pp. 49-65 https://dx.doi.org/10.4314/johasam.v7i2.5

Histological and Biochemical Evaluation of *Juglans Regia* L (Walnut) as Potential Remedial Properties on the Lungs and Liver Tissues of Albino Rats Pre-Treated with Methotrexate

Nosa Terry Omorodion[1], Godfrey Innocent Iyare[2], & Barisuka Kiofi[3]
Department of Medical Laboratory Sciences, College of Medicine University of Benin, Benin City, Nigeria[2]
Department of Medical Laboratory Science, Faculty of Applied Health Sciences, Edo State University, Uzairue[1]
River State College of Health Science and Management Technology, Port Harcourt, Rivers State, Nigeria[3]
Correspondence: Omorodion Nosa Terry / 08136742270 / Department of Medical Laboratory Science, School of Basic Medical Sciences, College of Medical Sciences, University of Benin, Benin City, Edo State, Nigeria/E-mail - terry.omorodion@uniben.edu/Orcid ID: 0000-0002-5500-5293

Abstract

A multipurpose tree species known for its nutritional, medicinal, and commercial worth is *Juglans regia Linn*. Higher dosages of the multipurpose medicine methotrexate can be hazardous to the liver. It is a widely used anti-rheumatic therapy. The liver and lungs of Wistar albino rats that had been pretreated with methotrexate were studied to see what effect Juglans regia Linn had and what benefits it might have. Five groups (control, negative, positive, and counter groups) of twenty-five Wistar albino rats were used in the study design. The assessment included histological alterations and hepatic biomarkers (globulin, albumin, TP, CB, ALP, and ALT)by which method. To evaluate the obtained data, SPSS version 20, a statistical programme for social sciences, was utilised. When comparing the groups, the biomarkers did not reveal any discernible differences at $p<0.05$. The experimental animals in group B showed modest structural abnormalities in their liver tissue and an inflammatory effect on their lung tissue in histopathological findings. On the hepatic and pulmonary tissues, groups A and C showed no effects. Notwithstanding, there were minor structural alterations noted in the liver tissue of group D. Since the liver and lungs in group E showed no reaction to the medication, it is possible that the extract could offset the drug's effects at greater dosages.This study suggests that Juglan regia Linn could amelioratethe liver and lung effect of methotrexate in Wistar albino rats.

Keywords: lung, liver, *Juglans regia*, methotrexate

Introduction

Walnuts (Juglans regia L.) are a valuable commodity that is native to the region of Eurasia that stretches from the Near East to the Himalayas and Western China. Walnuts are grown all over the world now, having started in Europe in the 1500s (Rogers, 2004). According to Rusu et al. (2019), walnuts (Juglans regia L.) are a significant crop grown for their nutritious nuts, which are essential to diets that promote long life. Furthermore, it was determined through characterization and demonstration that certain by-products of walnuts, such as leaves or green husks, are good sources of bioactive molecules, such as phenolic compounds and tocopherols (Santos, 2013). Walnuts are rich in phytochemicals; quinines, oils, phospholipids, sphingolipids, phenols, sterols, tannins, terpenes, and essential fatty acids are just a few of the many compounds found in walnut **(**Sajeeda et al., 2022**)**. There have been reports of an effective self-tanning agent found in the aqueous extract obtained from the fresh green walnut shells (Sajeeda et al., 2022). By lowering oxidative stress and preventing macromolecular oxidation,

phytochemicals like phenolic compounds are thought to be good for human health and reduce the risk of degenerative disorders (Pereira et al., 2007). Furthermore, it has been demonstrated that J. regia L. extracts prevent oxidative damage, inflammation, photoaging, and cancer formation (Sajeeda et al., 2022). There's a plentiful supply of leaves.

The lungs are two spongy, air-filled organs that are situated on either side of the chest (thorax). Two membranes called pleurae surround each lung, and a layer of pleural fluid separates them from one another. This permits the inner and outer membranes to glide over one another with little resistance when breathing. A portion of the foregut gives rise to the liver. During the fourth week of development, it begins as the hepatic diverticulum and is derived from endodermal cells. Forming inside the peritoneum, the falciform ligament, originating from the ventral mesentery, serves as its anchor to the abdominal wall.

For many rheumatic and non-rheumatic disorders, methotrexate is the first-line medication treatment. It is effective at reducing disease activity and averting disease-related damage, in addition to being significantly less expensive than many alternatives (Conway & Carey, 2017). In order to treat autoimmune diseases, methotrexate is a commonly used immunosuppressive and antineoplastic medication (Ozgocmen et al., 2022). Methotrexate is a chemotherapeutic and immunosuppressive drug that has nephrotoxicity and hepatotoxicity side effects that could be fatal. Because of this, it is not used as much to treat autoimmune diseases and other types of cancer. Cancer patients' overall prognosis may be jeopardised by this hepatotoxicity, which can cause liver damage. Thus, it is necessary to investigate alternative therapies that can improve patient safety during chemotherapy and lessen the hepatotoxic effects of methotrexate.

The fact that it has been used for a long time to treat other systemic immunoinflammatory rheumatic disorders is more proof that it is safe and works (Malaviya, 2020). The suppression of polyamines by methotrexate may also have anti-inflammatory effects. The way that methotrexate works against inflammatory disorders and produces some of its well-known side effects has been explained by the biological effects on inflammation linked to adenosine release (Chan & Crostein, 2010).

Bioactive substances with antioxidant qualities have been found in walnut leaves. The oxidative stress that the drug causes is one of the processes underlying methotrexate's side effects. Researching how methotrexate-treated rats' oxidative stress markers respond to walnut leaf extract can reveal information about the extract's possible antioxidant qualities. It could be helpful to mitigate the negative effects of methotrexate if the extract has antioxidant properties, as seen by a decrease in oxidative stress indicators. This study aimed to assess the possible therapeutic effects of Juglans regia L (walnut) on the lung and liver tissues of albino rats that had received methotrexate prior to treatment.

Materials and Methods

Location and Duration of the Study

The current study was carried out at the Histopathology Sub-Departmental Laboratory, which is housed under the School of Basic Medical Sciences at the College of Medical Sciences, University of Benin, Benin City. The laboratory is a part of the Department of Medical Laboratory Science. The study was conducted between January 31, 2023, and June 30, 2023.

Reagents and chemicals

Scott's water, distilled water, xylene, ethanol, dispersion plasticizer, 1% acid-alcohol, haematoxylin dye, eosin dye, 1% acid-alcohol, and normal saline were some of the chemicals used in this study. It is important to note that all reagents were distilled prior to their use in the experiment.

Equipment and apparatus

The laboratory equipment used in this experiment includes an analytical weighing balance, plastic cages, and various dissecting supplies like a dissecting board, dissecting set, cotton wool, gauze, and husks. The subject of discussion is glassware, measuring cylinder, conical flask, cover slip, slides, universal containers, and 5 mL syringes. A Hestion ATP7000 tissue processor from Germany, an embedding machine (Hestion E500), Leuckhartmoulds, a digital rotary microscope (Hestion ERM 4000 from Germany), a water bath (Gallenkamp), a hot plate, a muslin cloth, a staining rack, a soxhlet extractor, forceps, a Swift (R) binocular soxhlet extractor, an Olympus England microscope, and a digital electronic balance (Gilbertini, Italy; sensitivity = 0.001 g) are among the supplies used for tissue processing, spectrophotometer, cuvette.

Drug (Methotrexate) Procurement

The drug methotrexate was gotten from a pharmacy in Benin City with my ethical approval.

Collection and Preparation of Plant Materials

The leaves were from a tree in the Edo State local government area of Owan East, specifically in the hamlet of Sabongida Ora. Both the stems and the leaves were carefully cut off. The University of Benin's Plant Biology and Biotechnology (PBB) department identified the species Plukenetiaconophora (Mull. Arg), popularly known as walnut (Julgan regia L.). This particular species of walnut is native to West Africa. Prof. Aigbokhan Emmanuel Izaka was in charge of the identification. Specimens of the foliage were submitted to the herbarium of the department, where they were assigned a voucher number (UBH-P402). The leaves underwent a drying process in an oven set at a temperature of 40 degrees Celsius. The leaves were homogenised using a standard kitchen blender, resulting in the attainment of a consistent and homogeneous texture.

Extraction

Clamps and supports were used to build a rig that would support the extraction process. 250 ml of ethanol were added to a round-bottom flask, and this flask was then linked to a condenser and Soxhlet extraction devices that were both fixed on an isomantle. The combined leaves were placed into the thimble, a cylindrical container that is then placed within the Soxhlet extractor. Glass wool was used as insulation on the side arm. The isomantle device was used to apply heat to the solvent, causing it to evaporate and then pass through the apparatus and into the condenser. The condensate then flows downhill into the reservoir where the thimble is located. The solvent was then refilled into the flask to begin the cycle over again after it reached the syphon's level. The process was carried out for sixteen hours.

Freeze Drying

The ethanol that was obtained was sent to a research facility located at the University of Benin. The ethanol that was obtained was subjected to freeze-drying using a SCIENTZ-12N laboratory lyophilizer. The plant extracts were subjected to freezing, resulting in the formation of a semisolid state.

Animal Care

A total of twenty (20) rats This study utilised adult Wistar albino rats, characterised by an average weight ranging from 140 to 200 g. The rats were acquired and housed within the animal facility located in the anatomy department of the University of Benin. The rats were divided into five groups, with each group consisting of four rats. The rats were housed in sanitary, well-ventilated plastic enclosures and provided with uncontaminated water and rat feed (specifically, grower's mash feed obtained from a nearby supplier). The animals had a two-week acclimation phase in the laboratory, during which they were exposed to temperatures ranging from 25 to 30 degrees Celsius. This acclimation period took place prior to the initiation of the experiment at the animal house.

Acute Toxicity

The assessment of acute toxicity in this study was conducted using Lorke's approach. The rats were divided into five groups, with each group consisting of two rats. Each group was subjected to varying amounts of the extract, specifically 2000mg, 1000mg, 500mg, 350mg, and 250mg, during a 24-hour period. Various symptoms were monitored, including vomiting, weakness, and potentially fatal outcome.

Experimental Design

The animals were individually chosen using a hand towel and subsequently administered an appropriate dosage of Julgans regia L. leaf extract orally through an orogastric tube for a duration of 14 days in accordance with the LD50. Just food and purified water were given to Group A, which was supposed to be the control group. One dosage of 20 mg/kg of methotrexate was given once a day to the negative control group, also known as Group B.

250 mg/kg of the extract was given as a single dosage to the experimental group, designated as Group C, which functioned as the positive control. For the first 7 days, the participants in Group D received a dosage of 20 mg/kg of methotrexate once daily. For the following 7 days, they received a dosage of 350 mg/kg of extract. In Group E, the subjects were administered a dosage of 20 mg/kg of methotrexate once daily for a duration of 14 days, along with an oral intake of 500 mg/kg of extract for the same duration.

Excision of tissues

At the end of the experiment, the animals were observed to evaluate their physical characteristics, and a scale was used to determine their weight. Two animals per group were put down via cervical dislocation exactly twenty-four hours following the last day of treatment. The liver and lungs were surgically removed from all experimental groups, including the control group, using a sterile surgical blade. The removed organs were promptly immersed in a solution of 10% formal saline for fixation.

Histopathological Examination

Following a 24-hour fixation period, the tissues undergo processing, which involves the first fragmentation of each organ into smaller 5mm pieces.

Photomicrography

The sections were examined on a Leica DM750 research microscope that was outfitted with a Leica ICC50 digital camera. The tissue sample was imaged using magnifications of x40 and x100, resulting in a digital photomicrograph.

Biochemical analyses

The blood samples were collected from the heart chamber and placed in a conventional sample container. In order to get the serum for examination, the blood sample was subjected to centrifugation at a rate of 3000 revolutions per minute (rpm) for a period of 5 minutes.

The liver function test

The present study aims to evaluate liver enzymes. The Reitman-Frankel kit, containing all essential reagents, was employed in accordance with the recommended protocol. The experiment incorporated the use of Alanine aminotransferase (ALT). The experiment involved the combination of a reagent volume of 1 ml with a sample volume of 50 microliters, conducted at a temperature of 37 degrees Celsius. Subsequently, the resultant mixture was subjected to incubation following which the absorbance at a specific wavelength of 550nm was quantified. The experimental approach involved the preparation of a mixture by combining 1 ml of aspartate aminotransferase (AST) reagent (acting as the substrate) and 1 ml of reagent (providing colour) with 0.2 ml of the sample, along with 10 ml of NaOH. The resultant mixture was homogeneously mixed and subsequently subjected to incubation at a temperature of 37 degrees Celsius. Subsequently, the measurement of the mixture's absorbance was conducted at a wavelength of 550nm.

Statistical Analysis

The weight values were expressed using the statistical measure of the mean plus or minus the standard deviation. The statistical significance of the findings was evaluated by utilising analysis of variance (ANOVA). Statistical significance was established by employing a threshold of p-values below 0.05. The statistical analysis was performed using Statistical Packages for Social Sciences (SPSS) version 20.

RESULT

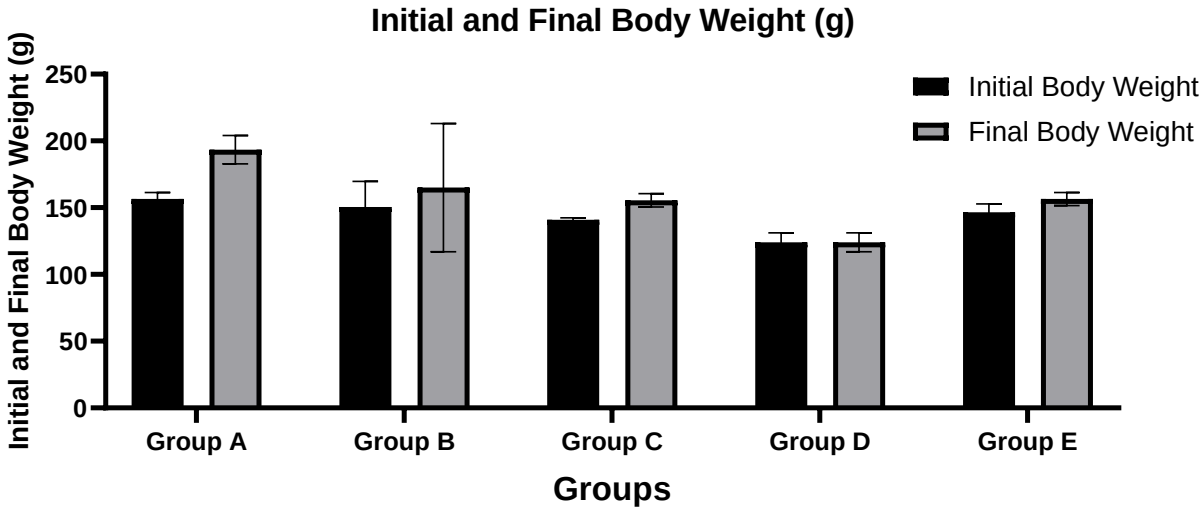
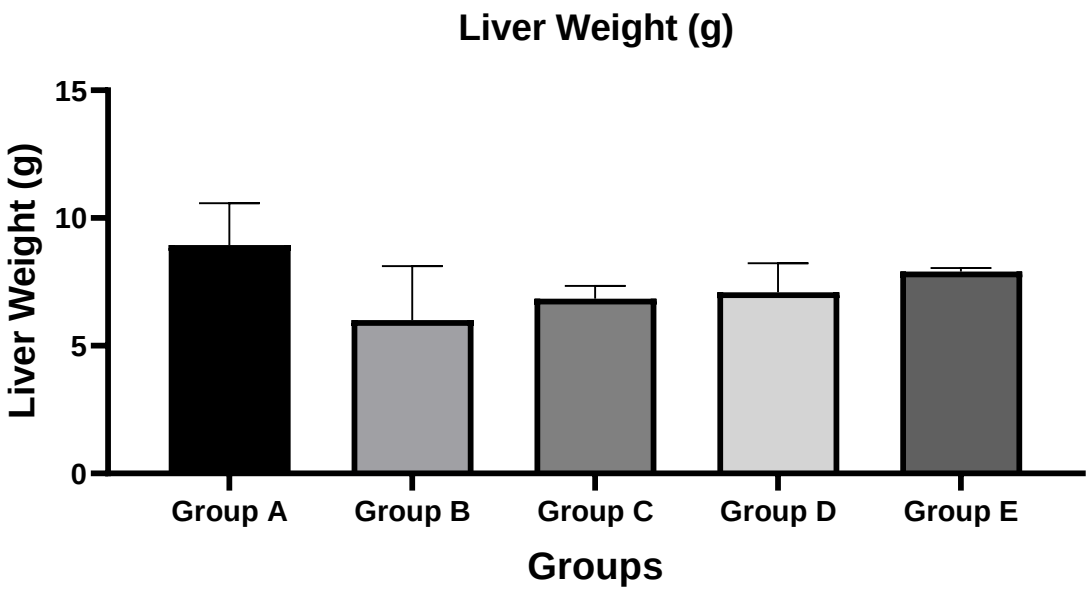
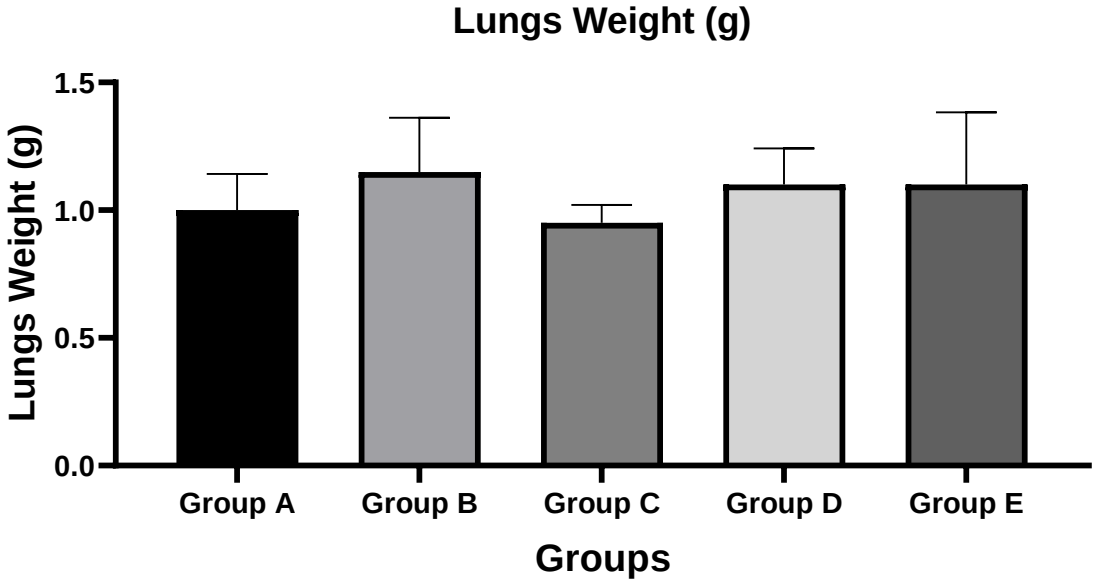


Figure 1: Comparing each group's starting and ending body weight. When comparing the starting and final body weights (g) of each group to the control group, there was no statistically significant difference ($P<0.05$).

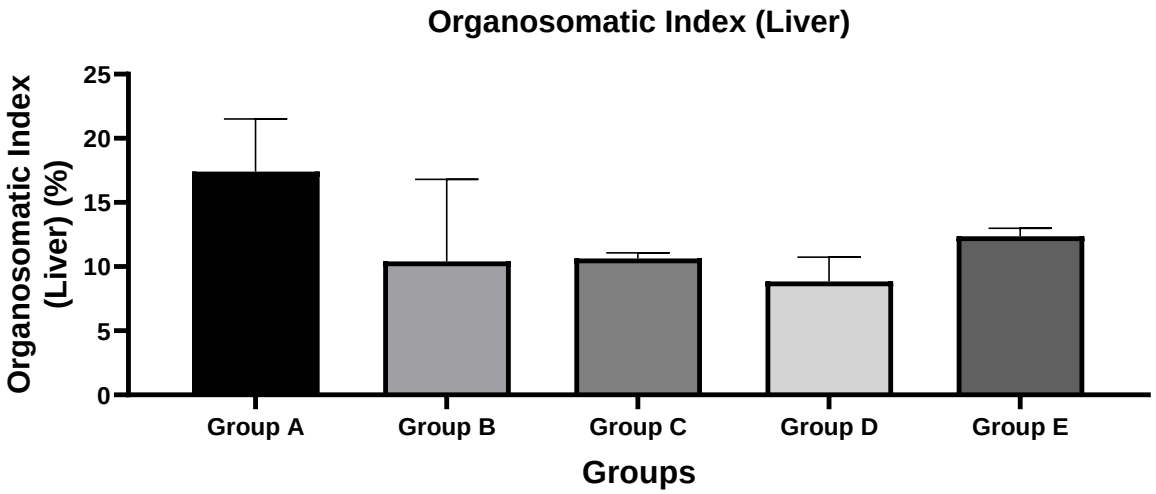


Liver weight for each group is displayed in Fig 2

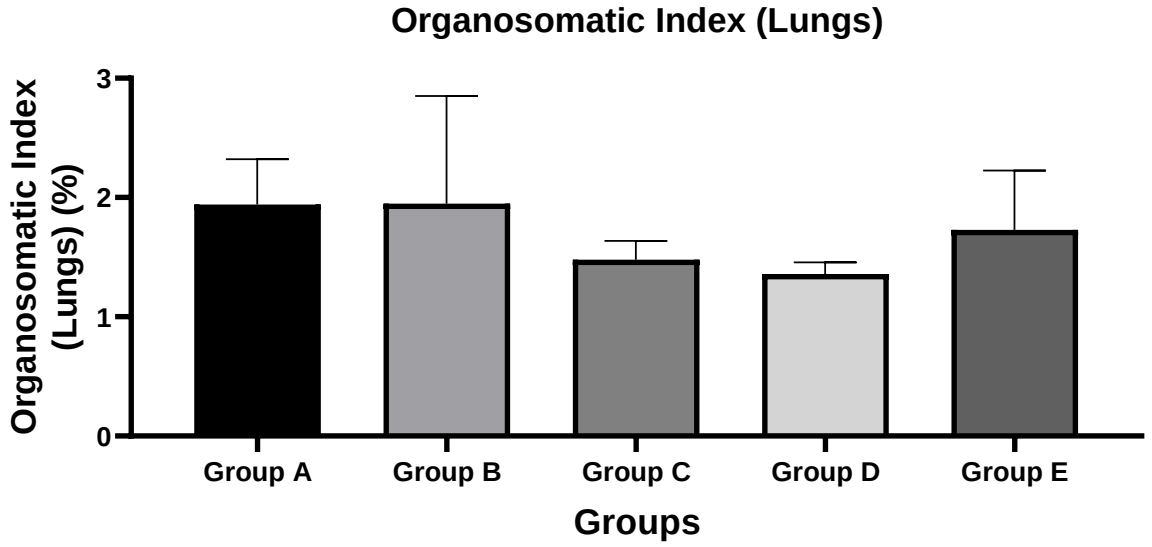
When the liver weight (g) of each group was compared to the control group, there was no statistically significant difference ($P<0.05$) seen.
Crucials:



Lung weight for each group is displayed in Fig. 3.
When comparing the lung weight (g) of each group to that of the control group, there was no statistically significant difference ($P<0.05$).



The liver organo-somatic index for each group is displayed in Figure 4.
When comparing the liver organo-somatic index (%) for each group to the control group, there was no statistically significant difference ($P<0.05$).



The organo-somatic index of the lungs for each group is displayed in Fig. 4.
When comparing each group's Lungs organo-somatic index (%) to the control group, there was no statistically significant difference ($P<0.05$) observed.

Liver function test

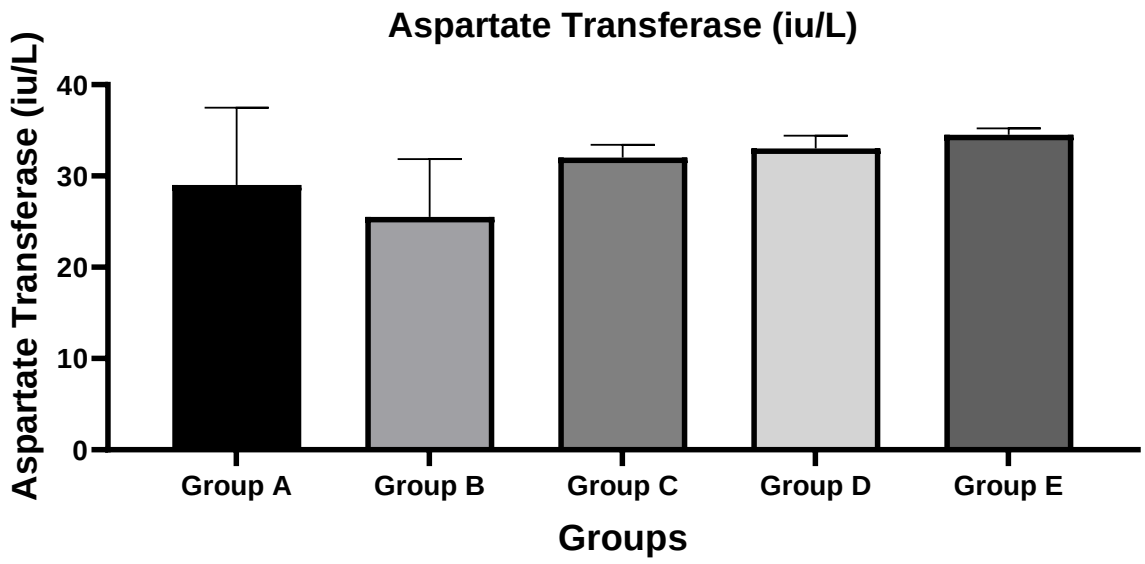
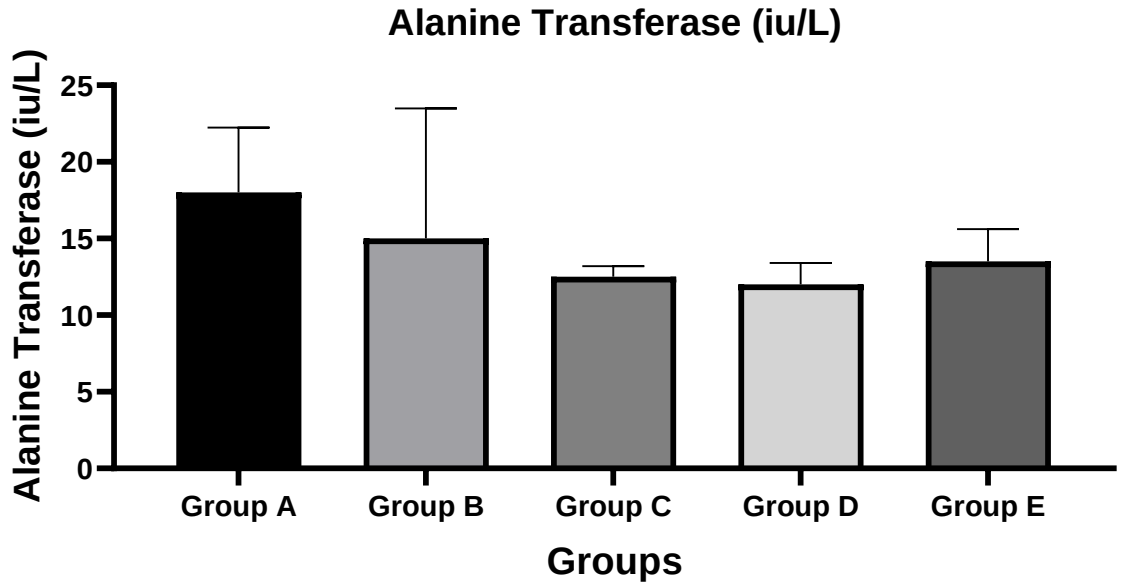
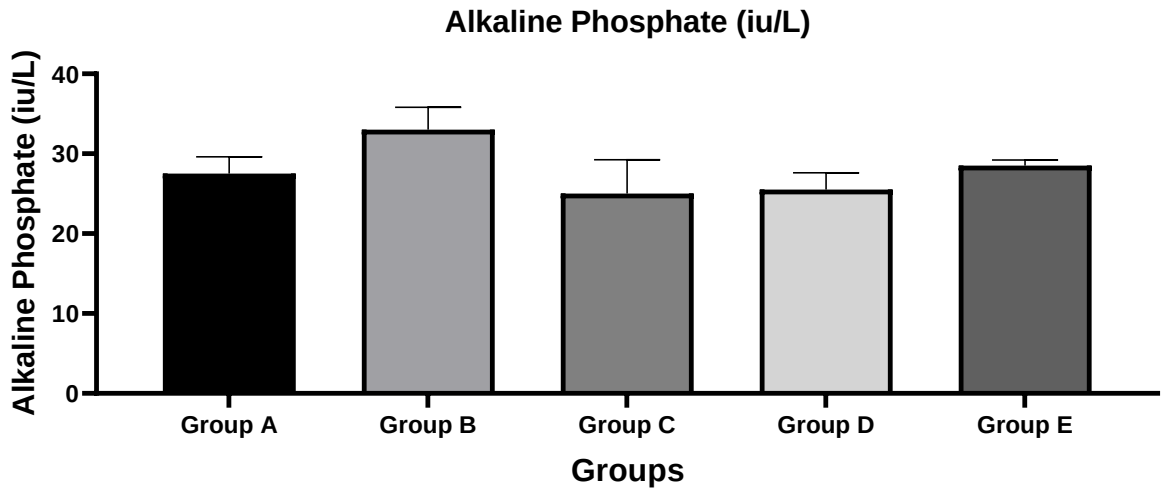


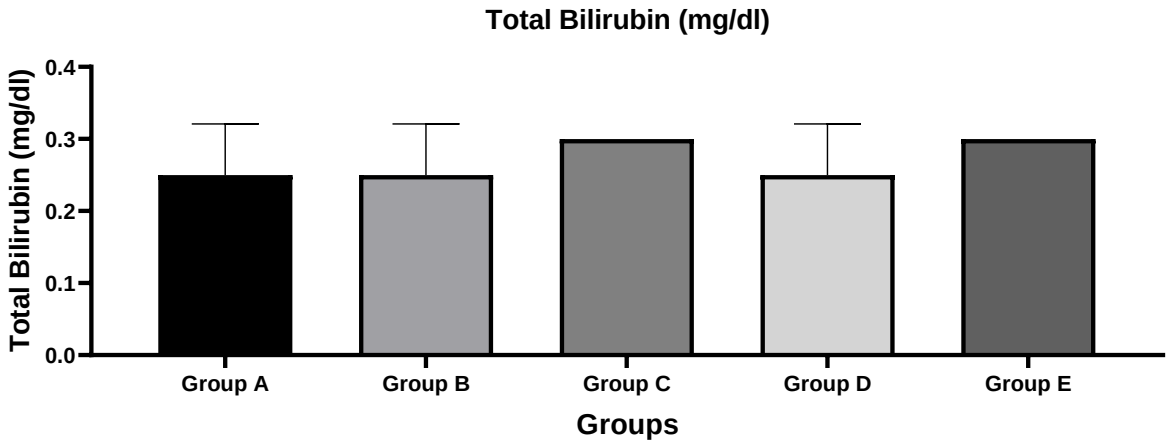
Figure 6 displays the levels of aspartate transferase in each group.
When comparing the Aspartate Transferase levels in each group to those in the control group, there was no statistically significant difference (P<0.05).
Group E will receive 500 mg/kg of extract orally for 14 days in addition to 20 mg/kg of methotrexate once daily.



All groups' levels of Alanine Transferase are displayed in Fig. 7.
Comparing all groups' levels of Alanine Transferase to those of the control group revealed no statistically significant difference (P<0.05).



All groups' alkaline phosphate levels are displayed in Fig. 8.
Comparing the Alkaline Phosphate levels of each group to those of the control group revealed no statistically significant difference ($P<0.05$).



The total bilirubin levels for each group are displayed in Figure 9.
When comparing the total bilirubin levels of each group to those of the control group, there was no statistically significant difference ($P<0.05$).

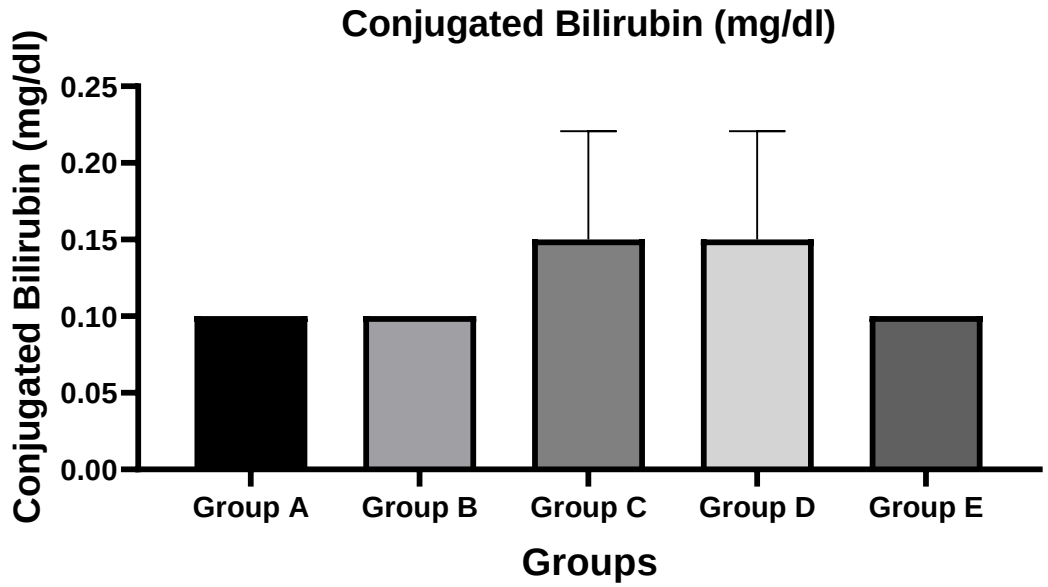


Figure 10 displays the levels of conjugated bilirubin in each group.
When compared to the control group, there was no statistically significant difference ($P<0.05$) in the levels of conjugated bilirubin in any of the categories.

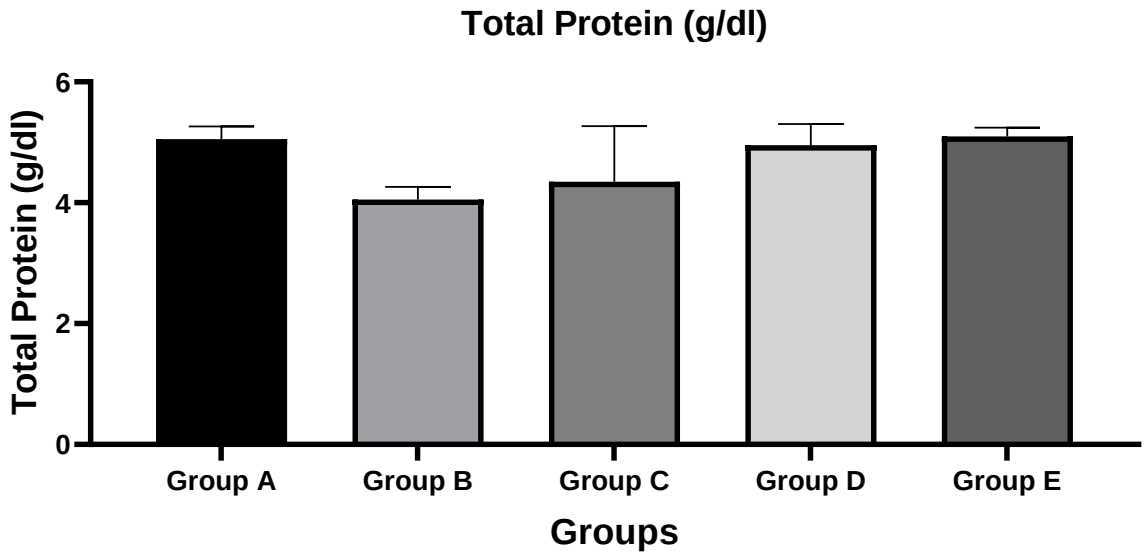
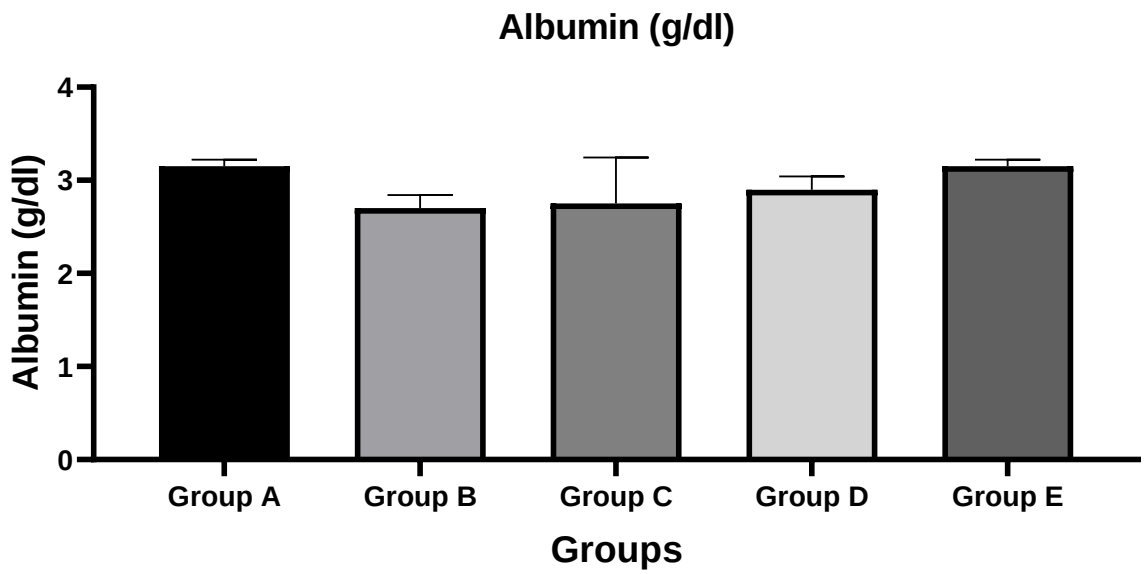


Figure 11: Total Protein Levels in Each Group.
When comparing the amounts of Total Protein in each group to those in the control group, there was no statistically significant difference ($P<0.05$).



All groups' albumin levels are displayed in Fig. 12.
When the albumin levels in each group were compared to the control group, there was no statistically significant difference ($P<0.05$) seen.

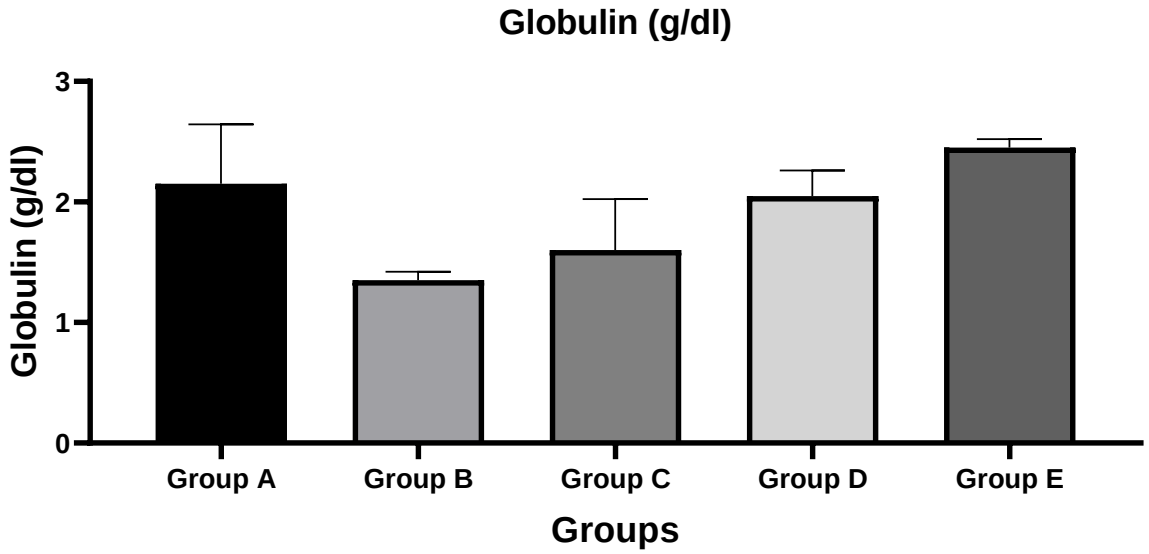


Figure 13: Globulin Levels for Every Group.
When comparing the levels of Globulin in each group to those in the control group, there was no statistically significant difference ($P<0.05$).

Histopathological Analysis

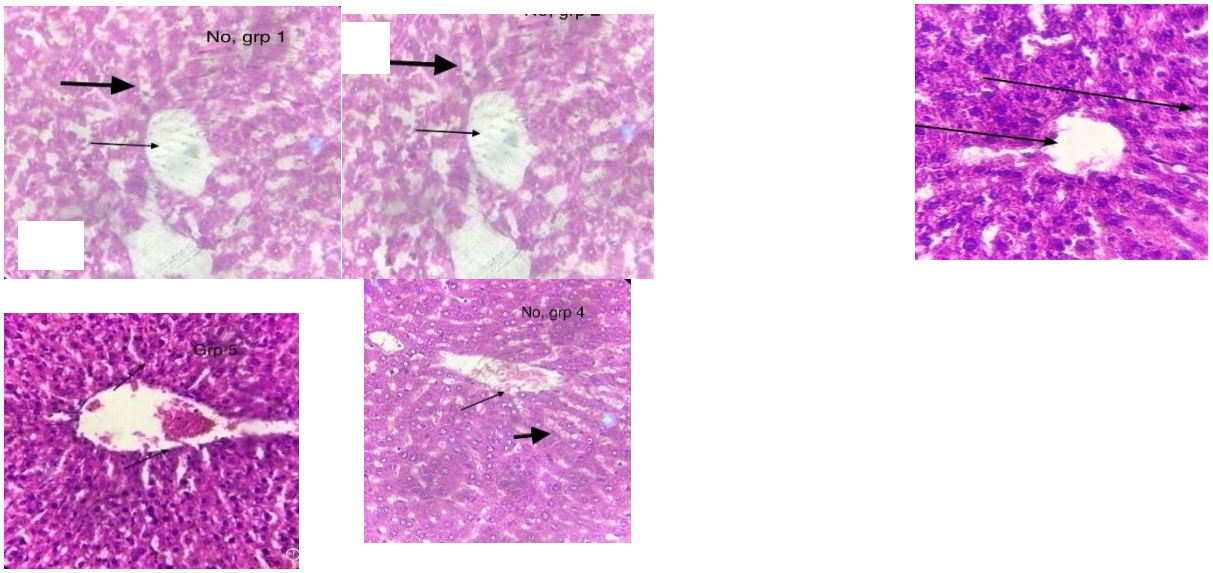
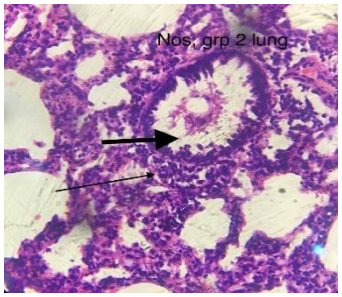


Plate 1: Photomicrographs illustrating the histological characteristics of liver tissue under different experimental conditions. Plate 1 depicts liver tissue samples subjected to various treatments, including rat pellet and distilled water, rat pellet, distilled water, and 20mg of methotrexate, a single dose of 250 mg/kg of Julgans regia extract, and a combination of 20 mg/kg of methotrexate for the first 7 days, followed by 350 mg/kg of Julgans regia extract for the next 7 days. In the first photomicrograph, the liver tissue fed with rat pellets and distilled water exhibits normal features, including a central vein (indicated by a thin arrow), hepatocytes (indicated by a thick arrow), and sinusoids. The second photomicrograph shows slight erosion of the sinusoids while maintaining a normal central vein when the liver tissue is fed rat pellet, distilled water, and 20mg of methotrexate. Moving on, the third photomicrograph demonstrates normal liver features, such as hepatocytes, sinusoids, and a central vein, when the liver tissue is fed a single dose of 250 mg/kg of Julgans regia extract. Finally, the fourth photomicrograph reveals normal central vein and sinusoids, along with microvacuolation of the hepatocytes (indicated by a thick dark arrow), when the liver tissue is fed 20 mg/kg of methotrexate once daily for the first 7 days and 350 mg/kg of Julgans regia extract for the next 7 days. Additionally, the fifth photomicrograph displays normal central vein, sinusoids, and other features (indicated by a thin dark arrow) when the liver tissue is fed 20 mg/kg of methotrexate once daily for 14 days and 500 mg/kg of Julgans regia extract orally for 14 days. The sample was observed under a microscope at a magnification of 400 times using the hematoxylin and eosin staining technique.



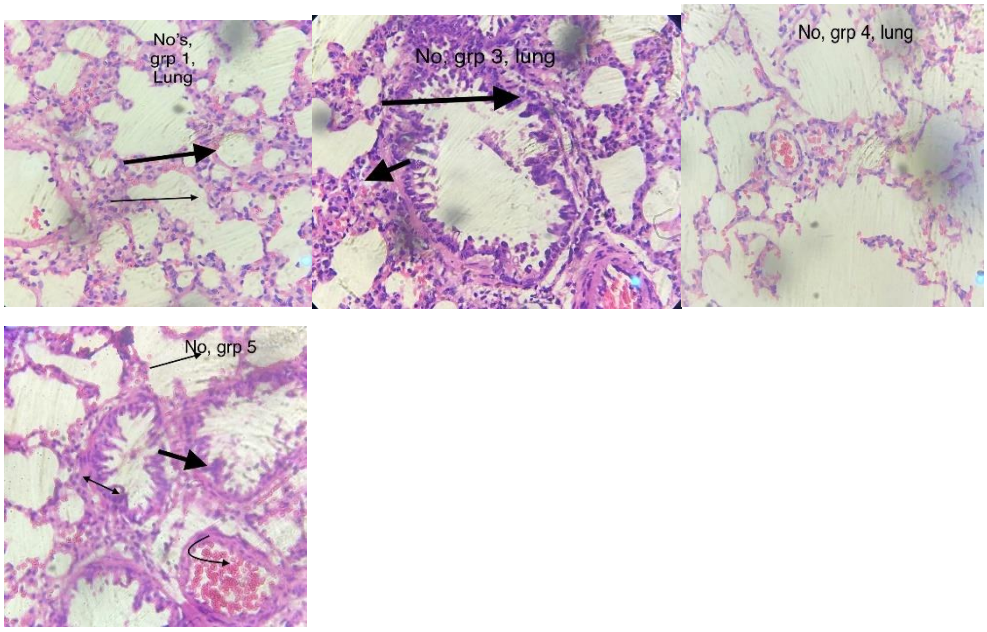


Plate 2: The photomicrograph shows lung tissue that has been fed rat pellets and distilled water. It shows that there are alveoli that look like other alveoli (slide 1). The photomicrograph depicts lung tissue that has been exposed to rat pellets, water, and a dosage of 20mg of methotrexate. The observed findings include mild inflammation of the bronchi, indicative of bronchitis, as well as blockage of the alveoli (slide 2). The photomicrograph presented in this study depicts lung tissue that has been administered a single dose of 250 mg/kg of Julgans regia extract. The image, captured at a magnification of X400 m, showcases the normal anatomical characteristics of the lung, specifically highlighting the bronchi with a long, dark arrow (slide 3). A photomicrograph at a magnification of 400X, utilising hematoxylin and eosin staining, depicts lung tissue that was administered a daily dose of 20 mg/kg of methotrexate for the initial 7 days, followed by a dose of 350 mg/kg of Julgans regia extract for the subsequent 7 days. The image reveals the presence of normal alveoli and bronchioles, as shown by a prominent dark arrow (slide 4). A photomicrograph of lung tissue, magnified at 400 times using hematoxylin and eosin staining, depicts the effects of administering methotrexate at a dosage of 20 mg/kg once daily for a duration of 14 days, along with oral administration of Julgans regia extract at a dosage of 500 mg/kg for the same duration. A thin, dark arrow in the image indicates the presence of normal bronchi, bronchioles, alveoli, and other anatomical features (slide 5). The sample was observed under a microscope at a magnification of 400x using the hematoxylin and eosin staining technique.

Discussion

Notably, discernible differences in the dimensions, pigmentation, and texture of organs were observed between the control group (Group A) and the experimental groups (Groups B, C, D, and E) during the excision process. The rats involved in the trial, specifically those assigned to Groups B and D, exhibited atypical behavioural manifestations, including lethargy, decreased locomotor activity, and agitation. The pulmonary surfaces of all the experimental groups exhibited a normal appearance, with the exception of Group B, which displayed mild inflammation and discoloration. With the exception of Group B, the hepatic surface appeared to be within normal parameters across all other groups. The appearance of Group B is characterised by nodular formations that have a yellowish-tan coloration. The weights of the organs had a rather equal distribution.

The study looked at what happened to the mice's body weight and organ weights when different amounts of methotrexate and Juglans regia L. extract were given to them. The liver, lungs, and beginning and end weight of the animals were assessed following a 14-day duration. The results shown in Statistical Table 4.1 show that there were no statistically significant differences between the treatment groups in the values of these variables. Both the medicine methotrexate and the extract of Juglans regia L. did not seem to change the body and organ weight of the animals used in the study.

The study of biomarkers in liver tissue (see statistical table 4.2) shows that there were no statistically significant differences between the groups in the amounts of aspartate aminotransferase (AST), Alanin aminotransferase (ALT), alkaline phosphatase (ALP), total bilirubin (TB), and conjugated bilirubin (CB) after 14 days of treatment. In addition, there were no statistically significant disparities observed across the groups with regards to the biomarkers globulin, total protein, and albumin. The lack of significant modifications found across the various groups may be related to the duration of the intervention period. It is conceivable that an extended period of time could have led to significant differences between the groups.

Salliot and van der Heijde, (2009), observed and found that 13% of these patients had transaminase levels above twice the upper limit of normal (Salliot and van der Heijde, 2009) which was at variant with our findings. In a recent meta-analysis conducted by Conway et al. (2015), findings revealed that patients treated with methotrexate exhibited a cumulative incidence of liver adverse events at a rate of 11.2%, whereas those receiving alternative treatments displayed a lower incidence of 6.3%. According to Conway et al. (2015), a significant proportion of the observed adverse events were characterised by mild increases in liver enzymes. The incidence rate of these events was found to be 16 per 100 patient years among individuals receiving methotrexate treatment, whereas it was 8 per 100 patient years in the comparison group however, our findings was not in concordance with the aforementioned.

The results of a literature review on liver problems that were confirmed by biopsy showed that 3% of patients who were treated with methotrexate had histological problems one year after getting the treatment. It's important to note that after 4 years of treatment, no histological problems were found in controlled studies with patients who had biopsies done before they were given methotrexate (Salliot and van der Heijde, 2009). As you can see in Plate 4.1, the histopathological slides from Group A show normal liver tissue. These slides show the central vein, hepatocytes, and sinusoids. Additionally, Plate 4.2 displays normal lung tissue, specifically showcasing the alveoli. Figure 4.3 illustrates the liver tissue exposed to a dosage of 20mg of methotrexate (Group B), exhibiting a central vein within normal parameters, however accompanied by erosions of the sinusoids. The alterations occurring in hepatic sinusoids have a pivotal role in the development of liver cirrhosis and portal hypertension, as highlighted by Greuter and Shah (2016). In Plate 4.4 of Group B, the observed lung specimens exhibited mild inflammation of the airways and blockage of the alveoli. According to Mammadov et al. (2019), the elevation of malondialdehyde (MDA) and myeloperoxidase (MPO) concentrations, along with the reduction in glutathione (GSH) levels, have been implicated in the development of lung injury induced by methotrexate. The examination of pulmonary biopsy specimens reveals the presence of alveolar destruction and pulmonary fibrosis, which are accompanied by cellular interstitial infiltrations, granulomas, and peribronchial inflammation. The aforementioned results are not exclusive to methotrexate-induced lung fibrosis, since similar patterns have been observed in instances of pulmonary toxicity linked to alternative medications (Imokawa et al., 2000).

Van Ede *et al*. (1998) examined the pathophysiology of methotrexate-associated pulmonary illness, which is still not fully understood. Several explanations have been put forth to explain the phenomenon, including hyperactive reactions, folate inadequacy, and idiosyncratic reactions. Clinical manifestations such as dyspnea, cough, fever, and shortness of breath may be observed in individuals who experience methotrexate-induced pulmonary toxicity. Symptoms often manifest during the subacute phase, with occasional reports of patients following methotrexate administration. Rales are commonly auscultated in the basal regions of the majority of individuals. Common observations identified on chest radiographs consist of interstitial or alveolar infiltrations in the basal region (Imokawa et al., 2000).

At the moment, methotrexate is widely used to treat psoriasis. However, the fact that pulmonary complications are so rare adds to the evidence for the idea of unique reactions (McKenna and Burrows, 2000). Plate 4.5 exhibits the presence of typical liver characteristics, including sinusoids, hepatocytes, and a central vein, within Group C. Group C exhibits typical characteristics of healthy lung tissue. Plate 4.7 reveals the presence of a normal hepatic central vein, sinusoids, and microvacuolization of the hepatocytes within Group D. According to Aravinthan et al. (2012), hepatocyte vacuolation is believed to be benign and associated with non-alcohol-related fatty liver disease. In their investigation, Eidi *et al*. (2013) documented the hepatoprotective effect of walnut extract in mitigating hepatic injury induced by CCl4 in rats. Also in their study, the administered dosage of CCl4 induced hepatic damage in the rat subjects. The rats that were administered an excessive amount of CCl4 were the ones who exhibited notable hepatic injury, as evidenced by a marked elevation in the levels of serum markers. The rats were subjected to co-treatment with walnut extract at doses of 0.1, 0.2, and 0.4 g/kg body weight, along with carbon tetrachloride (CCl4) for a duration of 28 days. This co-treatment demonstrated a notable protective effect against the rise of blood marker enzymes generated by CCl4. Carbon tetrachloride (CCl4) is a well-established hepatotoxic substance that induces expeditious hepatic injury, characterised by the progression from steatosis to centrilobular necrosis. The chronic infusion of carbon tetrachloride (CCl4) has been extensively recognised as a model for inducing hepatic fibrosis, resulting in long-term liver impairment (Hernandez-Munoz et al., 1990; Pierce et al., 1987). The hepatotoxicity of CCl4 is widely recognised to be a result of its metabolic activation, leading to the selective manifestation of toxicity in liver cells while preserving partial metabolic functionality.

The lung tissue of Group D exhibits a normal histological appearance, characterised by the presence of intact alveoli and bronchioles, as observed in Plate 42 Group E has histological sections of lungs and liver tissue that appear to be within the normal range, as depicted in Plates 2, slide 4. This observation suggests that the effects of methotrexate and walnut leaf extract counteract each other. Contemporary surveillance approaches and therapeutic protocols seem to exhibit notably reduced hazards compared to the past associations linked to the use of methotrexate. Two recent studies of excellent quality which were at variant with our findings have indicated. that 22% of individuals experienced higher levels of transaminases, with as few as 1% exhibiting transaminases that exceeded twice the upper limit of normal (Curtis et al., 2010; Dirven et al., 2013). According to Schmajuk et al. (2014), a greater frequency is observed when the treatment is administered in conjunction with other therapeutic approaches. Several other risk factors for hepatotoxicity have been identified, such as obesity and hypercholesterolemia (Schmajuk et al., 2014).

Conclusion

The protective effects of Julglan regia Linn leaf extract on the liver and lungs against methotrexate-induced damage can be attributed to its nutritional composition, anti-inflammatory properties, antioxidant properties, and bioactive compounds, such as polyphenols, tannins, vitamins, and minerals.

Recommendation

Juglans regia Linn has demonstrated potential effects on pulmonary and hepatic organs. Hence, it is imperative to do additional research in order to enhance comprehension and maybe unveil novel perspectives.

Acknowledgement:

We wish to acknowledge Mr Sam of Anatomy Department, Mr Fred of the Pathology Department, University of Benin

Conflict of interest: None declared

References

Chan, E. S., and Cronstein, B. N., (2010). Methotrexate—how does it really work?. *Nat Rev Rheumatology*, 6(3), 175–178.

Conway, R., Carey J.J. (2017). Methotrexate and lung disease in rheumatoid arthritis. *Panminerva Medicine.* 59:33–46.

Conway, R., Low, C., Coughlan, R. J., O'Donnell, M.J., Carey, J. J.,(2015). Risk of liver injury among methotrexate users: A meta-analysis of randomised controlled trials. *Semin Arthritis Rheum.* 45:156–162.

Conway, R., Carey, J. J., (2017). Methotrexate and lung disease in rheumatoid arthritis. *Panminerva Medicinal.* 59:33–46.

Curtis, J.R., Beukelman, T., Onofrei, A., Cassell, S.,Greenberg, J.D., Kavanaugh, A., Reed, G., Strand, V., Kremer, J.M. (2010). Elevated liver enzyme tests among patients with rheumatoid arthritis or psoriatic arthritis treated with methotrexate and/or leflunomide. *Annals of Rheumatoid Disease.* 69:43–47.

Dirven, L., Klarenbeek, N.B., van den Broek, M., van Groenendael, J.H., de Sonnaville P.B., Kerstens, P.J., Djerassi, I., Farber, S., Abir, E., Neikirk, W. (1967) Continuous infusion of methotrexate in children with acute leukemia. *Cancer.* 20,233–242.

Djerassi, I., Farber, S., Abir, E., Neikirk, W., (1967). Continuous infusion of methotrexate in children with acute leukemia. *Cancer.* 20:233–242.

Edem, C.A., Dosunmu, I.M. and Bassey F.I., (2009). 'Determination of proximate composition, ascorbic acid and heavy metal content of African walnut (*Tetracarpidiumconophorum*). *Pakistan Journal of Nutrition:* 8(3):225–226.

Eidi, A., Zarringhalam, J., Moghadam, P.J., Rezazadeh, S., Olamafar, S. (2013). Hepatoprotective effects of Juglans regiaextract against CCl_4-induced oxidative damage in rats, Phamaceutical biology. 013; 51(5): 558–565

Hernandez-Munoz R, Diaz-Munoz M, Suarez J, Chagoya de Sanches V., (1990). Adenosine partially prevents cirrhosis induced by carbontetrachloride in rats.Hepatology12:242–824

McKleroy, W., Lyn-Kew, K., (2018). 500 Million Alveoli from 30,000 Feet: A Brief Primer on Lung Anatomy. *Methods in Molecular Biology*. 1809:3-15.

Malaviya, A. N., (2020). Does methotrexate cause interstitial lung disease in rheumatoid arthritis: What is the evidence? *International Journal Rheumatic Diseases*: 23(6): 713– 716

Martínez, M., L., Labuckas, D., O., Lamarque, A., L., Maestri, D.,(2010). Walnut (*Juglans regia* L.): genetic resources, chemistry, byproducts. *Journal of the Science of Food and Agriculture 90 (12), 1959-1967.*

Mehrzadi, S., Fatemi, I., Esmaeilizadeh, M., Ghaznavi, H., Kalantar,h., Goudarzi, M., (2018). Hepatoprotective effect of berberine against methotrexate induced liver toxicity in rats. *Biomedicine and Pharmacotherapy*, 97, 233-239

Özgöçmen, M., Aşcı, H., Doğan, H. K., İlhan, İ., Pekgöz, Ş., Mustafaoğlu, A., (2022). A study on Wistar Albino rats: investigating protective role of ramelteon on liver damage caused by methotrexate. *Drug Chemical Toxicology*, 45(6):2678-2685.

Rogers R., (2004). Temperate ecosystems|Juglandaceae. *Encylopedia of Forest Sciences, Elsevier*, 1427-1430.

Rusu, M.E., Mocan, A., Ferreira, I.C.F.R., Popa, D. S., (2019). Health Benefits of Nut Consumption in Middle-Aged and Elderly Population. *Antioxidants (Basel), 8*, 302.

Santos, A., Barros, L., Calhelha, R.C., Dueñas, M., Carvalho, A.M., Santos-Buelga, C., Ferreira, I.C.F.R (2013). Leaves and decoction of *Juglans regia* L.: Different performances regarding bioactive compounds and in vitro antioxidant and antitumor effects. *Industrial Crop Production. 51*, 430–436.

Salliot, C., van der Heijde, D., (2009). Long-term safety of methotrexate immunotherapy in patients with rheumatoid arthritis: a systematic literature research. *Annals Rheumatoid Diseases.* 68:1100–1104.

Sánchez-González, C., Ciudad, C. J., Noé, V., and Izquierdo-Pulido, M., (2017). Health benefits of walnut polyphenols: An exploration beyond their lipid profile. *Critical reviews in food science and nutrition*, *57*(16), 3373–3383.

Schmajuk, G., Miao, Y., Yazdany, J., Boscardin, W.J., Daikh, D. I., Steinman, M.A., (2014). Identification of risk factors for elevated transaminases in methotrexate users through an electronic health record. *Arthritis Care Res (Hoboken).*66:1159–1166

Shah, R. A., Nwannunu, C. E., Limmer, A. L., Patel, R. R., Mui, U. N., and Tyring, S. K. (2019). Brief Update on Dermatologic Uses of Methotrexate. *Skin therapy letter*, 24(6):5–8.

Visser, K., van der Heijde, D. M., (2009). Risk and management of liver toxicity during methotrexate treatment in rheumatoid and psoriatic arthritis: a systematic review of the literature. *Clin ExpRheumatol.,* 27:1017–1025

Wilke, W.S., Biro, J.A., Segal, A. M., (1987). Methotrexate in the treatment of arthritisand connectivee tissuediseases. *Cleve Clinical Journal of Medicine.* 54:327–338.