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BIOCHEMICAL AND HISTOPATHOLOGICAL ANALYSIS OF AQUEOUS EXTRACT OF AN APHRODISIAC FORMULATION AGAINST ACETAMINOPHEN INDUCE HEPATOCYTE INJURIES IN WISTAR RATS

KABRUK CAROLINE EZIAFAKAEGO[1, 2], AMUZAT ALIYU OLALEKAN[1], IBRAHIM MOHAMMED IBRAHIM[1, 3], YAKUBU NDATSU[1]

[1]Department of Biochemistry, Faculty of Natural Sciences, Ibrahim Badamasi Babangida University, Lapai, Niger State of Nigeria.
[2]Federal polytechnic, Bida, Niger State.
[3]Sheda Science and Technology Complex, Abuja.

ABSTRACT

The aimed of this study was to examine the biochemical parameters and histopathological effects of aqueous extract of an aphrodisiac formulation against acetaminophen induce hepatocyte injuries in Wistar rats. Zainacin Dadin duniya (aphrodisiac herbs) extract was prepared by cold maceration. The animal study was done using 30 adults both male and female Wistar rats was grouped into 5 groups (n=6) and orally administered with five different treatments (saline, liver drug, acetaminophen, extract and extract/ acetaminophen) for the period of 28days. The blood is collected from each grouped rat for biochemical parameters test and all rats were later sacrificed for histologically processed investigation. The results showed that the oral administration of the acetaminophen in group 3 treated rats causes a significant ($P<0.05$) increased in the activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP), but, when administered with the extract in group 4 rats, there was a significantly ($p<0.05$) decreased in the activities of ALT, AST and ALP. Significantly ($p<0.05$) increased in the Kidney Function Markers (Creatinine, and Urea) and decreased in Glucose level in the group 3 rats treated with acetaminophen, when compared with the group 4 rats treated with the extract, there was a significant ($p<0.05$) decreased in Creatinine, and Urea level and increased in the Glucose level. The serum total protein, Albumin, and Globulin significantly ($p<0.05$) decreased in the group 3 rats treated with acetaminophen while the bilirubin increased significantly. While group 4 rats showed significant increase in the total protein, Albumin, and Globulin and decreased in the bilirubin levels. The Results of Histopathology findings revealed hepatic tissue with distorted hepatocytes, portal vein, and sinusoids accompanied with massive necrosis in the liver of the group 3 rats treated with acetaminophen. While the liver of the group 4 rats showed, hepatic tissue with preserved architecture composed of cords of normal hepatocytes, normal portal vein, and sinusoids. There are no features of acute or chronic damage. The histologically Results of the small intestines of the all five (5) grouped rats showed preserved layers of muscosa, submucosa, muscularis externa, and serosa. There are no features of acute or chronic toxicity. Conclusively, the prolonged oral consumption

of acetaminophen in animals resulted in hepatocellular deleterious effects and may be of a similar hazard in humans. Overall, administration of the extract in rats has significant ameliorative effect on the rats; this may be of immense benefits in the management of hepatocytes, necrosis in the liver and its associated biomarkers complications. Improved liver functions as well as improved antioxidant status are beneficial in the management of chronic diseases such as liver failure.

KEYWORDS: Acetaminophen, Biochemical parameters, histological techniques, wistar rats, Zainacin Dadin duniya extract.

INTRODUCTION

The conventional herb is defined as a plant whose organs contain substances that can be utilized for therapeutic, research, or as precursors for creating new drugs (Sofowora 1982). Numerous plants have been studied for their aphrodisiac effects and these plant extracts are commonly used in various cultures to improve sexual performance. Lately, there has been a growing focus on using natural products from medicinal plants as a preferred method for addressing liver damage, according to Almasi et al. (2017).

Acetaminophen, also known as APAP, is a commonly used over-the-counter drug worldwide. It is an analgesic and antipyretic medication considered safe when taken at recommended therapeutic doses (AlWahsh et al., 2019). When taken at recommended levels, APAP is generally safe with minimal side effects; however, prolonged use or overdose can result in oxidative stress, liver damage, and kidney failure, potentially leading to death in both humans and animals, with a mortality rate as high as 90%. APAP induces liver damage at high doses by producing reactive metabolites, which are transformed into harmless metabolites with the help of glutathione before being eliminated through urine (Ramachandran & Jaeschke, 2017). When taken in therapeutic doses over a long time or in high amounts, the levels of reactive metabolites rise. These metabolites bind to proteins in liver cells, causing depletion of glutathione, oxidative stress, mitochondrial dysfunction, and decreased ATP production. This process ultimately results in liver cell necrosis, apoptosis, DNA damage, and the release of membrane proteins into the cytosol, leading to liver injury, cell damage, and cell death. The word "aphrodisiac" originates from the Greek goddess of love and sensuality named "Aphrodite". Aphrodisiac herbs, from the Greek word "Aphrodisiakos" meaning sexual, are utilized for treating sexual dysfunction by impacting certain neurotransmitters or sex hormones. More precisely, certain herbs containing aphrodisiac qualities can lead to an increased blood flow that leads to a prolonged erection and can also irritate genital tissues to heighten pleasure during sexual intercourse (Jain, et. al, 2010). Aphrodisiac is described as something that can stimulate sexual desire, elicit venereal cravings, and enhance pleasure, libido, or potency according to various researchers (Soref, 2008; Shamloul, 2010; Kotta et al., 2013; Chauhan et al., 2014). In Hausa, it is also known as "Maganin Burantashi." Aphrodisiacs can be classified into three (3) groups based on how they work: those that boost libido, enhance sexual pleasure, and increase potency. The employment of aphrodisiac herbs to enhance sexual performance is a key component of classic medicine. The aim of this study was to investigate the biochemical parameters and histopathological analysis of aqueous extract of an aphrodisiac formulation against acetaminophen-induced hepatocyte injuries in Wistar rats.

MATERIALS AND METHODS

MATERIALS

Collection of Herbal Samples

The herbs sample was collected from Suleja, Suleja local government Area, Niger state.

Extraction of Herbal samples

The herbal samples were extracted using the cold maceration method with distilled water. Around 100 grams of the sample was measured and placed into new 2000mL conical flasks. 1000mL of distilled water was poured into the conical flasks and left to sit at room temperature for 72 hours while being continuously shaken with a magnetic stirrer. After 72 hours, the extracts were strained into a fresh beaker with the help of Whatman filter paper. The water-based extract was dehydrated with an LGJ-Freeze-dryer. The samples were placed in containers and stored for later use. The rats were given the preparations orally via gavage at a dosage of 5 ml/kg of body weight.

Approval for Ethical clearance.

Approval for this research was requested and granted by the Animal Ethics and Research Committee of IBBU, Lapai, Niger state of Nigeria, prior to initiating the experimental study.

Dosage, preparation, and administration of substances for testing.

Acetaminophens inform of Emzor paracetamol tablets (500mg) was bought from a registered pharmacy in Lapai, Niger State for use in this research. The concentrated solution for administering to the Wistar rats was made by mixing 500mg of paracetamol tablets with 50mL of distilled water, following the specified concentration based on their body weight

Animals

Experimental animals

Animals used in experiments

Approximately 60 Wistar rats, including both males and females, weighing around 180 ± 5.00g, were obtained from the Animal house of the Biochemistry Department of Federal University of Technology, Bosso Campus, Minna, Niger State of Nigeria. The animals were housed in polypropylene cages with temperature held at 27±2℃ and relative humidity ranging from 46-53%. The animals had unrestricted access to water and were provided with unlimited pelletized commercial grower feed from Vital Feeds, Jos Nigeria. The study was conducted in accordance with the review protocol (1997) established by the Canadian Council on Animal care and use guidelines.

Design of the experiment

A sum of 30 healthy adult wistar rats, including 15 males and 15 females, were randomly allocated into 5 groups consisting of 6 rats each. The dosing and method of delivery are outlined in (Table 1). Every animal in the group received treatment for 28 consecutive days. By the conclusion of this timeframe, blood samples were taken from every rat, and their sera were isolated and evaluated for biochemical metrics. Afterwards, the animals were slaughtered, with their liver and small intestine taken out for histopathological analysis.

Table 1: *(Experimental design showing the groups of Wistar rats and the treatments they were given.)*

GROUP	NO. OF ANIMALS	TREATMENT
Grp1	6	2mL of normal saline alone
Grp2	6	2mL of standard liver drug alone
Grp3	6	2mL of saline + 3ml of acetaminophen alone
Grp4	6	500kg/kg/day of aqueous extract alone
Grp5	6	500kg/kg/day of aqueous extract + 3ml/day of acetaminophen

Collection of serum and estimation of liver function

Blood samples were collected into a test tube without anticoagulant and left to clot for 10 minutes at room temperature. Following coagulation, the blood was spun at 0ºC for the serum to be obtained. The collected serum was kept at a temperature of 80ºC until it was needed in the future.

Biochemical analyses

Biochemical markers such as ALT, AST, ALP, total protein, albumin, globulin, bilirubin, glucose, creatinine, and urea were analyzed using a commercial kit (AGAPE, Switzerland) and a UV-visible spectrophotometer at specified wavelengths.

Histopathology Analysis

The animal studies utilized the standard histological technique with enhanced modification in histochemical methods (Avwioro, 2010). A thorough inspection was conducted on each organ of interest, including liver and small intestines. The tissues were sliced to a thickness of 3–5mm after being examined. The sliced tissues were treated in a Hestion-ATP7000 tissue processor from Germany for dehydration, clearing, and impregnation. Preparation of embedding was done using molten paraffin wax with the assistance of the embedding machine (Sakura Tissue-Tek 5). Serial ribbons were produced by obtaining sections at 3-5 microns with the digital rotary microtome (Hestion ERM 4000 Germany). Staining was performed following the Hematoxylin and Eosin technique.

Data analysis based on statistics.

The mean ± SD are used to present the results of biochemical parameters. Significance of mean discrepancies within all groups was assessed through one-way analysis of variance (ANOVA) with Duncan's test, where $P<0.05$ was deemed significant, utilizing the statistical analysis tool SPSS (SPSS, 2017).

RESULTS

Biochemical test

Table 2: *(Biochemical parameters of liver: ALP, AST, and ALT.)*

Group	ALT (U/L)	AST (U/L)	ALP (U/L)

G1	19.70±0.79[a]	32.24±1.20[a]	55.64±1.76[a]
G2	26.64±0.86[b]	38.87±0.88[b]	62.55±1.70[b]
G3	56.69±1.44[c]	56.48±1.15[c]	82.85±1.55[c]
G4	18.68±0.80[a]	31.06±1.02[a]	53.91±1.67[a]
G5	24.48±1.19[b]	38.80±0.78[b]	63.82±1.15[b]

Values are presented as mean ± standard error of mean (SEM) of three replicates. Values with different along column are significantly different at p < 0.05

Table 2 demonstrates a notable rise (P<0.05) in the plasma concentrations of the liver enzymes AST, ALT, and ALP in group 3 in comparison to the other groups. Group 3 showed a notable rise (P<0.05) in the plasma concentrations of the liver enzymes AST, ALT, and ALP when contrasted with group 1. The levels of AST, ALT, and ALP in group 2 showed a significant rise (P<0.05) compared to group 1 and a decrease when compared to group 3. There was no significant difference in the plasma AST, ALT, and ALP levels between group 1 and group 4, but they were significantly lower in comparison to group 3. There were no notable differences in the plasma levels of AST, ALT, and ALP between group 5 and group 2 rats. Groups 1 and 4 experienced a notable drop in plasma concentrations of AST, ALT, and ALP in comparison to group 5.

Table 3: *(Kidney Function Markers (Glucose, Creatinine, and Urea.)*

Group	Glucose (g/dL)	Creatinine (mg/dL)	Urea (mg/dL)
G1	90.04±0.78[b]	8.63±0.86[a]	33.65±1.13[a]
G2	88.95±0.82[b]	13.10±1.06[bc]	41.95±2.08[b]
G3	83.39±1.08[a]	17.10±1.10[c]	48.49±2.04[c]
G4	91.25±1.15[b]	11.92±1.24[ab]	32.35±1.92[a]
G5	88.03±1.36[b]	16.75±1.91[c]	41.63±1.76[b]

Values are presented as mean ± standard error of mean (SEM) of three replicates. Values with different along column are significantly different at p < 0.05.

Table 3 indicated a notable reduction (P<0.05) in the plasma glucose levels in group 3 in comparison to the other groups. No notable variance (P<0.05) was observed in the plasma glucose levels among groups 1, 2, 4, and 5 rats. Group 3 rats had significantly higher plasma Creatinine levels (P<0.05) than groups 1, 2, and 4 rats, but did not show a significant difference compared to group 5 rats. Table 3 also indicates that group 2 had similar plasma Urea levels as group 5, group 1 had comparable levels as group 4, but group 3 had significantly higher levels compared to the other groups.

Table 4: *(Serum Total Protein, Albumin, Globulin, and Bilirubin.)*

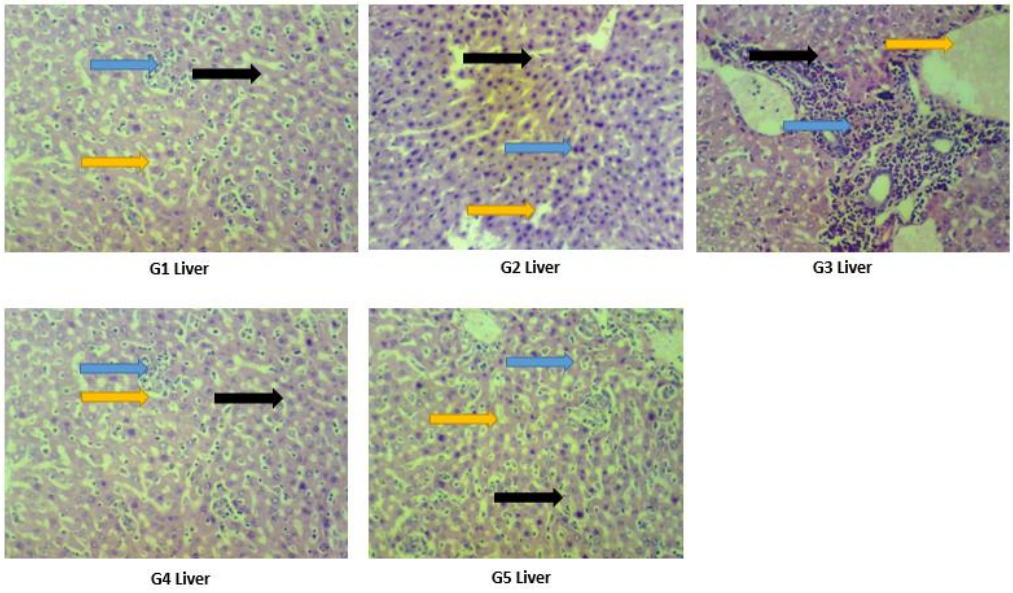
Group	Total protein (g/L)	Albumin (g/L)	Globulin (g/L)	Bilirubin (mg/dL)

G1	17.43±1.08^{c}	12.39±1.16^{c}	5.04±0.22^{b}	0.81±0.01^{b}
G2	9.43±0.26^{b}	6.78±0.13^{b}	2.65±0.14^{a}	0.95±0.02^{c}
G3	4.87±0.39^{a}	2.60±0.43^{a}	2.27±0.22^{a}	1.45±0.04^{d}
G4	22.13±1.24^{d}	16.95±0.92^{d}	5.19±0.32^{b}	0.67±0.03^{a}
G5	14.46±1.26^{c}	10.03±1.01^{c}	4.44±0.53^{b}	0.83±0.07^{b}

Values are presented as mean ± standard error of mean (SEM) of six replicates. Values with different along column are significantly different at p < 0.05.

Table 4 demonstrates a notable reduction (P<0.05) in the plasma concentrations of Total protein and Albumin in group 3 in comparison to the other groups. Nonetheless, no notable distinction was observed between group 1 and 5 rats. A notable discrepancy (P<0.05) was observed in the plasma concentrations of Globulin between group 2 and 3 rats, as well as among group 1, 4, and 5 rats. There was a notable distinction (P<0.05) between group 2 and 3 rats in comparison to group 1, 4, and 5 rats. In group 3, the levels of Bilirubin in the plasma were notably higher (P<0.05) compared to the other groups, while groups 1 and 5 did not display any significant variance.

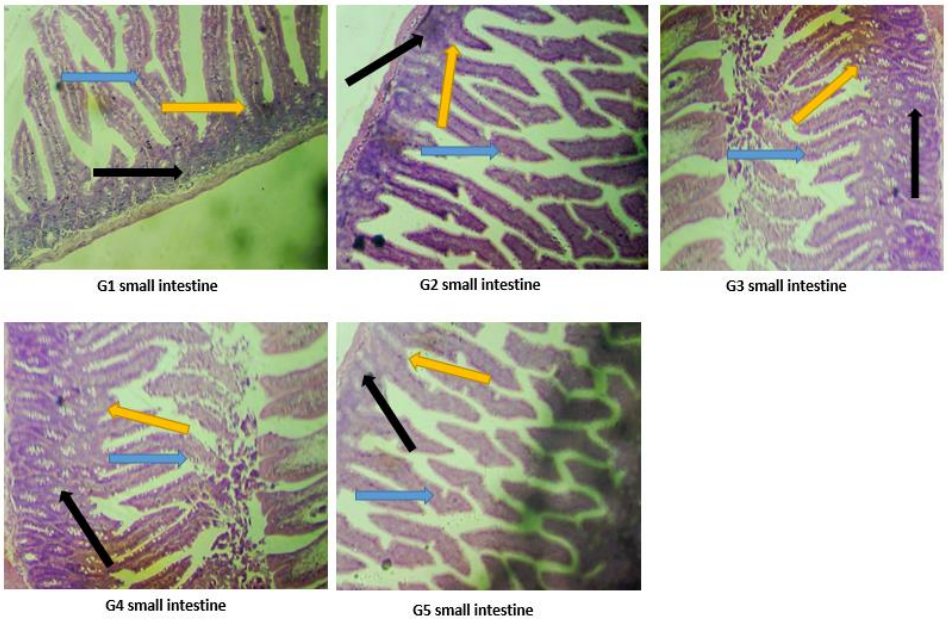
Histopathological analysis



(Blue arrow represents hepatocytes (HC), yellow arrow represents hepatocytes sinusoids (HS), and black arrow represents hepatic portal vein (HV).)

Figure1: Representative G1-G5 Histopathological analysis photomicrograph of liver tissue of Wistar rats administrated with different treatments. G1 shows hepatic tissue with preserved architecture composed of cords of normal hepatocytes, normal portal vein, and sinusoids. There are no features of acute or chronic damage. G2 shows hepatic tissue with preserved architecture composed of cords of normal hepatocytes, normal portal vein with mild enlargement of the sinusoids. G3 shows hepatic tissue

with distorted hepatocytes, portal vein, and sinusoids accompanied with massive necrosis. G4 shows hepatic tissue with preserved architecture composed of cords of normal hepatocytes, normal portal vein, and sinusoids. There are no features of acute or chronic damage. G5 shows hepatic tissue with preserved architecture composed of cords of normal hepatocytes, normal portal vein However, mild enlargement of sinusoids was observed.



(Blue arrow represents serosa (S), yellow arrow represents mucosa (M), and black arrow represents muscularis external (ME).)

Figures 2: Representative G1-G5 Histopathological analysis photomicrograph of small intestine of Wistar rats administrated with different treatments. G1 shows preserved layers of muscosa, submucosa, muscularis external, and serosa. There are no features of acute or chronic toxicity. G2 shows preserved layers of muscosa, submucosa, muscularis externa, and serosa. There are no features of acute or chronic toxicity. G3 shows preserved layers of muscosa, submucosa, muscularis externa, and serosa. There are no features of acute or chronic toxicity. G4 shows preserved layers of muscosa, submucosa, muscularis externa, and serosa. There are no features of acute or chronic toxicity. G5 shows preserved layers of muscosa, submucosa, muscularis externa, and serosa. There are no features of acute or chronic toxicity.

DISCUSSIONS

Table 2: Group 3 rats showed a noticeable increase in serum liver marker enzyme levels. Multiple research studies have noted a comparable increase in the levels of serum liver enzymes when paracetamol is administered (Rawi, et al, 2011) (Gometi, et al, 2014). The increased liver enzyme levels in group 3 rats could be caused by damage to the liver cells from paracetamol toxicity, as liver damage is frequently linked to changes in enzyme levels in the blood. Furthermore, a rise in ALP activity could suggest cell membrane peroxidation and compromised membrane integrity. Miikue-Yobe et al. (2015) stated that ALT plays a role in gluconeogenesis and its expression is inhibited by insulin, so elevated levels could indicate issues with insulin signalling rather than liver damage. The extract treatment led to a notable reduction in transaminases and ALP activities, with statistical significance at $p<0.05$. The

intensity of this impact did not depend on the dosage. According to Patrick et al. (2008), plant extracts have been found to impede transaminase activity. The decline could be linked to the liver-protecting and antioxidant properties of the extract (Patrick, et al, 2008). Hassan et al. (2015) discovered that antioxidants can decrease the occurrence of chemically induced liver injury. The enhanced levels of antioxidants in group 3 rats treated with the extract may have played a role in these outcomes. The rise in serum protein levels may indicate hepatoprotective effects, as it accelerates the regeneration and production of liver cells by stimulating protein synthesis (Patrick, et al, 2008). This study's findings agree with a previous study by Chen and Yen (2007) that showed how the ethanol extracts of N. laevis stem and leaves can affect serum liver marker enzymes and antioxidant activities in diabetic rats.

Table 3: The kidneys are the primary organs responsible for excretion, and tests to assess renal function are used to identify potential kidney damage. Elevated levels of urea and creatinine in the bloodstream are some of the most reliable markers for kidney damage. The study found that rats in group 3 had markedly higher levels of urea and creatinine ($p< 0.05$), suggesting kidney damage. Elevations are caused by hyperglycemia. Alarcon et al. (2002) found that elevated plasma urea and creatinine levels were observed as a result of paracetamol-induced hyperglycemia. Abdulazeez et al. (2013) proposed that the elevated serum urea in group 3 rats may be attributed to the activation of gluconeogenesis in the absence of insulin, serving as a substitute for glucose. Increased protein breakdown in the liver provides glucogenic amino acids for gluconeogenesis, leading to elevated urea levels (Abdulazeez, et al, 2013). Giving the extracts to rats in group 4 for 28 days caused a significant and dose-dependent decrease in these indicators ($p<0.05$). The stabilization of these factors shows betterment in kidney function, which may be due to the extract's antihyperglycemic properties.

Table 4: The total protein in serum indicates how the liver is functioning and helps in determining the extent of liver damage (Imo, et al, 2014). Lower serum protein levels in group 3 rats could be due to hepatocyte damage caused by paracetamol and prolonged high blood sugar levels. The cause could also be a higher rate of amino acid transforming into glucose and decreased production of ribosomal proteins because of the lack of insulin. Protein can also be harmed directly by the specific interaction of oxidants or free radicals with amino acids that are particularly prone to damage (Narasmihanaidu, and Ponnaian, 2006). Rawi, et al (2011) reported a strong correlation between insulin levels and protein synthesis. Even though insulin levels were not specifically analysed in this research, the notable decrease in blood sugar levels can be seen as clear proof of heightened insulin levels in group 3 rats that were treated. Administration of the extract to group 4 rats for 28 days resulted in a noticeable enhancement of serum total protein levels, correlating with the dosage ($p < 0.05$). The level of total bilirubin is also a significant indicator of liver function as it is processed in the liver to be potentially eliminated through the kidneys or bile. Group 3 rats showed elevated total bilirubin levels, suggesting impaired liver function. This finding aligns with a previous study by Shah and Khan in 2014. Liver damage can lead to an increase in plasma bilirubin due to decreased liver uptake, conjugation, or an increase in bilirubin formation (Rana, et al, 1996). Giving group 4 rats the extract for 28 days resulted in a significant decrease ($p<0.05$) in total bilirubin levels in a dose-dependent manner, indicating enhanced liver function. Sunmonu and Afolayan (2013) also noted a notable decrease in bilirubin levels in STZ-induced diabetic rats after receiving Artemisia afra aqueous extract treatment for 15 days.

Assessing tissues through histopathology is a crucial technique in biomedical studies, especially in research involving experimental animals studying the effects of medications or chemicals on human diseases. Histochemical staining is a crucial technique for examining tissues. Multiple histological and

histochemical staining methods are employed to identify the chemical constituents of tissues. An illustration of this is that H&E is a common stain utilized for examining tissues in histology. Additional unique histochemical techniques include periodic acid Schiff (PAS), which can attach to particular biochemical components and act as useful markers utilized to assess histological changes. PAS is clearly a specialized dye that is utilized to detect glycogen in the liver and neutral mucins in other organs (Meyerholz et al., 2018). Treatment of rats with therapeutic dose of Acetaminophen for a period of 28 days resulted in significant depletion of glycogen in hepatocytes in the liver. The histochemical alteration may result from Acetaminophen's direct impact on the enzymes responsible for glycogenesis, glycolysis, or glucose absorption. The liver cells located near blood vessels showed a greater impact. This alteration pertains to glycolysis, which experienced a greater impact compared to glycogenesis following Acetaminophen treatment. Depletion of glycogen in rats treated with Acetaminophen was linked to the damaging impact of reactive metabolites. These results align with the conclusions of a prior study conducted on mice that were administered Acetaminophen at a dosage of 500 mg/kg intraperitoneally. (Hinson et al. 1983). Treatment with G5 aqueous extract in rats prevents glycogen depletion induced by Acetaminophen by enhancing detoxification through scavenging reactive metabolites (Hinson et al. 1983). Glycogen is a quick source of fuel located in the cytoplasm of cardiac cells (2%), skeletal muscle cells (1-2%), and liver cells (5-6%). The glycogen particles in liver cells may be ten times bigger than the ones in muscle cells (Murray & Rosenbloom, 2018).

CONCLUSION

Our findings show that administering a therapeutic dosage of acetaminophen to rats for 28 days can cause significant changes in liver histology and biochemistry. Moreover, the current findings offer compelling proof that combining Zainacin Dadin duniya extract can prevent and improve liver damage caused by acetaminophen, potentially through scavenging free radicals or blocking their production. Additional research is needed to evaluate the hepatoprotective benefits of plant extracts against acetaminophen toxicity in order to verify their protective properties.

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