https://doi.org/10.48047/AFJBS.6.8.2024.1732-1752

African Journal of Biological Sciences

Research Paper Open Access

PROTECTIVE EFFECT OF THE RHIZOME EXTRACT OF *Drynaria quercifolia* (L.) J. Smith. ON CADMIUM CHLORIDE INDUCED HEPATOTOXIC RATS

Prasanna Gopinathan* and Anuradha Ramamoorthy

PG and Research Department of Biochemistry, Sengamala Thayaar Educational Trust Women's College (Autonomous) (Affiliated to Bharathidasan University), Sundarakkottai-614 016, Mannargudi, Thiruvarur Dt, Tamil Nadu, India

*Corresponding Author mail ID: gprasannakerthi08@gmail.com

Article History

Volume 6,Issue 8, 2024
Received:15 Mar 2024
Accepted : 29 Apr 2024
doi: 10.33472/AFJBS.6.8.2024.1732-1752

ABSTRACT

Liver diseases as one of the fatal diseases worldwide, second most common cause of death. Cadmium (Cd) is a heavy metal that naturally occurs, and many methods have suggested that it may be hazardous to the liver. The current study's objective was to assess the potential hepatoprotective effects of *Drynaria quercifolia* rhizome extract using an animal model. Six rats were randomly assigned to each of the five groups: Group I received saline (0.5 ml/kg) as a normal control, Group II received cadmium chloride (3.5 mg/kg/IP/single dose) as a disease control, and Group III received cadmium chloride (3.5 mg/kg/IP/single dosage) intoxicated rats pretreated with rhizome extract of *Drynaria quercifolia* orally (300mg/kg) for 30 days, Group IV- Cadmium chloride intoxicated rats received standard drug silymarin (25 mg/kg), Rats in Group V were given an oral dose of *Drynaria quercifolia* rhizome extract (300mg/kg) for 30 days. The results of biochemical assays and histopathological studies revealed that the plant extract was found to have possible protective effect against the liver damage produced by cadmium chloride at a dosage level of 300 mg/kg, and the results were equivalent to those of the widely used drug silymarin and how well *Drynaria quercifolia* rhizome extract (L) works. Future opportunities of the plant would undoubtedly be numerous. Hence, it can be recommended therapeutically for treating liver disorders. To identify the hepatoprotective bioactive elements in plant extract, more investigation is needed.

Keywords: Cadmium chloride, *Drynaria quercifolia*, Liver diseases, Rhizome extract, Silymarin

INTRODCUTION

Liver diseases are considered as one of the fatal diseases worldwide from 1990 to 2010, number of annual liver cancer and cirrhosis death rate rose by 1.25 to 1.75 million (Asrani *et al*., 2019). In 2010, 45%, and 25% of death occurs due to liver cancer and hepatitis C respectively. Over a million deaths worldwide, or about 2% of all deaths in 2010, were attributed to liver cirrhosis, according to estimates (Asrani *et al*., 2019). In 2012, liver cancer caused 745,000 fatalities (WHO, 2016) (Ferlay *et al*., 2015). Liver cancer, which is second only to lung cancer in terms of causes of mortality worldwide. Alcohol and hepatitis B were less common causes (Iida-Ueno et al., 2017). In India, It is estimated that liver diseases are among the top ten killer diseases (Mondal *et al*., 2022). Lakhs of death may occur due to liver diseases (Asrani *et al*., 2019). Indian deaths from liver disease totaled 216,865 in May 2014, accounting for 2.44% of all deaths, placing India in 61st place globally (Asrani *et al*., 2019).

According to Ferguson (1956), heavy metals are often referred to as metallic elements with high atomic weights that, when measured against water, have a comparatively high density. Examples of such elements are arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb), and mercury (HgAccording to Tchounwou et al. (2012), environmental contamination caused by these metals is becoming a bigger issue for ecological health and worldwide public health. A naturally occurring heavy metal called cadmium (Cd) has been linked to hepatotoxicity through a number of methods (Genchi *et al*., 2020). Reactive oxygen species (ROS) such hydroxyl groups, superoxide radicals, and hydrogen peroxide radicals damage lipid membranes by oxidation, which is one of the potential pathways (Nita *et al*., 2016). Oxidative stress is often brought on by an excess of ROS (Pizzino *et al*., 2017). Because generated Reactive Oxygen Species may deplete antioxidant status, resulting in cell death and tissue damage, they are a significant contributor to the cadmium-induced hepatotoxicity (Branca *et al*., 2020).

Drynaria quercifolia contains a variety of phytochemicals, including phenols, tannins, alkaloids, proteins, xanthoproteins, carboxylic acid, coumarins, saponins, catechin, flavonoids, steroids, and triterpenes (Ramesh *et al*., 2001). According to Mizushina *et al*. (2006), dried rhizomes include the flavones glycoside naringin, aglycone naringinin, beta-amyrin, beta-sitosterol, beta-sitosterol 3-beta-D-glucopyranoside, 3, 4 dihydroxyl benzoic acid, acetyl lupeol, and beta-sitosterol. Several extractives contained high concentrations of phenolic compounds, ranging from 103.43 to 132.23 mg of GAE/g of extractive (Aryal *et al*., 2019). According to Anuja *et al*. (2010), Drynaria quercifolia contained 0.048% naringin and 244 mg/g of total phenolics.

Reported in vitro pharmacological studies were antimicrobial (Balouiri *et al*., 2016). Anti-inflammatory pharmacological actions in vivo were reported (Azza *et al*., 2017). Till date, there is no scientifically proven data to validate the liver protective potential of *Drynaria quercifolia* (L.) J. Smith. rhizome extract. Therefore, the current study was conducted to evaluate the hepatoprotective effect of *Drynaria quercifolia* rhizome extract utilizing an animal model.

MATERIALS AND METHODS

Collection of Plant Material

In the Kollimalai, Namakkal district of the Indian state of Tamil Nadu, rhizomes of *Drynaria quercifolia* (L.) J. Smith was collected. The samples were appropriately stored in polythene bags after being collected. The director of the RAPINAT Herbarium at St. Joseph's College in Tiruchirappalli, Dr. S. John Britto, inspected these plant samples. He then placed a voucher specimen (Voucher No. 001) in the biochemistry department of S.T.E.T Women's College in Mannargudi.

Preparation of the Extract

Brown hair-like microscopic structures cover the rhizome. They were taken out using a sterile scalpel and sterile distilled water. They were reduced to tiny bits, air dried in the shade, pounded into a coarse powder, and then kept in airtight jars. *Drynaria quercifolia* Weighed 500g of rhizome powder was macerated 1:6 in methanol. They were kept at room temperature for 72 hours. A sterile glass rod was used to stir the liquid every 24 hours. Then, Whatmann No. 1 filter paper was used to further filter the mixture. The extraction process was done twice to obtain all bioactive compounds. The resultant filtrate was blended and concentrated under vacuum using a rotary evaporator. The dried residue was stored in a refrigerator for subsequent use.

Maintenance of animals

Male adult Albino Wistar rats weighing 150–200g were selected for this study. They were kept in a tidy polypropylene cage under typical laboratory settings (25 20 C, 12 12 h of darkness and light). They were given a regular pellet diet from Hindustan Lever in Kolkata, India, along with unlimited access to water. Prior to the trial, the animals spent a month becoming used to the laboratory environment. The research was carried out at the Srimad Andavan Arts and Science College in Tiruchirappalli, Tamil Nadu, India. Institutional Animal Ethical Committee (IAEC) examined and approved all of the mentioned procedures (Reg. No. SAC/IAEC/BC/2015/Ph.D.003).

EXPERIMENTAL DESIGN

By randomly assigning rats to each group, five groups of six rats were generated.

Group I: Rats in the control group were given saline (0.5ml/kg).

Group II: Rats used in disease control received a single dosage of 3.5 mg/kg/IP of cadmium chloride (Elgaml *et al*., 2014).

Group III: Rats were given a 300 mg/kg dose of *Drynaria quercifolia* rhizome extract orally for 30 days before receiving a single dose of cadmium chloride (3.5 mg/kg/IP).

Group IV: Rats exposed to cadmium chloride were given the conventional medication silymarin (25 mg/kg) (Senthilkumar *et al*., 2014)

Group V: Rats were administered 300 mg/kg of *Drynaria quercifolia* rhizome extract orally for 30 days.

30 days after the plant extract treatment, Animals were sacrificed by injecting with sodium pentabarbitone and blood was collected in plain and heparinized tubes immediately after sacrifice. The collected blood was centrifuged for 10min at 2500rpm and the serum separated was stored at 4^{o}C until and used for biochemical assays such as determination of body weight and liver weight, blood glucose (Folin – Wu, 1991), glycogen (Seifter *et al*.,1950), protein (Lowry, 1951), A/G ratio (Reichold, 1953), bilirubin (Malloy and

Evelyn,1937); AST (King, 1965), ALT (King, 1965), ALP (King, 1965), ACP (Tennis Wood *et al*., 1976), γ GGT (Rosalki and Rau, 1972). Lactate Dehydrogenase (King, 1965), lipid profile such as Cholesterol (Parekh and Jung, 1970); triglyceride (Foster and Dunn, 1973). Free Fatty Acid (Falholt *et al.,* 1973), phospholipids (Bartlette, 1959). HDL (Friedewald *et al.,* 1972), serum LDL and VLDL (Ohkawa *et al*., 1979), Urea (Natelson *et al.,* 1951), serum uric acid (Caraway and Seligson, 1963), creatinine (Bonsnes and Taussky, 1945).

Liver and kidney were removed and washed with saline and the liver weight of all rats was measured. Liver and kidney tissues were used for histopathological study.

RESULTS AND DISCUSSION

In India, the use of plants as a source of medication is a crucial part of the country's healthcare system. Approximately 45,000 medicinal plants have been used in India, among them, 3,000 are officially documented with medicinal potential. However, some 6,000 botanicals have been employed traditionally by Ayurvedic practitioners. Rural residents rely on the conventional medical system 70% of the time.

The extensive, complex understanding of plants and their products has developed into its own Shastra, known as Dravya Guna Shastra. Rasa (taste), Vipaka (metabolic characteristic), Guna (quality), Prabhava (biological effect), and Virya (potency) are well-defined biological elements in the study of plants and their products. These studies have resulted in the codification of about 25,000 plant extract mixtures. Additionally, it is estimated that there are over 50,000 formulas in folk and tribal traditions. All of these indicate a strong desire to learn everything there is to know about the medicinal plants that have existed in this country since the beginning of time.

Drynaria quecifolia (L.) J. Smith. was gathered from Kollimalai, Namakkal District, Tamil Nadu, for the suggested study. The acquired components were carefully authenticated and identified. The chosen plant, *Drynaria quecifolia*, is a member of the Polypodiaceae family and one of 16 Drynaria species. It is an epiphytic fern that is indigenous to tropical regions of Australia, Asia, and Africa. The plant's rhizomes are 2 cm thick, dark brown in colour, and range in size from 20 to 25 mm and 0.7 to 2.5 mm. Medicinally, this fern is used as carminative, expectorant and tonic, relieve fever, headache and joint pain. Abdominal pain and intestinal worms are treated with the rhizomes and leaves (Nath *et al*., 2016).

Effect of plant extract on body weight and liver weight

During the experiment, the rats' weight gain is solely determined by the availability of foods. Weight loss was seen in the cadmium chloride-treated rats in the current study, which was related to a decrease in food intake (Rafati Rahimzadeh *et al*., 2017). The general increased breakdown of lipids and proteins caused by CdCl2 toxicity resulted in further weight loss (Rafati Rahimzadeh *et al*., 2017). Furthermore, CdCl2 therapy led in selective Cd accumulation in various tissues, particularly the liver. Our findings agreed with certain previously published reports (Bernhoft *et al*., 2013). Adding plant extract to the diet, on the other hand, boosted feed consumption and body weight. Regeneration of liver cell activity was also detected, which normalised liver weight, indicating the efficacy

of the test drug in regulating some aspects such as hunger, digestion, and assimilation in cadmium chloride intoxicated rats. There was no discernible difference between groups I (control) and V (rats treated with plant extract alone).

Effect of plant extract on biochemical parameters

In cadmium chloride-intoxicated rats compared to control, the blood glucose concentration was substantially higher and the liver's glycogen content was much lower. This increased level of glucose may be brought on by cadmium-mediated decrease of insulin production and glucose tolerance (Buha *et al*., 1991). Excess glucose enters hepatocytes and binds to the regulatory site of glycogen phosphorylase a, key enzyme of glycogenolysis causing conformational changes. As a result, breakdown of glycogen to glucose and thereby reduced the level of liver glycogen content and also further enhancement of glucose. In cadmium-intoxicated rats, oral administration of *Drynaria quercifolia* rhizome extract resulted in a significant (P 0.05) increase in liver glycogen content and a decrease in plasma glucose level that was practically comparable to control rats. Rats given the standard therapy had a similar outcome.

Proteins carry out a wide range of processes, including as metabolic processes, DNA replication, and transporting chemicals, among others. The blood total protein/albumin globulin ratio (A/G ratio) in rats treated with CdCl2 decreased significantly (P 0.05). Group II. This was linked to a deficiency in protein synthesis as well as polyribosome disruption and disassociation from the endoplasmic reticulum (Schwarz *et al*., 2016). Pretreatment of plant extract resulted in a significant increase in protein and albumin globulin ratio (A/G ratio), which could be attributed to the assembly of ribosomes on endoplasmic reticulum, which accelerates protein synthesis (Schwarz *et al*., 2016). Rats in the control and plant extract alone treated groups showed no discernible differences.

Effect of plant extract on bilirubin

Bilirubin is an endogenous anion that is released from the liver in the bile as a result of the normal breakdown of haemoglobin from red blood cells. It is a chemical that the liver needs to generate bile and is only detected in trace amounts in blood. When the liver cells are injured, bilirubin can accumulate in the blood and extracellular fluid because they may be unable to discharge it appropriately. If the serum albumin rises and the bilirubin moves from the tissue sites to the circulation, the serum bilirubin level may rise. Jaundice and associated signs of hepatotoxicity can develop from increased levels of bilirubin brought on by a decrease in liver clearance (Grigorian *et al*., 2014).

Total bilirubin, as opposed to ALT, may serve as a more accurate gauge of disease severity in cases of acute human hepatic damage (Torruellas *et al*., 2014). The levels of total and direct bilirubin in group II rats were considerably higher (p 0.05) than those in group I control rats due to hepatocyte degeneration and bile duct obstruction in the current study's rats. Pretreatment of cadmium chloride-intoxicated rats with plant extract restored total and direct bilirubin levels. This response was almost identical to that shown in rats

given the standard medication silymarin (Freitag *et al*., 2015). Bilirubin levels in the plant extract alone treatment group were similar to those in control rats.

Effect of plant extract on liver marker enzymes

Transaminases are key indicators of hepatocellular injury. Alanine Transaminase (ALT) (SGPT) was previously known as Serum Glutamate Pyruvate Transaminase. It is widely dispersed in the liver. In the clinic, ALT levels are determined as part of a diagnostic assessment of hepatocellular damage (Lala *et al*., 2022). Aspartate Transaminase (AST) is a transaminase similar to Alanine Transaminase. Additionally, it aids in the clinical diagnosis of liver conditions. A release of enzymes into circulation also takes place if liver cell injury or necrosis occurs. Increased blood enzyme levels are indicators of cellular leakage and a deterioration of the liver cell membrane's functional integrity (Contreras-Zentella *et al*., 2016). Hence, a high ALT and AST level indicates liver damage (Lala *et al*., 2022). According to the current study, cadmium chloride poisoning may be caused by oxidative damage to liver cells or tissues.

Oxidative damage can be exacerbated by high levels of reactive oxygen species (ROS) and inadequate antioxidant capacity in cells (Pizzino *et al*., 2017). An imbalance between ROS and antioxidant status causes cellular leakage of ALT and AST, which raises their levels in the blood. Plant extract treatment reduced cadmium-induced hepatotoxicity, as evidenced by lower levels (p0.05) of ALT and AST intoxication by cadmium chloride rats. This could be because plant extracts can protect the liver by protecting the structural integrity of the plasma membrane, which prevents AST from seeping over the membrane. The rats given plant extract alone had similar results to the rats in the control group.

Phosphatases are enzymes that are found in many types of tissues. Alkaline phosphatase (ALP) and acid phosphatase (ACP), like transaminases, are used to identify liver injury. When the liver is damaged, the damaged liver cell releases an increased amount of ALP and ACP into the blood. This leakage could be caused by changes in membrane permeability (El-Nakeeb *et al*., 2011). Furthermore, an increase in serum ALP levels results from an increase in synthesis in the context of an increase in biliary pressure (Lowe *et al*., 2022). Because ACP is found in the cytoplasm, it is released into the blood upon cellular damage (Lowe et al., 2022). In this study, rats given cadmium chloride showed considerably greater levels of phosphatases than control rats (p 0.05). This could be due to increased membrane permeability. Meanwhile, *Drynaria quercifolia* rhizome extract considerably reduced the elevated ALP and ACP levels, which was comparable to the silymarin-treated group rats.

Lactate dehydrogenase (LDH) is an enzyme found in nearly all bodily tissues, but its concentration in the blood is low. It is essential for cellular respiration. When tissues are injured, they release more LDH into the bloodstream. LDH is most commonly discovered in the liver. When liver tissues are injured by heavy metals such as cadmium, LDH leaks into the blood (Fatima *et al*., 2019). This could be due to cadmium chloride-induced oxidative stress failing to stabilise the hepatocyte membrane. According to the current study, providing cadmium chloride (group II) enhanced the level of LDH in

plasma considerably (P 0.05) compared to the control group (Aziz *et al*., 2014). (This is group I). The plant extract's hepatoprotective activity was demonstrated by a considerable reduction in the high level of LDH caused by cadmium chloride. On the level of LDH, plant extract has similar effects to conventional medications. Another indicator of liver function is serum Gamma Glutamyl Transferase (GGT).

Several researches have recently indicated that GGT may be effective in oxidative stress studies. Cadmium chloride induces the production of free radicals, which causes oxidative stress (Liu *et al*., 2008). Several studies have recently indicated that GGT may be effective in oxidative stress research. Oxidative stress is principally generated by the production of free radicals, which is triggered by cadmium chloride (Liu *et al*., 2008). The serum GGT level was significantly (P 0.05) normalised by oral administration of plant extract, showing that the plant extract prevents cadmium chloride-induced liver damage and reduces GGT leakage in blood (Koenig *et al*., 2015). In the current study, the effect of plant extract on GGT levels is comparable to that of rats getting standard pharmaceutical treatment. There was no noticeable difference between rats treated with simply plant extract and control rats.

Effect of plant extract on lipid profile

In comparison to control rats, the cadmium chloride-treated rats had considerably higher levels of elevated cholesterol. Some hepatic enzymes' gene expression is altered by cadmium. The expression of Hydroxyl-Methyl-Glutaryl Co A reductase is increased by cadmium (HMG – COA), an anabolic enzyme and suppression of 7á hydroxylase, a catabolic enzyme of cholesterol metabolism. As a result, level of cholesterol was increased in blood and also peripheral tissues (Huff *et al*., 2022). Increased cholesterol (Hypercholesterolemia) is a major risk of Coronary Heart Diseases (CHD). Extract of D. quercifolia rhizome significantly reduced ($P < 0.05$) the cholesterol level which indicatesd the hypo cholesterolemic activity of rhizome extract. The impact of plant extract on cholesterol levels was comparable to those of common drugs. In rats treated with plant extract alone vs control rats, no discernible change was found.

Triglycerides are the primary components of human body fat. In the current study, rats given cadmium chloride had significantly higher levels of triglycerides and free fatty acids than the control group. Cadmium's negative effects on cell membranes and disturbance of lipid metabolism, which results in an increase in hepatic synthesis of triglycerides and/or a lower clearance rate of triglyceride-rich lipoproteins, may be to blame (Alves-Bezerra *et al*., 2017). Cadmium exposure causes mitochondrial dysfunction in the liver (Branca et al., 2003), which inhibits FFA oxidation and resulting in buildup (Talley *et al*., 2022). Furthermore, cadmium toxicity increased the rate of cholesterol production while decreasing phospholipid concentration by increasing the rate of phospholipid breakdown. When cadmium-intoxicated rats were given a plant extract similar to silymarin-treated animals, the change in triglyceride, free fatty acid, and phospholipid levels was significantly (P 0.05) returned to near normal (group IV).

Effect of plant extract on lipoproteins

In the current study, cadmium chloride significantly increased plasma triglyceride and cholesterol levels, which increased the concentration of triglyceride-rich lipoproteins such as LDL-C and VLDL-C (p 0.05) (group II) compared to the control. The new

findings are comparable to those of the previous report (Samarghandian *et al*., 2015). The acquired data clearly reflected the cadmium chloride-induced changes in lipoprotein metabolism. In contrast, rats exposed to cadmium chloride had significantly lower HDL levels. High Density Lipoprotein Cholesterol (HDL-C) is good cholesterol. It is significant in lowering the risk of Cardiovascular Disease (CVD).

HDL-C mediates its effect by acting as a carrier for free cholesterol from the liver processes and excretes cholesterol from peripheral tissues, lowering blood cholesterol levels (Ouimet *et al*., 2019). It has been hypothesised that a liver problem brought on by cadmium exposure will lower HDL-C levels, lead to dyslipidemia, and interfere with the biological processes that make HDL-C. (Samarghandian *et al*., 2015). In the current investigation, group II rats were exposed to cadmium chloride, which changed their livers and caused tissue damage. Reduced HDL production was caused by liver injury. Oral treatment with plant extract significantly (P 0.05) reduced the severity of liver damage, resulting in a significant decrease in LDL and VLDL levels and an increase in HDL levels (group III). A comparable result was observed in the group receiving standard pharmaceutical therapy (IV). The results of rats given only plant extract were comparable to those of control rats.

Effect of plant extract on kidney markers

In animals, the primary nitrogenous byproduct of protein metabolism is urea. In the course of protein breakdown, amino groups are removed and converted into ammonia which in turn converted into urea by liver. The formed urea is then passes into kidney and eventually excreted. The principal organs affected by cadmium exposure are the liver and kidneys (Rafati Rahimzadeh *et al*., 2017). Cadmium accumulation was discovered in the current study by an increased amount of urea in serum compared to the healthy control (P 0.05). This discovery demonstrated that the kidneys' ability to remove urea was compromised (Pizzorno *et al*., 2015). The results were significantly returned to near normal following oral administration of rhizome extract and silymarin (p 0.05), providing evidence for the preventive effects of plant extract and silymarin on the kidney.

The metabolic breakdown of purine nucleotides produces uric acid. In the current investigation, cadmium injection (group II) significantly elevated uric acid levels (p 0.05) as compared to the healthy control group. The increased amount could be linked to the kidney's oxidative imbalance, which has been associated to cadmium toxicity (Yan *et al*., 2021). However, as compared to the cadmium chloride-treated rats, the animals pretreated with plant extract had a considerable drop in uric acid levels. The outcomes of those treated with standard drugs and those treated just with plant extract were comparable. The study report was compared to a few previously published studies (Ranganathan *et al*., 2018).

Serum creatinine, a basic consequence of muscle metabolism that is removed unchanged by the kidneys, is an important biomarker of renal health. The kidneys are the primary organs in charge of eliminating creatinine from the blood, primarily via glomerular filtration but also via proximal tubular secretion. In the current investigation, rats exposed to cadmium chloride had substantially higher levels of creatinine (p0.05) than controls. This could be due to cadmium's direct toxic effects on the kidney, which result in insufficient renal filtration and a rise in blood creatinine levels (Prozialeck *et al*., 2012).

Our findings were consistent with previous research on cadmium nephrotoxicity (Satarug *et al*., 2020). Creatinine levels in rats treated with standard medications were significantly lowered after administration of an oral plant extract.

Histopathological studies of Liver

Toxins and chemicals are mostly detoxified in the liver (Gu X *et al*., 2012). Toxic metal accumulation, on the other hand, may harm the normal structure of hepatocytes (Balali-Mood *et al*, 2021). It is the most vulnerable organ to cadmium (Rafati Rahimzadeh et al, 2017). In the current study, Nikon microscopy was utilised to analyse the histopathological results in the liver tissues of control and experimental animals. According to the architecture (H & E stain and 100x), hepatocytes (HC) in the rat control liver (group I) were normal. Lymphocytic infiltration (LI) in the portal and periportal areas, as well as cell debris in the circulation sinusoids (arrow mark), can be seen in the livers of group II cadmium chloride-exposed rats. Blood sinusoids include cell debris, and hepatocytes have eosinophilic and ground-glass-like cytoplasm. Necrosis (N) affects a subset of hepatocytes. Previous research has also revealed some histological abnormalities in the liver tissue of rats exposed to Cd for 22 days. The loss of the normal architecture of the parenchymatous tissue, cytoplasmic vacuolization, cellular degeneration and necrosis, blocked blood vessels, destroyed mitochondrial cristae, fat globules, severe glycogen depletion, lipofuscin pigments, and the formation of collagenous fibres are all histological changes that can cause both apoptosis and necrosis (Miller *et al*., 2017).Previous research has also revealed some histological abnormalities in the liver tissue of rats exposed to Cd for 22 days. The loss of the normal architecture of the parenchymatous tissue, cytoplasmic vacuolization, cellular degeneration and necrosis, blocked blood vessels, destroyed mitochondrial cristae, fat globules, severe glycogen depletion, lipofuscin pigments, and the formation of collagenous fibres are all histological changes that can cause both apoptosis and necrosis (Miller *et al*., 2017).

Furthermore, Cd is a non-redox metal that may cause oxidative stress indirectly by lowering GSH levels in cells. Competition with important metals including Zn, selenium (Se), copper (Cu), and calcium impedes a variety of cellular activities, including metal membrane transport and energy metabolism (Samet *et al*., 2020). It also has an impact on a number of biological functions, including enzyme activity, DNA repair mechanisms, and signal transmission (Sever et al., 2015). In order to restore the pathological changes caused by cadmium chloride to normal, we used a plant extract that significantly decreased (p0.05) the widening of the blood sinusoid (S), less fragmentation of the hepatocyte nuclei (HN), less lighting of the cytoplasm, and less infiltration of mononuclear cells. A poisonous rat's liver (group IV) revealed little indications of nuclei fragmentation, very little infiltration, or necrotic development. Rats given only plant extract had no significant liver symptoms (group V). This finding was consistent with previous research (Abdulaziz Bardi *et al*., 2014).

Histopathological studies of Kidney

The cadmium metallothionein (Cd-MT) complex, which is generated in the liver, released into the bloodstream, and absorbed by renal proximal tubule cells, mediates cadmium chloride nephrotoxicity (Almeer et al., 2019). Cadmium was not bound to MT when the cadmium level was high and the MT synthesis was insufficient, causing

hepatocyte damage and the release of a Cd-MT complex into the blood. After passing through the kidney's glomeruli, the complex in the plasma is then taken up by the proximal tubular cells, causing harm, primarily in the cortical region, before reaching the proximal tubule and gradually impairing the function of the organ (Basile *et al*., 2012).

Moreover, oxidative stress was clearly linked to the mechanism through which CdCl2 caused kidney injury. Increased free radical production and poor antioxidant status are the main causes of tissue damage brought on by oxidative stress (Pizzino *et al*., 2017). In the current study, kidney tissues from healthy controls showed a typical thick cubic epithelium and normal architecture of the distal convoluted tubule (group I). The Bowman's capsule was coated with flat epithelium in CdCl2-induced kidney tissues (group II), which had larger vascular glomeruli (G) tightly packed within the glomerular capsular space (CP) (BC). A few proximal (X) and distal convoluted tubular epithelial cells exhibit oedema-like features. Capillaries are filled with blood cells, and some tubules contain single desquamated cells.

The proximal (X) and distal (D) convoluted tubular epithelial oedema in poisoned rats (group III) was considerably reduced (p0.05) following pretreatment with plant extract, confirming the antioxidant and hepatoprotective properties of plant extract. The kidney of rats exposed to CdCl2 and silymarin (group IV) showed no blood cells in the capillaries and no tight filling of the capsular space. Plant extract alone treated rats had the same renal tissue architecture as the control group. Our findings are consistent with earlier research (Sonfack *et al*., 2009).

CONCLUSION

The data from the results showed that at a dose level of 300 mg/kg body weight, the chosen plant extract displayed protective effect against cadmium chloride-induced liver damage, and the results were comparable to those of the silymarin standard medicine. *Drynaria quercifolia* rhizome extract (L) efficacy. J. Smith would very certainly have numerous possibilities in the future. As a result, it can be used therapeutically to treat liver problems. Future research is needed to isolate hepatoprotective bioactive components from plant extract. To establish the hepatoprotective potential of isolated lead compounds, preclinical and clinical investigations are required.

REFERENCES

Asrani SK, Devarbhavi H, Eaton J, Kamath PS (2019). Burden of liver diseases in the world. J Hepatol, 70:151-171.

Abdulaziz Bardi D, Halabi MF, Hassandarvish P, Rouhollahi E, PM,Moghadamtousi SZ, Al-Wajeeh NS, Ablat, A, Abdullah NA, Abdulla, Almeer RS, AlBasher GI, Alarifi S, Alkahtani S, Ali D, Abdel Moneim AE, (2019). Royal jelly attenuates cadmium-induced nephrotoxicity in male mice. Sci Rep. 9, 5825.

Alves-Bezerra M, Cohen DE, (2017). Triglyceride Metabolism in the Liver. Compr Physiol. 8:1-8.

Anuja GI, Latha PG, Suja SR, Shyamal S, Shine VJ, Sini S, Pradeep S, Shikha P, Rajasekharan S, (2010). Anti-inflammatory and analgesic properties of Drynaria quercifolia (L) J, Smith J Ethnopharmacol. 11;132(2):456-60.

Aryal S, Baniya MK, Danekhu K, Kunwar P, Gurung R., Koirala N, (2019). Total Phenolic Content, Flavonoid Content and Antioxidant Potential of Wild Vegetables from Western Nepal. Plants (Basel). 11;8(4)-96.

Aziz R, Rafiq MT, Yang J, Liu D, Lu L, He Z, Daud MK, Li T, Yang X, (2014). Impact assessment of cadmium toxicity and its bioavailability in human cell lines (Caco-2 and HL-7702). Biomed Res Int. 2014:839538.

Azza Z, Oudghiri M, (2015). In vivo anti-inflammatory and antiarthritic activities of aqueous extracts from Thymelaeahirsuta. Pharmacognosy Res. 7(2):213-6.

Balali-Mood M, Naseri K, Tahergorabi Z, Khazdair MR, Sadeghi M, (2021). Toxic Mechanisms of Five Heavy Metals: Mercury, Lead, Chromium, Cadmium, and Arsenic. Front Pharmacol. 12, 643972.

Balouiri M, Sadiki M, Ibnsouda SK, (2016). Methods for in vitro evaluating antimicrobial activity: A review. J Pharm Anal. 6(2):71-79.

Bartlette GR, (1959). Phosphorus assay in column chromatography J. Biol. Chem., 234:466-468.

Basile DP, Anderson MD, Sutton TA, (2011). Pathophysiology of acute kidney injury. Compr Physiol. 2, 1303-53.

Bernhoft RA, (2013). Cadmium toxicity and treatment. Scientific World Journal. Jun 3; 2013: 394652.

Bonsnes RW, Taussky HA, (1945). On the colorimetric determination of creatinine by the Jaffe reaction J. Biol. Chem., 158:581-591.

Branca JJV, Fiorillo C, Carrino D, Paternostro F, Taddei N, Gulisano M, Pacini A, Becatti M, (2020). Cadmium-Induced Oxidative Stress: Focus on the Central Nervous System. Antioxidants (Basel). 5; 9(6):492.

Branca JJV, Pacini A, Gulisano M, Taddei N, Fiorillo C, Becatti M, (2020). Cadmium-Induced Cytotoxicity: Effects on Mitochondrial Electron Transport Chain. Front Cell Dev Biol. 8:604377.

Buha A, Đukić-Ćosić D, Ćurčić M, Bulat Z, Antonijević B, Moulis JM, Goumenou M, Wallace D, (2020). Emerging Links between Cadmium Exposure and Insulin Resistance: Human, Animal, and Cell Study Data. Toxics. 8(3):63.

Caraway WT, Seligson D, (1963). Uric acid. In: Standard methods of Clinical Chemistry. vol 4. [Seligson, D. ed.] New York: Academic Press, pp.239-247.

Contreras-Zentella ML, Hernández-Muñoz R., (2016). Is Liver Enzyme Release Really Associated with Cell Necrosis Induced by Oxidant Stress? Oxid Med Cell Longev. 3529149.

Elgaml SA, and Hashish EA, (2014). Clinicopathological studies of Thymus vulgaris extract against cadmium induced hepatotoxicity in albino rats. Global J. Pharmacol., 8:501-509.

El-Nakeeb MA, Abou-Shleib HM, Khalil AM, Omar HG, El-Halfawy OM, (2011).Membrane permeability alteration of some bacterial clinical isolates by selected antihistaminics. Braz J Microbiol. 42, 992-1000.

Falholt K, Falholt W, Lund B, (1973). An easy colorimetric method for routine determination of free fattry acids in plasma. Clin. Chim. Acta., 46:105-111.

Fatima G, Raza AM, Hadi N, Nigam N, Mahdi AA, (2019). Cadmium in Human Diseases: It's More than Just a Mere Metal. Indian J Clin Biochem. 34:371-378.

Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM., Forman D, Bray F, (2012). Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer. 1,136.

Folin Wu, (1991). Estimation of Blood Glucose. J. Biol. Chem, 38, 106.

Foster LB, Dunn RT, (1973). Stable reagents for determination of serum triglycerides by a colorimetric Hantzsch condensation method. Clin. Chem., 19, 338-340.

Freitag AF, Cardia GF, da Rocha BA, Aguiar RP, Silva-Comar FM, Spironello RA Grespan R, Caparroz-Assef SM, Bersani-Amado CA, Cuman RK, (2015). Hepatoprotective Effect of Silymarin (Silybum marianum) on Hepatotoxicity Induced by Acetaminophen in Spontaneously Hypertensive Rats. Evid Based Complement Alternat Med. 2015:538317.

Friedewald WT, Levy RI, Fredrickson DS, (1972). Estimation of the concentration of low density lipoprotein cholesterol in plasma without the use of proparative centrifuge. J. Clin. Chem., 18:499-502.

Genchi G, Sinicropi MS, Lauria G, Carocci A, Catalano A, (2020). The Effects of Cadmium Toxicity. Int J Environ Res Public Health. 17(11):3782 .

Grigorian A, O'Brien CB, (2014). Hepatotoxicity Secondary to Chemotherapy. J Clin Transl Hepatol. 95-102.

Gu X, Manautou, JE, (2012). Molecular mechanisms underlying chemical liver injury. Expert Rev Mol Med. 14, e4.

Huff T, Boyd B, Jialal I, (2022). Physiology, Cholesterol. [Updated 2022 Mar 9]. In: Stat Pearls [Internet]. Treasure Island (FL): StatPearls Publishing.

JanIida-Ueno A, Enomoto M, Tamori A, Kawada N, Hepatitis B, (2017). virus infection and alcohol consumption. World J Gastroenterol. 23(15):2651-2659.

King J, (1965). The dehydrogenase or Oxido reductase-lactate dehydrogenase. In Practical Clinical Enzymology. [King, J. ed.] London: Van Nostrand Company Ltd., pp.83-95.

Koenig G, Seneff S, (2015). Gamma-Glutamyltransferase: A Predictive Biomarker of Cellular Antioxidant Inadequacy and Disease Risk. Dis Markers. 818570.

Lala V, Zubair M, Minter DA, (2022). Liver Function Tests. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing.

Liu J, Qian SY, Guo Q, Jiang J, Waalkes MP, Mason RP, Kadiiska MB, (2008). Cadmium generates reactive oxygen- and carbon-centered radical species in rats: insights from in vivo spin-trapping studies. Free Radic Biol Med. 45:475-81.

Lowe D, Sanvictores T, Zubair M, Alkaline Phosphatase, (2022). [Updated 2022 Nov 4]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing.

Lowry OH, Rosenbrough NJ, Farr AL, Randall R, (1951). Protein determination using folin-ciocalteu reagent. J. Biol. Chem., 193:265-275.

MA, (2014). Andrographis paniculata leaf extract prevents thioacetamide-induced liver cirrhosis in rats. PLoS One. 9, e109424.

Malloy HT, Evelyn KA, (1937). The Determination of bilirubin with the photoelectric colorimeter J. Biol. Chem., 119:481-490.

Miller MA, Zachary JF, (2017). Mechanisms and Morphology of Cellular Injury, Adaptation, and Death. Pathologic Basis of Veterinary Disease. 2–43, e19.

Mizushina Y, Nakanishi R, Kuriyama I, Kamiya K, Satake T, Shimazaki N, Koiwai O, Uchiyama Y, Yonezawa Y, Takemura M, Sakaguchi K, Yoshida H, (2006). Beta-sitosterol-3-O-beta-D-glucopyranoside: a eukaryotic DNA polymerase lambda inhibitor. J Steroid Biochem Mol Biol. 99(2-3):100-7.

Mondal D, Das K, Chowdhury A, (2022). Epidemiology of Liver Diseases in India. Clin Liver Dis (Hoboken). 19(3):114-117.

Natelson S, Scott ML, Beffa D, (1951). A rapid method for the estimation of urea in biological fluids by means of the reaction between diacetyl and urea. Am. J. Chem. Pathol., 21:275-281.

Nath P, Yadav AK, (2016). Anthelmintic activity of a standardized extract from the rhizomes of Acorus calamus Linn. (Acoraceae) against experimentally induced cestodiasis in rats. J Intercult Ethnopharmacol. 5:390-395.

Nita M, Grzybowski A, (2016). The Role of the Reactive Oxygen Species and Oxidative Stress in the Pathomechanism of the Age-Related Ocular Diseases and Other Pathologies of the Anterior and Posterior Eye Segments in Adults. Oxid Med Cell Longev. 2016:3164734.

Ohkawa H, Ohishi N, Yagi K, (1979). Assay of lipid peroxides in animal tissues for thiobarbituric acid reaction. Annual Biochem., 95:351-358.

Ouimet M, Barret TJ, Fisher EA, (2019). HDL and Reverse Cholesterol Transport. Circ Res.124, 1505-1518.

Parekh AC, Jung DH, (1970). Cholesterol determination with ferric acetate, uranium acetate and sulphuric acid-ferrous sulphate reagents. Anal. Chem., 42:1423-1427.

Pizzino G, Irrera N, Cucinotta M, Pallio G, Mannino F, Arcoraci V, Squadrito F, Altavilla D, Bitto A, (2017). Oxidative Stress: Harms and Benefits for Human Health. Oxid Med Cell Longev. 2017:8416763. .

Pizzorno J, (2015). The Kidney Dysfunction Epidemic, Part 1: Causes. Integr Med (Encinitas). 14:8-13.

Prozialeck WC, Edwards JR, (2012). Mechanisms of cadmium-induced proximal tubule injury: new insights with implications for biomonitoring and therapeutic interventions. J Pharmacol Exp Ther. 343, 2-12.

Rafati Rahimzadeh M, Kazemi S, Moghadamnia AA, (2017). Cadmium toxicity and treatment: An update. Caspian J Intern Med. (2017) Summer; 8:135-145.

Ramesh N, Viswanathan MB, Saraswathy A, Balakrishna K, Brindha P, Lakshmanaperumalsamy P, (2001). Phytochemical and antimicrobial studies on Drynaria quercifolia. Fitoterapia. 72(8):934-6.

Ranganathan P, Aggarwal R, (2018). Study designs: Part 1 - An overview and classification. Perspect Clin Res. 9:184-186. .

Reichold JG, (1953). Standard Methods of Clinical Chemistry. 1st ed. [Reiner, M. ed.]. New York: Academic Press, pp.88

Rosalki SB, Rau D, (1972). Serum gamma glutamyl transpeptidase activity in alcoholism. Clin. Chem. Acta, 39:41-47.

Samarghandian S, Azimi-Nezhad M, Shabestari MM, Azad, FJ, Farkhondeh T, Bafandeh F, (2015). Effect of chronic exposure to cadmium on serum lipid, lipoprotein and oxidative stress indices in male rats. InterdiscipToxicol. 151-4.

Samet JM, Chen H, Pennington ER, Bromberg PA, (2020). Non-redox cycling mechanisms of oxidative stress induced by PM metals. Free Radic Biol Med. 1;151:26-37.

Satarug S, Gobe G, Vesey D, Phelps KR, (2020). Cadmium and Lead Exposure, Nephrotoxicity, and Mortality. Toxics. 8, 86.

Schwarz DS, Blower MD, (2016). The endoplasmic reticulum: structure, function and response to cellular signaling. Cell Mol Life Sci. (2016) Jan;73(1):79-94.

Seifter S, Dayton S, et al., (1950). The estimation of glycogen with the anthrone reagent. Arch. Biochem., 25:191-200.

Senthilkumar R, Chandran R, Thangaraj Parimelazhagan, (2014). Hepatoprotective effect of Rhodiola imbricata rhizome against paracetamol induced liver toxicity in rats. Saudi J. Biol. Sci., 21:409-416.

Sever R, Brugge JS, (2015). Signal transduction in cancer. Cold Spring Harb Perspect Med. 5(4), a006098.

SonfackCS, Nguelefack-Mbuyo EP, Kojom JJ, Lappa EL, Peyembouo FP, Fofié CK, Nolé T, Nguelefack TB, Dongmo AB, (2021). The Aqueous Extract from the Stem Bark of Garcinia lucida Vesque (Clusiaceae) Exhibits Cardioprotective and Nephroprotective Effects in Adenine-Induced Chronic Kidney Disease in Rats. Evid Based Complement Alternat Med. 2021, 5581041.

Talley JT, Mohiuddin SS, (2022). Biochemistry, Fatty Acid Oxidation. [Updated 2022 Jan 24]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022.

Tchounwou PB, Yedjou CG, Patlolla AK, Sutton DJ, (2012). Heavy metal toxicity and the environment. Exp Suppl. 101:133-64.

Torruellas C, French SW, Medici V, (2014). Diagnosis of alcoholic liver disease. World J Gastroenterol. 20, 11684-99.

Yan LJ, Allen DC, (2021). Cadmium-Induced Kidney Injury: Oxidative Damage as a Unifying Mechanism. Biomolecules. 11, 1575.

Figure Legend

Fig. 1. Effect of plant drug on body weight and liver weight of experimental animals

Results were expressed as mean ± S.E.M, n = 6. Values not sharing the common superscript were statistically significant with each other (a–c) Values that do not share a common superscript letter between groups different significance. b significantly different from group 1 at $p < 0.01$; c significantly different from group 1 at $p < 0.05$ (DMRT).

Fig. 2. Effect of plant drug on glucose, protein, and A/G ratio of experimental animals

Results were expressed as mean ± S.E.M, n = 6. Values not sharing the common superscript were statistically significant with each other (a–c) Values that do not share a common superscript letter between groups different significance. b significantly different from group 1 at $p < 0.01$; c significantly different from group 1 at $p < 0.05$ (DMRT).

Fig. 3. Effect of plant drug on direct and total bilurubin of experimental animals

Results were expressed as mean ± S.E.M, n = 6. Values not sharing the common superscript were statistically significant with each other (a–c) Values that do not share a common superscript letter between groups different significance. b significantly different from group 1 at $p < 0.01$; c significantly different from group 1 at $p < 0.05$ (DMRT).

Fig. 4. Effect of plant drug on AST, ALT, ALP, ACP, GGT and LDH of experimental animals.

Results were expressed as mean ± S.E.M, n = 6. Values not sharing the common superscript were statistically significant with each other (a–c) Values that do not share a common superscript letter between groups different significance. b significantly different from group 1 at $p < 0.01$; c significantly different from group 1 at $p < 0.05$ (DMRT).

Fig. 5. Effect of plant drug on cholesterol, triglyceride, FFA, and phospholipids of experimental animals.

Results were expressed as mean ± S.E.M, n = 6. Values not sharing the common superscript were statistically significant with each other (a–c) Values that do not share a common superscript letter between groups different significance. b significantly different from group 1 at $p < 0.01$; c significantly different from group 1 at $p < 0.05$ (DMRT).

Fig. 6. Effect of plant drug on lipoproteins of experimental animals.

Results were expressed as mean ± S.E.M, n = 6. Values not sharing the common superscript were statistically significant with each other (a–c) Values that do not share a common superscript letter between groups different significance. b significantly different from group 1 at $p < 0.01$; c significantly different from group 1 at $p < 0.05$ (DMRT).

Fig. 7. Effect of plant drug on urea, uric acid and creatinine of experimental animals

Results were expressed as mean ± S.E.M, n = 6. Values not sharing the common superscript were statistically significant with each other (a–c) Values that do not share a common superscript letter between groups different significance. b significantly different from group 1 at $p < 0.01$; c significantly different from group 1 at $p < 0.05$ (DMRT).

Fig. 8. Histopathological studies of Liver.

Group I: Control rat liver showed normal hepatocytes (HC) (H & E stain and 10 0x); Group II: shows lymphocytic infiltration (LI) in the portal and periportal presence of cell debris in the blood sinusoids (arrow mark). The cytoplasm of the hepatocytes has a ground glass and eosinophilic appearance, necrosis (N) of some hepatocyte, presence of cell debris in the blood sinusoids; Group III: shows decreased widening of blood sinusoid (S), less frag mentation of hepatocytes nuclei (HN), less lighting of the cytoplasm and de crease the mononuclear cells infiltration (arrow mark); Gro up IV: shows no fragmentation of nuclei, very less in filtration and no necrotic forma ion; Group : shows nor al hepatocytes and pathological signs were not noted.

Fig. 9. Histopathological studies of kidney (100X).

Group I show typical thick cubic epithelium and normal organization of distal convoluted tubule; Group II shows vascular glomeruli (G) are enlarged, tightly filling the glomerular capsular space (CP), with flat epithelium lining the Bowman's capsule (BC). Some cells of the proximal (X) and distal convoluted tubular epithelium show features of oedema. Capillaries are filled with blood cells; some tubules contain single desquamated cells (black arrow m ark) H&E, and 100x; Group III shows decreased oedema of both the proximal (X), and distal (D) convoluted tubular epithelium; Group IV shows an absence of blood cells in the capillaries and no tight filling of capsular space; Group V shows typical thick cubic epithelium a d normal organization of distal convoluted tubule as in control.

Figures

Fig. 1.

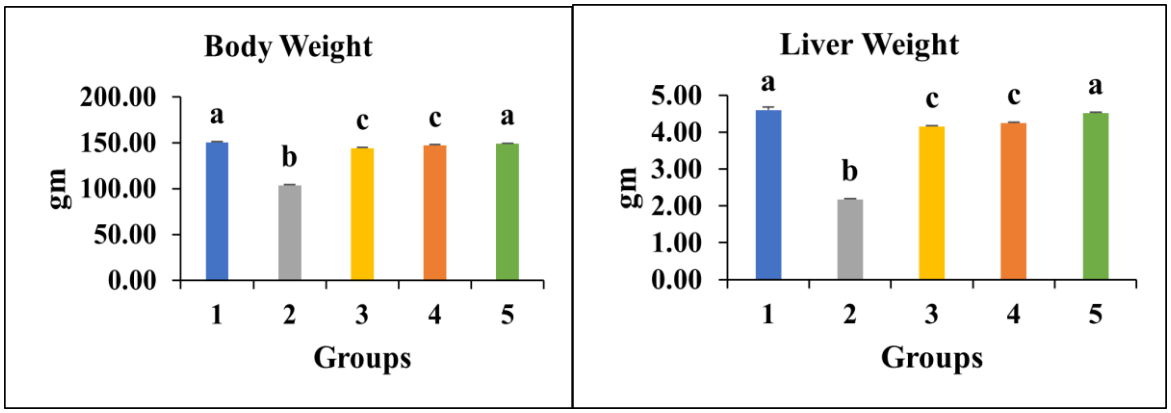
Body Weight
gm
200.00
150.00
100.00
50.00
0.00
a
b
c
c
a
1
2
3
4
5
Groups
Liver Weight
gm
5.00
4.00
3.00
2.00
1.00
0.00
a
b
c
c
a
1
2
3
4
5
Groups

Fig. 2.

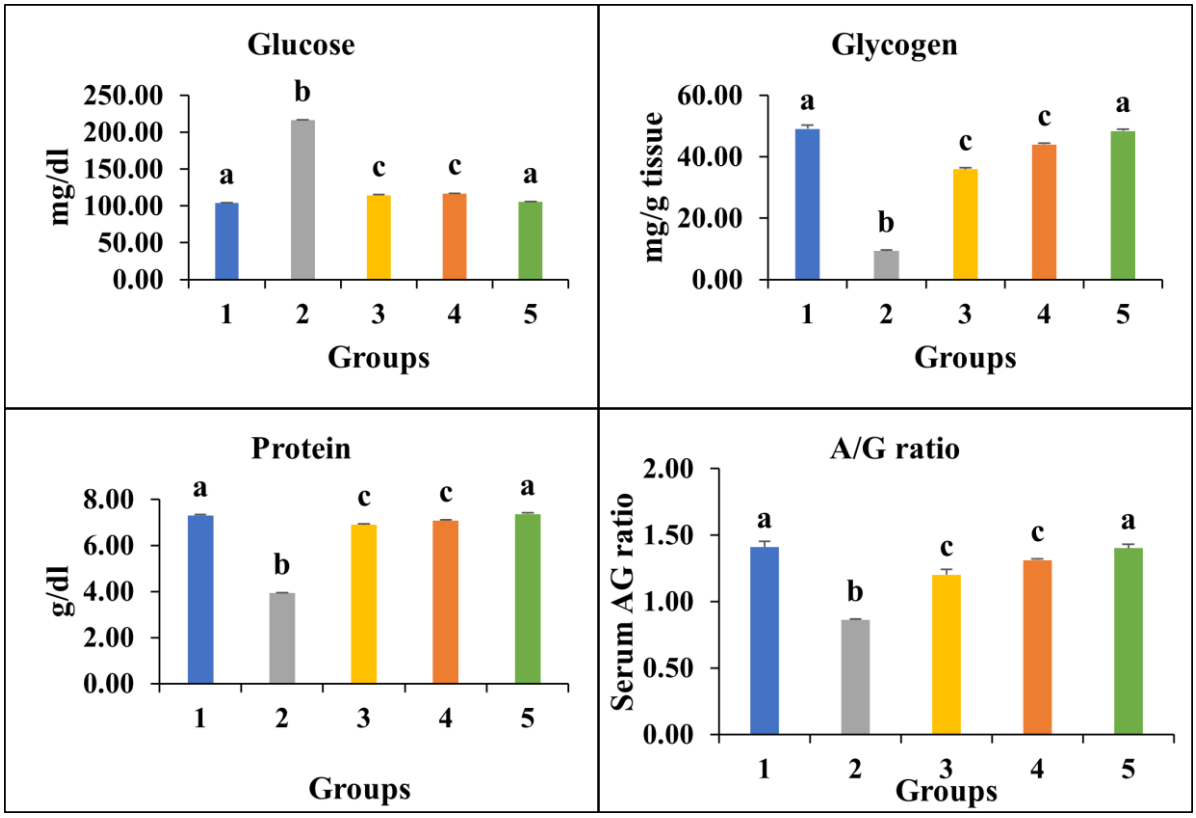
Glucose
mg/dl
250.00
200.00
150.00
100.00
50.00
0.00
a
b
c
c
a
1
2
3
4
5
Groups
Glycogen
mg/g tissue
60.00
40.00
20.00
0.00
a
b
c
c
a
1
2
3
4
5
Groups
Protein
g/dl
8.00
6.00
4.00
2.00
0.00
a
b
c
c
a
1
2
3
4
5
Groups
A/G ratio
Serum AG ratio
2.00
1.50
1.00
0.50
0.00
a
b
c
c
a
1
2
3
4
5
Groups

Fig. 3.

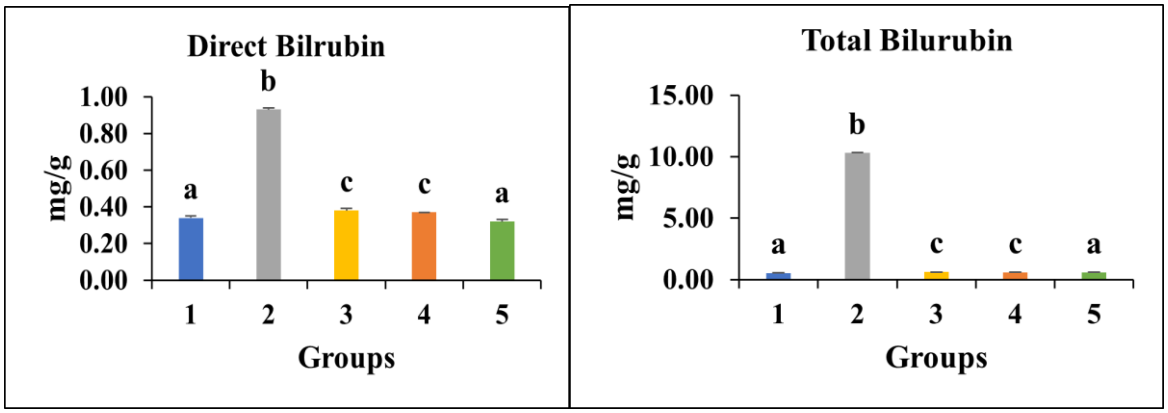
Direct Bilrubin
mg/g
1.00
0.80
0.60
0.40
0.20
0.00
a
b
c
c
a
1
2
3
4
5
Groups
Total Bilurubin
mg/g
15.00
10.00
5.00
0.00
a
b
c
c
a
1
2
3
4
5
Groups

Fig. 4.

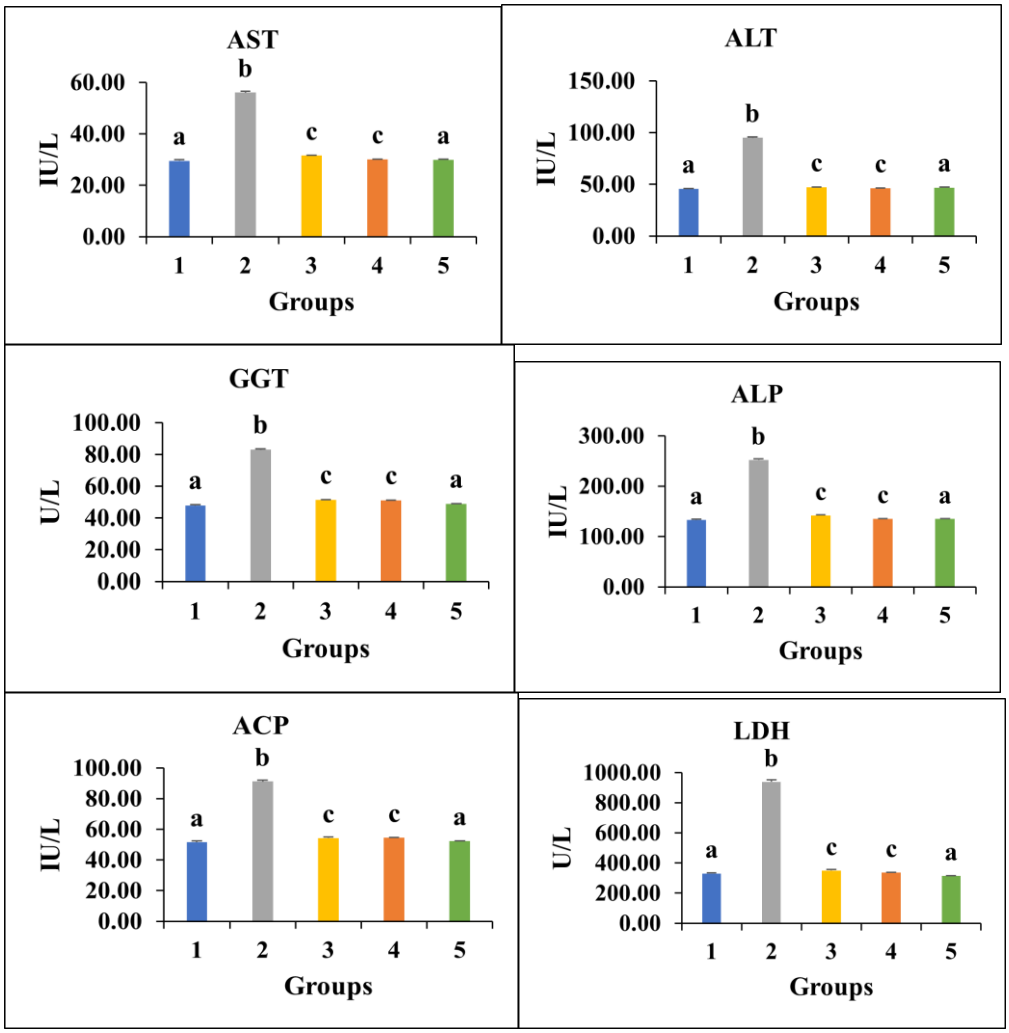
AST
IU/L
60.00
40.00
20.00
0.00
a
b
c
c
a
1
2
3
4
5
Groups
ALT
IU/L
150.00
100.00
50.00
0.00
a
b
c
c
a
1
2
3
4
5
Groups
GGT
U/L
100.00
80.00
60.00
40.00
20.00
0.00
a
b
c
c
a
1
2
3
4
5
Groups
ALP
IU/L
300.00
200.00
100.00
0.00
a
b
c
c
a
1
2
3
4
5
Groups
ACP
IU/L
100.00
80.00
60.00
40.00
20.00
0.00
a
b
c
c
a
1
2
3
4
5
Groups
LDH
U/L
1000.00
800.00
600.00
400.00
200.00
0.00
a
b
c
c
a
1
2
3
4
5
Groups

Fig. 5.

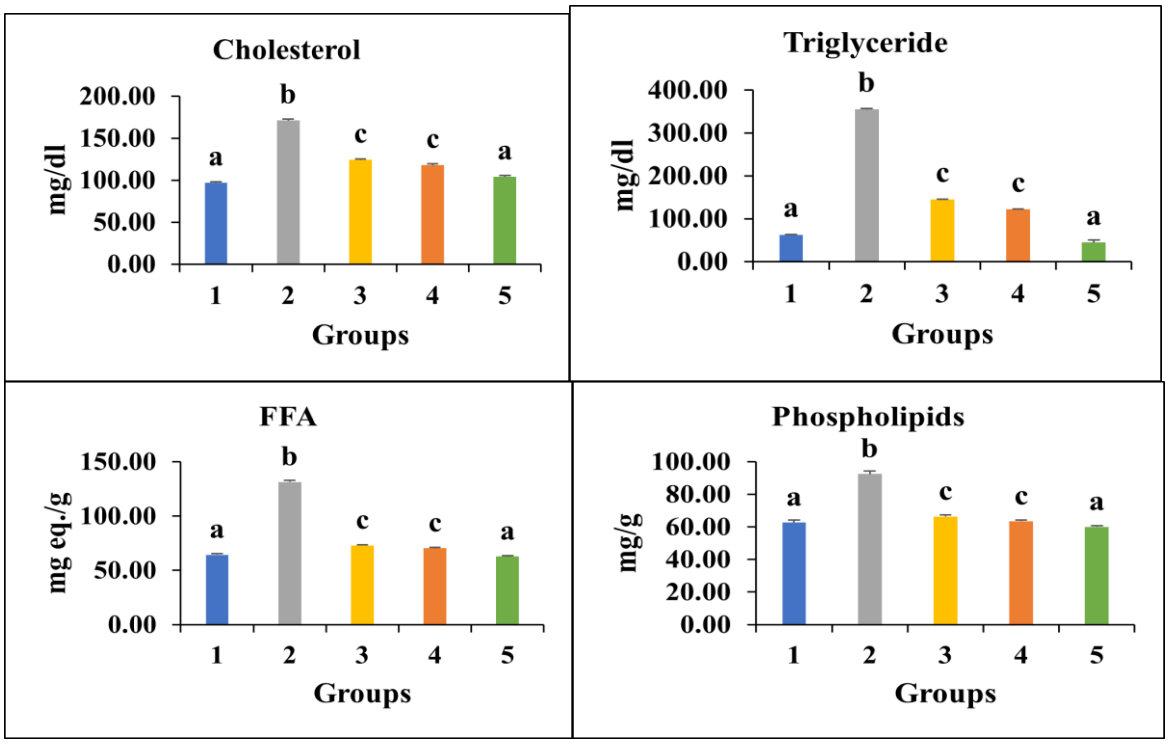
Cholesterol
mg/dl
200.00
150.00
100.00
50.00
0.00
a
b
c
c
a
1
2
3
4
5
Groups
Triglyceride
mg/dl
400.00
300.00
200.00
100.00
0.00
a
b
c
c
a
1
2
3
4
5
Groups
FFA
mg eq./g
150.00
100.00
50.00
0.00
a
b
c
c
a
1
2
3
4
5
Groups
Phospholipids
mg/g
100.00
80.00
60.00
40.00
20.00
0.00
a
b
c
c
a
1
2
3
4
5
Groups

Fig. 6.

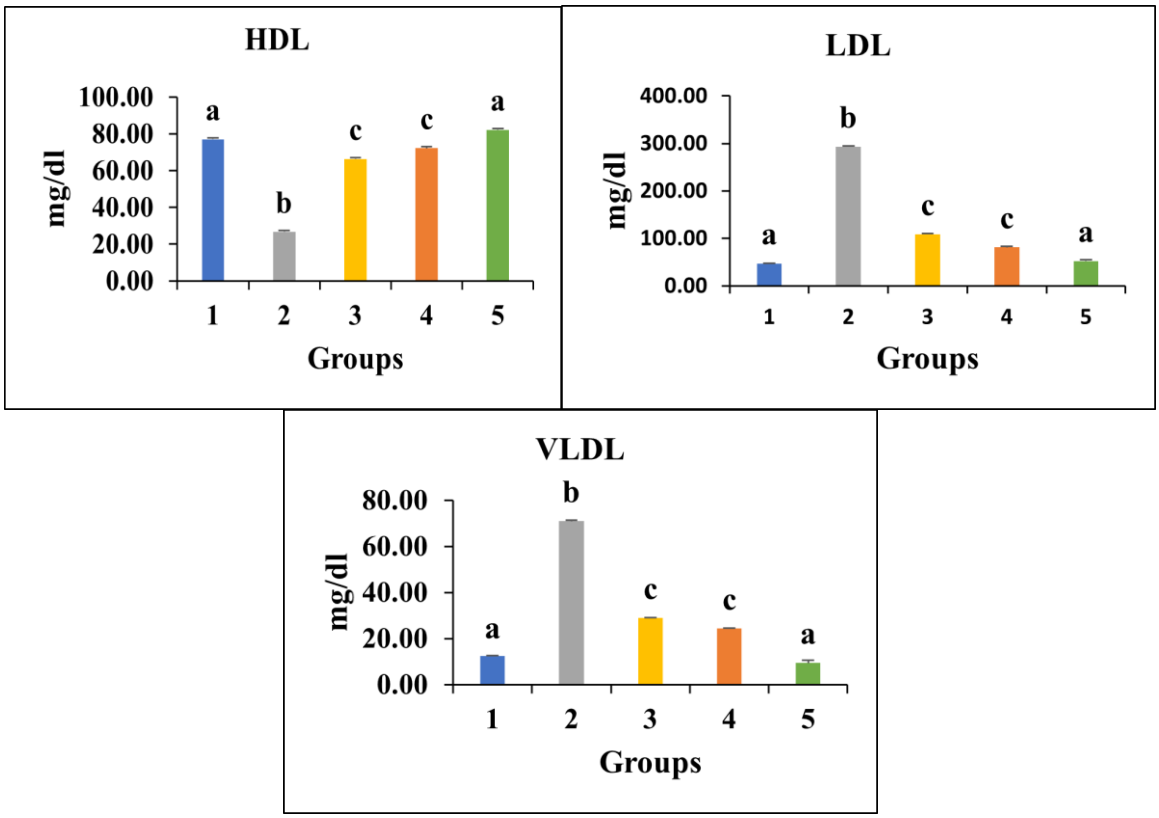
HDL
mg/dl
100.00
80.00
60.00
40.00
20.00
0.00
a
b
c
c
a
1
2
3
4
5
Groups
LDL
mg/dl
400.00
300.00
200.00
100.00
0.00
a
b
c
c
a
1
2
3
4
5
Groups
VLDL
mg/dl
80.00
60.00
40.00
20.00
0.00
a
b
c
c
a
1
2
3
4
5
Groups

Fig. 7.

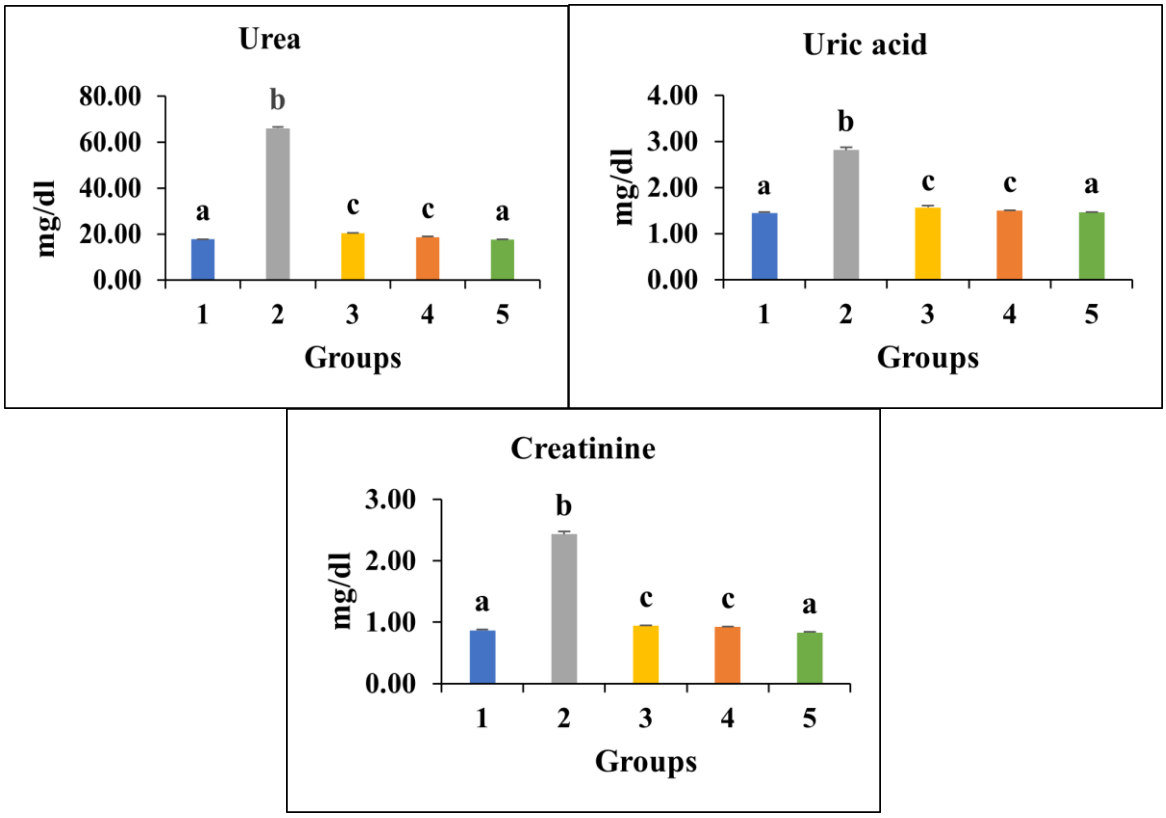
Urea
mg/dl
80.00
60.00
40.00
20.00
0.00
a
b
c
c
a
1
2
3
4
5
Groups
Uric acid
mg/dl
4.00
3.00
2.00
1.00
0.00
a
b
c
c
a
1
2
3
4
5
Groups
Creatinine
mg/dl
3.00
2.00
1.00
0.00
a
b
c
c
a
1
2
3
4
5
Groups

Fig. 8.

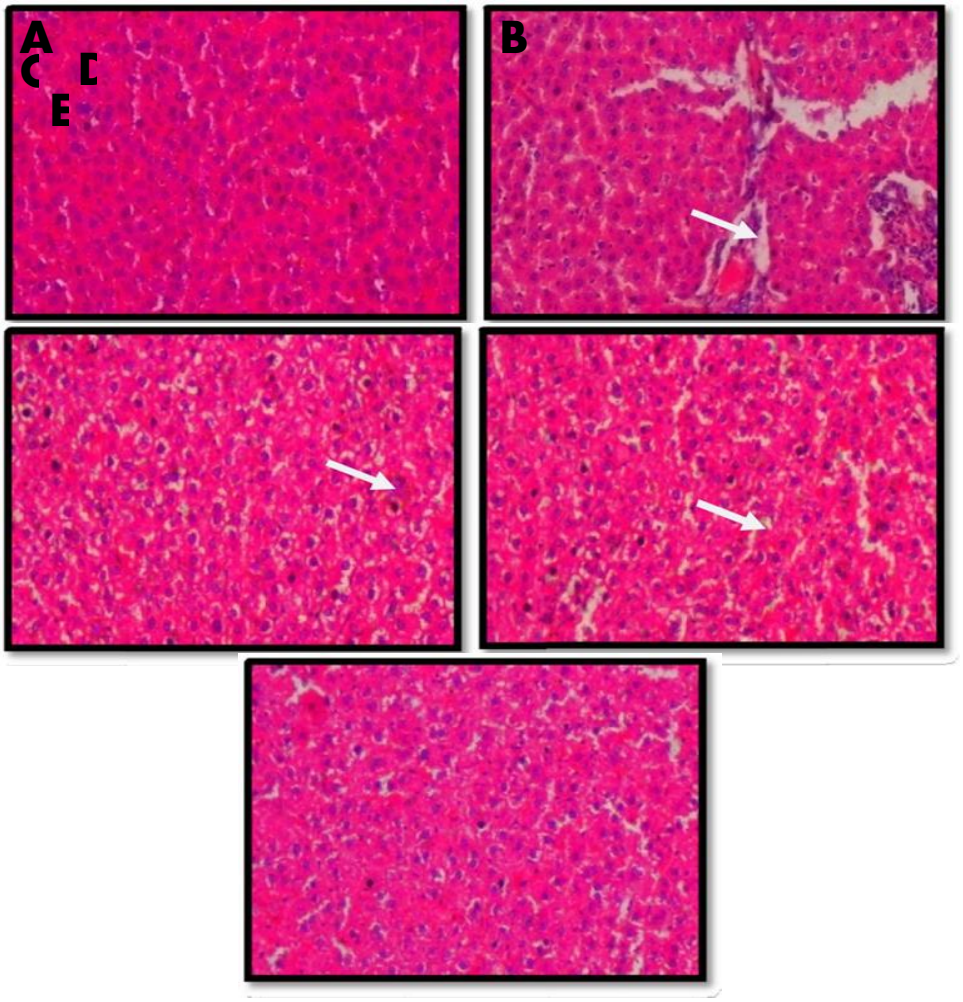
A
C
D
E
B

Fig. 9.