658	.03844	1.0413	1.3721
Total	9	1.2567	.07211	.02404	1.2012	1.3121
A1	3	7.9700	.77544	.44770	6.0437	9.8963
A2	3	8.8633	.04041	.02333	8.7629	8.9637
A3	3	6.8633	.40550	.23412	5.8560	7.8707
Total	9	7.8989	.97195	.32398	7.1518	8.6460

A1	3	52.8567	.67988	.39253	51.1678	54.5456
A2	3	56.5467	1.14269	.65973	53.7081	59.3853
A3	3	62.5633	1.74792	1.00916	58.2213	66.9054
Total	9	57.3222	4.38295	1.46098	53.9532	60.6913

Descriptives

		Minimum	Maximum
MC	A1	9.70	11.20
	A2	13.00	14.70
	A3	14.70	18.10
	Total	9.70	18.10
AC	A1	11.30	12.10
	A2	4.40	6.10
	A3	8.50	10.40
	Total	4.40	12.10

CFIBREC	A1	14.90	16.10
	A2	13.90	14.80
	A3	4.00	4.40
	Total	4.00	16.10
CFATC	A1	1.23	1.35
	A2	1.18	1.30
	A3	1.13	1.25
	Total	1.13	1.35
CPC	A1	7.44	8.86
	A2	8.84	8.91
	A3	6.47	7.28
	Total	6.47	8.91
CHC	A1	52.42	53.64
	A2	55.28	57.50
	A3	60.61	63.98
	Total	52.42	63.98

ANOVA						
		Sum of Squares	df	Mean Square	F	Sig.
MC	Between Groups	42.669	2	21.334	12.965	.007
	Within Groups	9.873	6	1.646		
	Total	52.542	8			
AC	Between Groups	63.902	2	31.951	49.751	.000
	Within Groups	3.853	6	.642		
	Total	67.756	8			
CFIBREC	Between Groups	231.976	2	115.988	567.332	.000
	Within Groups	1.227	6	.204		
	Total	233.202	8			
CFATC	Between Groups	.015	2	.008	1.692	.261
	Within Groups	.027	6	.004		
	Total	.042	8			
CPC	Between Groups	6.023	2	3.011	11.773	.008

	Within Groups	1.535	6	.256		
	Total	7.557	8			
CHC	Between Groups	144.036	2	72.018	44.795	.000
	Within Groups	9.646	6	1.608		
	Total	153.682	8			

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Apbwbt	CONTROL	3	137.7333	10.31392	5.95475	112.1121	163.3545	129.72	149.37
	10mg/kg	3	115.3600	10.49800	6.06103	89.2815	141.4385	106.11	126.77

	100mg/kg	3	138.5533	5.10206	2.94568	125.8791	151.2276	133.03	143.09
	1000mg/kg	3	148.0633	3.94229	2.27608	138.2701	157.8565	145.11	152.54
	1500mg/kg	3	175.4700	.97340	.56199	173.0519	177.8881	174.37	176.22
	2900mg/kg	3	186.3767	.91871	.53042	184.0945	188.6589	185.34	187.09
	5000mg/kg	3	186.7667	1.13072	.65282	183.9578	189.5755	185.66	187.92
	Total	21	155.4748	26.75805	5.83909	143.2946	167.6549	106.11	187.92
Apwtat	CONTROL	3	140.2033	11.32037	6.53582	112.0820	168.3247	131.57	153.02
	10mg/kg	3	130.8767	6.45500	3.72680	114.8416	146.9118	123.50	135.49
	100mg/kg	3	141.2167	4.16098	2.40234	130.8802	151.5531	136.43	143.97

	1000mg/kg	3	152.0467	4.28628	2.47469	141.3989	162.6944	147.26	155.53
	1500mg/kg	3	.0000	.00000	.00000	.0000	.0000	.00	.00
	2900mg/kg	3	175.3000	1.03591	.59808	172.7267	177.8733	174.11	176.00
	5000mg/kg	3	187.3100	.85035	.49095	185.1976	189.4224	186.36	188.00
	Total	21	132.4219	58.80104	12.83144	105.6560	159.1878	.00	188.00
Apdwt	CONTROL	3	2.4700	1.02235	.59025	-.0697	5.0097	1.85	3.65
	10mg/kg	3	15.5167	12.12787	7.00203	-14.6106	45.6440	6.87	29.38
	100mg/kg	3	2.6633	2.22828	1.28650	-2.8720	8.1987	.16	4.43
	1000mg/kg	3	3.9833	5.57450	3.21844	-9.8645	17.8312	.72	10.42
	1500mg/kg	3	.0000	.00000	.00000	.0000	.0000	.00	.00

	2900mg/kg	3	11.0767	.16042	.09262	10.6782	11.4752	10.91	11.23
	5000mg/kg	3	1.0167	1.14605	.66167	-1.8303	3.8636	.35	2.34
	Total	21	5.2467	6.97712	1.52253	2.0707	8.4226	.00	29.38
CBWBT	CONTROL	3	137.7333	10.31392	5.95475	112.1121	163.3545	129.72	149.37
	10mg/kg	3	158.6667	.49662	.28672	157.4330	159.9003	158.37	159.24
	100mg/kg	3	159.6367	.20108	.11609	159.1372	160.1362	159.47	159.86
	1000mg/kg	3	162.0267	7.56739	4.36904	143.2282	180.8251	153.52	168.01
	1500mg/kg	3	198.1600	.43278	.24987	197.0849	199.2351	197.67	198.49
	2900mg/kg	3	203.3533	.60583	.34978	201.8484	204.8583	202.73	203.94
	5000mg/kg	3	207.4667	1.00162	.57828	204.9785	209.9548	206.33	208.22

	Total	21	175.2919	26.16495	5.70966	163.3818	187.2020	129.72	208.22
CBWAT	CONTROL	3	140.2033	11.32037	6.53582	112.0820	168.3247	131.57	153.02
	10mg/kg	3	166.6167	2.99807	1.73094	159.1690	174.0643	164.29	170.00
	100mg/kg	3	162.6167	11.35938	6.55834	134.3984	190.8349	149.50	169.19
	1000mg/kg	3	174.8833	5.43597	3.13846	161.3796	188.3870	170.17	180.83
	1500mg/kg	3	210.3833	1.09646	.63304	207.6596	213.1071	209.53	211.62
	2900mg/kg	3	219.3433	.78360	.45241	217.3968	221.2899	218.79	220.24
	5000mg/kg	3	213.4633	.62308	.35974	211.9155	215.0112	212.78	214.00
	Total	21	183.9300	29.42599	6.42128	170.5354	197.3246	131.57	220.24

CDWT	CONTROL	3	2.4700	1.02235	.59025	-.0697	5.0097	1.85	3.65
	10mg/kg	3	8.1300	2.44718	1.41288	2.0509	14.2091	5.92	10.76
	100mg/kg	3	9.8400	.22517	.13000	9.2807	10.3993	9.58	9.97
	1000mg/kg	3	12.8567	3.77513	2.17957	3.4787	22.2346	9.10	16.65
	1500mg/kg	3	12.2233	.96443	.55682	9.8275	14.6191	11.21	13.13
	2900mg/kg	3	15.9900	1.02898	.59408	13.4339	18.5461	14.85	16.85
	5000mg/kg	3	5.9967	1.56845	.90554	2.1004	9.8929	4.56	7.67
	Total	21	9.6438	4.60765	1.00547	7.5464	11.7412	1.85	16.85
TBWBT	CONTROL	3	137.7333	10.31392	5.95475	112.1121	163.3545	129.72	149.37
	10mg/kg	3	170.0300	.53113	.30665	168.7106	171.3494	169.67	170.64

	100mg/kg	3	174.3400	3.43393	1.98258	165.8096	182.8704	171.31	178.07
	1000mg/kg	3	177.6933	.52880	.30530	176.3797	179.0070	177.33	178.30
	1500mg/kg	3	162.4500	.69087	.39887	160.7338	164.1662	161.86	163.21
	2900mg/kg	3	189.1000	1.59603	.92147	185.1353	193.0647	187.26	190.11
	5000mg/kg	3	184.2233	.92116	.53183	181.9350	186.5116	183.21	185.01
	Total	21	170.7957	16.52535	3.60613	163.2735	178.3180	129.72	190.11
TBWAT	CONTROL	3	140.2033	11.32037	6.53582	112.0820	168.3247	131.57	153.02
	10mg/kg	3	178.0167	4.99666	2.88482	165.6043	190.4291	172.26	181.23
	100mg/kg	3	183.8467	2.86709	1.65532	176.7244	190.9689	181.55	187.06

	1000mg/kg	3	183.9333	4.55533	2.63002	172.6173	195.2494	180.63	189.13
	1500mg/kg	3	167.4700	.76740	.44306	165.5637	169.3763	166.59	168.00
	2900mg/kg	3	195.6167	1.07784	.62229	192.9392	198.2942	194.77	196.83
	5000mg/kg	3	189.2067	.99551	.57476	186.7337	191.6796	188.23	190.22
	Total	21	176.8990	17.99804	3.92749	168.7064	185.0917	131.57	196.83
TDWT	CONTROL	3	2.4700	1.02235	.59025	-.0697	5.0097	1.85	3.65
	10mg/kg	3	7.9867	4.77127	2.75469	-3.8658	19.8392	2.48	10.89
	100mg/kg	3	9.5067	.65256	.37676	7.8856	11.1277	8.99	10.24
	1000mg/kg	3	6.2400	4.94592	2.85553	-6.0463	18.5263	2.33	11.80
	1500mg/kg	3	5.0200	.84870	.49000	2.9117	7.1283	4.31	5.96

	2900mg/kg	3	6.5167	2.66478	1.53851	-.1030	13.1363	4.66	9.57
	5000mg/kg	3	4.9833	1.90867	1.10197	.2419	9.7247	3.22	7.01
	Total	21	6.1033	3.26473	.71242	4.6172	7.5894	1.85	11.80

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Apbwbt	Between Groups	13797.411	6	2299.568	61.620	.000
	Within Groups	522.456	14	37.318		
	Total	14319.867	20			
Apwtat	Between Groups	68736.637	6	11456.106	386.844	.000
	Within Groups	414.600	14	29.614		
	Total	69151.237	20			
Apdwt	Between Groups	602.585	6	100.431	3.790	.019

	Within Groups	371.020	14	26.501		
	Total	973.605	20			
CBWBT	Between Groups	13361.120	6	2226.853	94.194	.000
	Within Groups	330.974	14	23.641		
	Total	13692.094	20			
CBWAT	Between Groups	16721.922	6	2786.987	65.482	.000
	Within Groups	595.858	14	42.561		
	Total	17317.780	20			
CDWT	Between Groups	373.037	6	62.173	16.878	.000
	Within Groups	51.570	14	3.684		
	Total	424.608	20			
TBWBT	Between Groups	5216.536	6	869.423	49.639	.000
	Within Groups	245.208	14	17.515		
	Total	5461.743	20			
TBWAT	Between Groups	6108.926	6	1018.154	38.560	.000
	Within Groups	369.661	14	26.404		

	Total	6478.587	20			
TDWT	Between Groups	92.845	6	15.474	1.800	.171
	Within Groups	120.325	14	8.595		
	Total	213.170	20			

Post Hoc Tests

Homogeneous Subsets

Apbwbt					
Duncan[a]					
Group	N	Subset for alpha = 0.05			
		1	2	3	4
10mg/kg	3	115.3600			
CONTROL	3		137.7333		
100mg/kg	3		138.5533		

1000mg/kg	3		148.0633		
1500mg/kg	3			175.4700	
2900mg/kg	3				186.3767
5000mg/kg	3				186.7667
Sig.		1.000	.068	1.000	.939

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 3.000.

Apwtat

Duncan[a]

Group	N	Subset for alpha = 0.05					
		1	2	3	4	5	6
1500mg/kg	3	.0000					
10mg/kg	3		130.8767				
CONTROL	3		140.2033	140.2033			

100mg/kg	3			141.2167			
1000mg/kg	3				152.0467		
2900mg/kg	3					175.3000	
5000mg/kg	3						187.3100
Sig.		1.000	.054	.823	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 3.000.

Apdwt

Duncan[a]

Group	N	Subset for alpha = 0.05		
		1	2	3
1500mg/kg	3	.0000		
5000mg/kg	3	1.0167		
CONTROL	3	2.4700	2.4700	

100mg/kg	3	2.6633	2.6633	
1000mg/kg	3	3.9833	3.9833	
2900mg/kg	3		11.0767	11.0767
10mg/kg	3			15.5167
Sig.		.404	.079	.309