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EVALUATION OF METHANOLIC EXTRACT OF SILYBUM MARIANUM FOR HEPATOPROTECTIVE ACTIVITY IN RATS.

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Abstract:

The liver is a vital organ involved in metabolism, detoxification, protein synthesis, and maintenance of physiological homeostasis. Excessive exposure to hepatotoxic agents such as paracetamol can produce hepatic injury primarily through the formation of reactive metabolites and increased oxidative stress. The present study was undertaken to evaluate the hepatoprotective and antioxidant potential of a methanolic plant extract against paracetamol-induced hepatotoxicity in experimental animals. The animals were divided into five groups, consisting of six animals in each group. Group I served as the normal control and received distilled water orally. Group II served as the toxic control and received paracetamol at a dose of 2 g/kg, orally, to induce hepatotoxicity. Group III received the standard hepatoprotective drug silymarin at 50 mg/kg, orally. Groups IV and V received the methanolic extract at doses of 200 and 400 mg/kg, orally, respectively. Following treatment, biochemical parameters were evaluated to determine the protective effect of the test extract against paracetamol-induced hepatic injury.

Paracetamol administration produced marked alterations in hepatic biochemical parameters, characterized by an increase in serum SGOT (AST), SGPT (ALT), ALP, and total bilirubin, together with a reduction in total protein, albumin, and globulin levels compared with the normal control group. Paracetamol also produced considerable oxidative stress, as demonstrated by a significant elevation of malondialdehyde (MDA) and depletion of endogenous antioxidant defenses, including reduced glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx).

Treatment with silymarin significantly attenuated the paracetamol-induced alterations and restored the biochemical and antioxidant parameters toward normal values. The methanolic extract at both 200 and 400 mg/kg produced significant hepatoprotective effects, as evidenced by reduction of elevated hepatic enzyme and bilirubin levels and improvement in serum protein parameters. The extract also significantly reduced MDA levels and restored GSH, SOD, CAT, and GPx activities. The protective effect was more pronounced at 400 mg/kg than at 200 mg/kg, indicating a dose-related response.

The hepatoprotective effect of the methanolic extract may be associated with its phytochemical constituents, particularly phenolic and flavonoid compounds, which may contribute to free-radical scavenging, inhibition of lipid peroxidation, and preservation of endogenous antioxidant defenses. Overall, the findings suggest that the methanolic extract possesses significant hepatoprotective and antioxidant activity against paracetamol-induced

hepatic injury. Further studies involving histopathological evaluation, isolation of active phytoconstituents, and molecular investigations are warranted to establish the precise mechanisms responsible for the observed protective effects.

Keywords: *Paracetamol, hepatotoxicity, hepatoprotective activity, methanolic extract, silymarin, oxidative stress, MDA, GSH, SOD, catalase, glutathione peroxidase.*

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INTRODUCTION:

The liver is the largest glandular organ in the body, and has more functions than any other human organ. A person's entire blood supply passes through the liver several times a day; The Liver has a pivotal role in human metabolism. Liver produces and secretes bile, it also produces prothrombin and fibrinogen, both blood clotting factors, and heparin, a mucopolysaccharide sulfuric acid ester that helps keep blood from clotting within the circulatory system. The liver converts sugar into glycogen, Liver diseases have become one of the major causes of morbidity and mortality in man and animals all over globe and hepatotoxicity due to drugs appears to be the most common contributing factor [1]. Among the many diseases that can affect the liver the most common is 'viral hepatitis' (Inflammation of liver caused by viral infection). Hepatitis can be caused by drugs, viruses, bacteria, mushrooms, parasites like amoebas or giardiasis. About 20,000 deaths found every year due to liver disorders. The use of natural remedies for the treatment of liver diseases has a long history and medicinal plants and their derivatives are still used all over the world in one form or the other for this purpose. Scientific evaluation of plants has often shown that active principles in these are responsible for therapeutic success. A large number of medicinal plants have been tested and found to contain active principles with curative properties against a variety of diseases [2]. Liver protective plants contain a variety of chemical constituents like phenols, coumarins, lignans, essential oil, monoterpenes, carotinoids, glycosides, flavanoids, organic acids, lipids, alkaloids and xanthenes.

In body, such as metabolism, secretion, storage and detoxifying. Liver damage is usually associated with the distortion of some of these functions. The liver is continuously exposed to an elevated number of toxic agents, because the portal vein supplies blood to this organ after intestinal absorption.

The World Health Organization (WHO) determined that around 2.4 million deaths yearly are linked to some liver disease, and that around 800 thousand of these deaths are attributable to cirrhosis [3] On the other hand, epidemiological studies conducted by the National Institute of Statistics and Geography (INEGI by its Spanish acronym) indicate that in 2013 in Mexico, over 600 thousand deaths were recorded. The main causes were diabetes mellitus (14.25%), followed by ischemic heart diseases (12.63%), cerebrovascular diseases (5.29%) and liver diseases (4.79%). Despite the advances in modern medicine and the development of new hepatoprotective drugs the incidence of hepatic diseases has not decreased or stopped; on the contrary, statistics suggest that these continue to increase.

Metabolism or biotransformation of hepatotoxic agents is a detoxifying process where molecules are surgically modified into less toxic shapes by different enzymatic systems. These modifications can generate metabolic products with varying degrees of pharmacological activity or inactive metabolites. There are different types of metabolic reactions: phase 1 reactions are usually oxidations, reductions or hydrolysis (modifying the structure of the reactive group); phase 2 reactions are those in which the drug conjugates with glucuronic acid, sulfates, acetates, methyl groups, glutathione or amino acids, generally to increase its solubility and be excreted. The liver's ability to be able to carry out the different oxidative metabolisms is associated with the high cytochrome P450 cell content.

Due to the high metabolite biotransformation rate, free radicals can be generated continuously. Most hepatotoxic substances, mainly damage the liver because of the generated oxidative stress; oxygen reactive species induced a rise in lipid peroxidation, a reduction of ATP and oxidative damage in the DNA and proteins

Protecting the liver from the harmful effects of hepatotoxins- which may be ingested- or counteracting the alterations in the antirradical defense mechanisms, is very important; the agents capable of doing this are called hepatoprotective.

For this reason, researches have been developed in the search of natural and/or synthetic compounds with hepatoprotective activity. The development of new pharmaceuticals consists of a variety of steps, going from the discovery of pharmacological side effects in cellular and animal models, to finally prove its efficacy and safety in human beings [4].

Plants and plant extracts have been used for thousands of years for the treatment of disease. More recently1 there has been renewed interest in the use of natural products because of their reduced side effects on the human body in comparison to synthetic medicinal drugs. Plants from the Cruciferae family have been widely used in folk medicine. Many of the plants belonging to this family are of economic importance, being either a very common food produce, or constitute important articles in oil4 production. Schouwia thebica Webb, a member of this family, has been used by Bedouins for the treatment of liver disorders. Our study has been carried out to validate this activity and to isolate the compounds responsible for such activity.

Medicinal plants play a key role in the human health care. About 80% of the world population rely on the use of traditional medicine which is predominantly based on plant materials [5]. The

traditional medicine refers to a broad range of ancient natural health care practices including folk/tribal practices as well as Ayurveda, Siddha, Amchi and Unani. These medical practices originated from time immemorial and developed gradually, to a large extent, by relying or based on practical experiences without significant references to modern scientific principles. These practices incorporated ancient beliefs and were passed on from one generation to another by oral tradition and/or guarded literature. Although herbal medicines are effective in the treatment of various ailments very often these drugs are unscientifically exploited and/or improperly used.

Therefore, these plant drugs deserve detailed studies in the light of modern science. It is estimated that about 7,500 plants are used in local health traditions in, mostly, rural and tribal villages of India. Out of these, the real medicinal value of over 4,000 plants is either little known or hitherto unknown to the mainstream population. The classical systems of medicine such as Ayurveda, Siddha, Amchi, Unani and Tibetan use about 1,200 plants [6]. A detailed investigation and documentation of plants used in local health traditions and pharmacological evaluation of these plants and their taxonomical relatives can lead to the development of invaluable plant drugs for many dreaded diseases. Random screening of plants has not proved economically effective [7].

Liver diseases and medicinal plants: Liver has a pivotal role in regulation of physiological processes. It is involved in several vital functions such as metabolism, secretion and storage. Furthermore, detoxification of a variety of drugs and xenobiotics occurs in liver. The bile secreted by the liver has, among other things, an important role in digestion. Liver diseases are among the most serious ailment. They may be classified as acute or chronic hepatitis (inflammatory liver diseases), hepatosis (non inflammatory diseases) and cirrhosis (degenerative disorder resulting in fibrosis of the liver). Liver diseases are mainly caused by toxic chemicals (certain antibiotics, chemotherapeutics, peroxidised oil, aflatoxin, carbon-tetrachloride, chlorinated hydrocarbons, etc.), excess consumption of alcohol, infections and autoimmune/disorder. Most of the hepatotoxic chemicals damage liver cells mainly by inducing lipid peroxidation and other oxidative damages in liver. Enhanced lipid peroxidation produced during the liver microsomal metabolism of ethanol may result in hepatitis and cirrhosis [8]. It has been estimated that about 90% of the acute hepatitis is due to viruses. The major viral agents involved are Hepatitis B, A, C, D (delta agents), E and G. Of these, Hepatitis B infection often results in chronic liver diseases and cirrhosis of liver. Primary liver cancer has also been shown to be produced by these viruses. It has been estimated that approximately 14- 16 million people are infected with this virus in South East Asia region and about 6% of the total population in the region are carriers of this virus. A vaccine has become available for immunization against Hepatitis B virus. Hepatitis C and Hepatitis E infections are also common in countries of South East Asia region [9-10].

Hepatoprotective agents are substances that protect the liver from damage and help maintain normal hepatic function. The liver plays a vital role in metabolism, detoxification, and synthesis of essential biomolecules, making it highly susceptible to injury from drugs, toxins, alcohol, infections, and metabolic disorders. Hepatoprotective agents help safeguard liver cells and support overall liver health.

These agents exert their protective effects through various mechanisms, including antioxidant activity, which reduces oxidative stress and neutralizes free radicals, and inhibition of lipid peroxidation, which prevents damage to hepatocyte membranes. They also stabilize liver cell membranes, enhance endogenous antioxidant defenses such as glutathione, and modulate detoxifying enzymes involved in hepatic metabolism.

In addition, hepatoprotective agents possess anti-inflammatory and anti-fibrotic properties that reduce liver inflammation and prevent the progression of fibrosis. By inhibiting inflammatory mediators and collagen deposition, they help limit chronic liver damage. Some agents also prevent apoptosis and improve bile secretion, contributing to better liver function.

Hepatoprotective drugs and herbal medicines are widely used in the treatment and prevention of liver disorders such as hepatitis, drug-induced hepatotoxicity, alcoholic liver disease, and fatty liver disease. Their ability to protect and regenerate liver tissue makes them an essential component in the management of both acute and chronic liver conditions [11-13].

MATERIALS AND METHODS

Collection and Identification of Plant Material

The Fresh Silybum Marianum are collected and identified was purchased from local market Pradesh, India, and authenticated by Dr. K. Madhava Chetty, Assistant Professor, Department of Botany, S.V University, Tirupati.

Preparation of the Extract

The Collected plant was wasted, air dried at room temp 7-14 days. were pulverized with the help of electric grinder the powder was further Sieved to get a fine powder and stored in air tight Container. The powder was extracted using Soxhlet apparatus.

Extraction procedure

Coarsely powdered Silybum Marianum were used for extraction with Ethanol by using Soxhlet method for 6-8 hours. 50g of dried powder was

weighed and 500mL of solvent Ethanol is used for the extraction. The extracts were evaporated by using a rotary evaporator and dried at room temperature. The obtained crude extracts were weighed and stored at 4°C for further analysis.

Acute toxicity [47]

The acute oral toxicity study of the methanolic extract of Silybum marianum was carried out in Wistar rats according to the guidelines of the Organisation for Economic Co-operation and Development (OECD 423). A single oral dose of the extract (300 mg/kg followed by 2000 mg/kg) was administered after overnight fasting, and the animals were observed continuously for the first 4 hours, periodically during the first 24 hours, and daily for 14 days for signs of toxicity and mortality. Behavioral changes such as tremors, convulsions, salivation, lethargy, and changes in food and water intake were monitored along with body weight variations. At the end of the observation period, animals were examined for gross pathological changes in vital organs. The absence of mortality or significant toxic symptoms at 2000 mg/kg indicated that the extract is relatively safe, with an LD_{50} greater than 2000 mg/kg, and doses for further pharmacological studies were selected accordingly.

Induction of Hepatotoxicity [14]

Paracetamol (acetaminophen) is commonly used as an analgesic and antipyretic, but at high doses it induces hepatotoxicity in experimental animals. Overdose of paracetamol leads to the formation of the reactive metabolite N-acetyl-p-benzoquinone imine (NAPQI), which depletes hepatic glutathione, binds to cellular proteins, and causes oxidative stress, resulting in centrilobular necrosis and hepatocyte degeneration. In experimental studies, hepatotoxicity is typically induced in adult Wistar rats (150–200 g) by administering a single oral dose of paracetamol, commonly 2 g/kg, after overnight fasting. Following administration, rats are observed for 24–48 hours for signs of liver damage, including behavioral changes and alterations in food and water intake. Biochemical parameters such as SGOT (AST), SGPT (ALT), ALP, total and direct bilirubin, and total protein are measured to assess liver function, while oxidative stress markers like malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH) are evaluated. Histopathological examination of liver tissue further confirms hepatocellular damage, and hepatoprotective activity is assessed by comparing rats treated with the test extract (Silybum marianum) or standard silymarin against the paracetamol-induced control group.

Animals [15]

In this study, healthy adult Wistar rats of either sex, weighing between 150–200 g, were used to evaluate the hepatoprotective activity of the methanolic extract of *Silybum marianum.* The animals were procured from a recognized laboratory animal facility and housed under standard laboratory conditions, including a temperature of 22 ± 2 °C, relative humidity of 50–60%, and a 12-hour light/dark cycle. They were provided with standard laboratory pellet diet and water ad libitum throughout the study. Prior to the experiment, the rats were acclimatized for 7 days to minimize stress and ensure uniform physiological conditions. All experimental protocols were conducted in accordance with institutional animal ethics committee guidelines and the principles of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), ensuring humane care and use of laboratory animals.

Pre-treatment [16]

In hepatoprotective studies, pre-treatment refers to the administration of the test extract or standard drug prior to the induction of hepatotoxicity, in order to evaluate its protective effect against liver injury. For Silybum marianum, rats are typically pre-treated with the methanolic extract at selected doses (e.g., 200 mg/kg and 400 mg/kg) orally once daily for 7–14 days. On the final day of pre-treatment, hepatotoxicity is induced using agents such as paracetamol (2 g/kg, orally) or CCl_4 (1 mL/kg, 50% in olive oil). After 24–48 hours of hepatotoxin administration, animals are assessed for biochemical markers (SGOT, SGPT, ALP, bilirubin), oxidative stress parameters (MDA, SOD, CAT, GSH), and histopathological changes in the liver. Pre-treatment allows the extract to exert its antioxidant and cytoprotective effects, stabilizing hepatocyte membranes, scavenging free radicals, and preventing the depletion of endogenous protective molecules, thereby mitigating liver injury compared to untreated controls.

Selection of the Doses for Animal Study [17]

The selection of appropriate doses is a critical step in designing animal studies to evaluate the hepatoprotective activity of plant extracts such as *Silybum marianum.* Initially, an acute toxicity study is conducted according to OECD guidelines (OECD 423 or 425) to determine the safety and maximum tolerable dose (LD_{50}) of the extract. Typically, no mortality or significant toxic effects are observed at doses up to 2000 mg/kg body weight, indicating that the extract is relatively safe. Based on these findings, sub-therapeutic and therapeutic doses are selected for pharmacological evaluation, commonly 1/10th and 1/5th of the maximum safe dose. For example, doses of 200 mg/kg and 400 mg/kg are often used for

hepatoprotective studies in rats. These doses are administered orally, either as a pre-treatment to evaluate preventive activity or as a post-treatment to assess curative effects against hepatotoxin-induced liver injury. Dose selection ensures efficacy while minimizing the risk of toxicity and allows a dose-dependent evaluation of pharmacological effects.

Animal grouping [18]

Group	Treatment	Dose	Route
I Group	Normal Control	Distilled water	Oral
II Group	Toxic Control	Paracetamol 2 g/kg, orally	
III Group	Standard Drug	Silymarin 50 mg/kg, orally	
IV Group	Test Extract	Methanolic extract 200 mg/kg, orally	
V Group	Test Extract	Methanolic extract 400 mg/kg, orally	

Parameters

Serum Biochemical Parameters (Liver Function Tests)

SGOT (AST – Aspartate Aminotransferase) [19]

Take three clean, dry test tubes and label them as Test (T), Control (C), and Standard (S). Add 0.5 ml of substrate reagent (L-aspartate and α-ketoglutarate in buffer) into the Test and Control tubes. To the Test tube, add 0.1 ml of serum sample, mix gently, and incubate at 37°C for 60 minutes. The Control tube is incubated without serum initially; the serum is added only after the addition of DNPH to stop enzymatic activity immediately. After incubation, add 0.5 ml of 2,4-dinitrophenylhydrazine (DNPH) reagent to all tubes and allow them to stand at room temperature for 20 minutes for color development. Then add 5 ml of 0.4 N NaOH to each tube and keep for 10 minutes to stabilize the brown-colored complex formed. Measure the absorbance of the Test and Standard against the reagent blank at 505 nm using a spectrophotometer. The SGOT (AST) activity is calculated in IU/L using the standard calibration or kit-provided factor.

SGPT (ALT – Alanine Aminotransferase)

SGPT (ALT – Alanine Aminotransferase) is an important biochemical marker used to assess liver function and hepatocellular damage in hepatoprotective studies. ALT is a cytoplasmic enzyme predominantly present in liver cells and catalyzes the conversion of L-alanine and α-ketoglutarate into pyruvate and L-glutamate. During liver injury, such as paracetamol-induced hepatotoxicity, damage to hepatocytes causes leakage of ALT into the bloodstream, resulting in elevated serum levels. Since ALT is more specific to the liver compared to AST, its elevation is considered a reliable indicator of hepatic injury. In experimental studies, a significant increase in ALT is observed in the toxic control group, while treatment with a standard drug like Silymarin or a test extract reduces ALT levels toward normal, indicating hepatoprotective activity.

ALP (Alkaline Phosphatase) [20]

Alkaline Phosphatase (ALP) is an important enzyme used as a biochemical marker in the assessment of liver function, particularly in hepatobiliary disorders. It is a membrane-bound enzyme mainly present in the liver, bile ducts, bone, intestine, and placenta. In the liver, ALP is associated with the canalicular and sinusoidal membranes of hepatocytes. In cases of liver injury such as paracetamol-induced hepatotoxicity, damage to the hepatobiliary system or obstruction of bile flow leads to increased synthesis and release of ALP into the bloodstream. Therefore, elevated serum ALP levels indicate cholestasis or biliary dysfunction. In hepatoprotective studies, the toxic control group typically shows a significant rise in ALP levels, while treatment with a standard drug such as Silymarin or a plant extract reduces ALP toward normal values, indicating protective activity against liver damage.

Total Bilirubin [21]

Total Bilirubin is an important biochemical parameter used to assess liver function and the liver's ability to metabolize and excrete bile pigments. Bilirubin is a yellow pigment formed from the breakdown of hemoglobin in red blood cells. It exists in two forms: unconjugated (indirect) bilirubin and conjugated (direct) bilirubin. Total bilirubin represents the sum of both forms in serum. In the liver, unconjugated bilirubin is taken up by hepatocytes, conjugated with glucuronic acid, and excreted into bile. During liver injury, such as paracetamol-induced hepatotoxicity, hepatocellular damage or bile duct obstruction impairs bilirubin metabolism and excretion, leading to elevated serum total bilirubin levels. Increased bilirubin indicates liver dysfunction and may be associated with jaundice. In hepatoprotective studies, the toxic control group typically shows a significant rise in total bilirubin, whereas treatment with a standard drug like Silymarin or a test extract reduces bilirubin levels toward normal, indicating restoration of liver function.

Direct Bilirubin

Direct Bilirubin refers to the conjugated form of bilirubin that has been processed in the liver. After the breakdown of hemoglobin, unconjugated (indirect) bilirubin is transported to the liver, where it is conjugated with glucuronic acid by hepatocytes to form water-soluble conjugated bilirubin. This conjugated bilirubin is then excreted into bile.In liver disorders such as paracetamol-induced hepatotoxicity, damage to hepatocytes or obstruction of bile flow impairs the excretion of conjugated bilirubin, leading to increased serum direct bilirubin levels. Elevated direct bilirubin is mainly associated with cholestasis, biliary obstruction, or severe hepatocellular injury. In

hepatoprotective studies, the toxic control group typically shows a significant rise in direct bilirubin, whereas treatment with a standard drug like Silymarin or a test extract reduces its level toward normal, indicating improvement in hepatic excretory function.

Total Protein [22]

Total Protein is an important biochemical parameter used to evaluate the synthetic function of the liver in hepatoprotective studies. Serum total protein mainly consists of albumin and globulins, which are synthesized primarily in the liver. Albumin maintains oncotic pressure and transports various substances, while globulins are involved in immune responses and transport functions.

In conditions such as paracetamol-induced hepatotoxicity, damage to hepatocytes impairs the liver's protein synthesis ability, leading to a decrease in serum total protein levels. Therefore, reduced total protein indicates compromised liver function. In hepatoprotective studies, the toxic control group typically shows a significant reduction in total protein levels, whereas treatment with a standard drug like Silymarin or a test extract restores protein levels toward normal, indicating improvement in hepatic synthetic capacity.

Albumin [23]

Albumin is the major plasma protein synthesized exclusively by the liver and serves as an important indicator of hepatic synthetic function in hepatoprotective studies. It plays a vital role in maintaining colloidal osmotic pressure and acts as a carrier protein for hormones, fatty acids, bilirubin, and various drugs. In conditions such as paracetamol-induced hepatotoxicity, hepatocellular damage impairs protein synthesis, leading to decreased serum albumin levels. A reduction in albumin indicates compromised liver function and reduced synthetic capacity. In experimental hepatoprotective models, the toxic control group generally shows a significant decline in albumin levels, whereas treatment with a standard drug like Silymarin or a test extract restores albumin concentration toward normal values, indicating improvement in liver function.

Globulin

Globulins are a group of serum proteins that, along with albumin, constitute total serum protein. They are synthesized partly in the liver and partly by the immune system (plasma cells). Globulins include different fractions such as α_1, α_2, β, and γ globulins, which are involved in immune responses, transport of lipids and metal ions, blood clotting, and inflammatory processes. In liver disorders such as paracetamol-induced hepatotoxicity, alterations in globulin levels may occur due to impaired protein synthesis or inflammatory responses. While albumin typically decreases in hepatocellular damage, globulin levels may increase due to immune activation, resulting in a decreased albumin/globulin (A/G) ratio. In hepatoprotective studies, restoration of normal globulin levels and improvement of the A/G ratio in treatment groups (e.g., Silymarin or test extract) indicate recovery of liver function and reduced inflammatory response.

Oxidative Stress / Antioxidant Parameters (Liver Tissue) [24]

MDA (Malondialdehyde) – Lipid peroxidation marker

Malondialdehyde (MDA) is a widely used biochemical marker for assessing lipid peroxidation and oxidative stress in hepatoprotective studies. It is a reactive aldehyde formed as a by-product during the peroxidation of polyunsaturated fatty acids in cell membranes. In conditions such as paracetamol-induced hepatotoxicity, the toxic metabolite (NAPQI) generates excessive reactive oxygen species (ROS), which attack membrane lipids and initiate lipid peroxidation. This process results in increased formation of MDA. Elevated MDA levels in liver tissue indicate oxidative damage and membrane disruption. Therefore, MDA estimation is commonly performed to evaluate the antioxidant and hepatoprotective potential of plant extracts or standard drugs like Silymarin. A significant reduction in MDA levels in treated groups compared to the toxic control indicates protective activity against oxidative stress. MDA is commonly measured using the Thiobarbituric Acid Reactive Substances (TBARS) method, where MDA reacts with thiobarbituric acid (TBA) to form a pink-colored complex that is measured spectrophotometrically at 532 nm.

GSH (Reduced Glutathione) [25]

Reduced Glutathione (GSH) is a major intracellular antioxidant present in liver cells and plays a crucial role in protecting cells against oxidative stress. It is a tripeptide composed of glutamic acid, cysteine, and glycine, and functions as a primary defense molecule against reactive oxygen species (ROS). In paracetamol-induced hepatotoxicity, the toxic metabolite NAPQI binds to and depletes hepatic GSH stores, leading to oxidative damage and cell death. Therefore, a significant decrease in GSH levels is observed in the toxic control group. Restoration of GSH levels in treatment groups (such as those receiving Silymarin or plant extracts) indicates antioxidant and hepatoprotective activity. Thus, estimation of GSH is an important parameter in evaluating protection against oxidative liver injury's is commonly estimated using Ellman's method, where GSH reacts with 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) to form a yellow-colored compound measured spectrophotometrically at 412 nm.

SOD (Superoxide Dismutase) [26]

Superoxide Dismutase (SOD) is an important antioxidant enzyme that protects cells from oxidative damage by catalysing the dismutation of

superoxide radicals (O_2^{-}•) into hydrogen peroxide (H_2O_2) and oxygen (O_2). It serves as the first line of defense against reactive oxygen species (ROS) generated during oxidative stress. In paracetamol-induced hepatotoxicity, excessive formation of reactive oxygen species leads to depletion or inhibition of antioxidant enzymes like SOD. As a result, SOD activity significantly decreases in the toxic control group. Restoration or elevation of SOD levels in treatment groups (such as those receiving Silymarin or plant extracts) indicates antioxidant and hepatoprotective potential. Therefore, SOD estimation is an essential parameter in evaluating protection against oxidative liver damage. SOD activity is commonly measured by its ability to inhibit the reduction of nitroblue tetrazolium (NBT) by superoxide radicals generated in the reaction system, and the absorbance is measured spectrophotometrically at 560 nm.

Catalase (CAT) [27]

Catalase (CAT) is an important antioxidant enzyme present in liver cells that protects tissues from oxidative damage by decomposing hydrogen peroxide (H_2O_2) into water and oxygen. Since hydrogen peroxide is a reactive oxygen species generated during metabolic processes and oxidative stress, catalase plays a crucial role in preventing cellular injury.In paracetamol-induced hepatotoxicity, excessive production of reactive oxygen species leads to depletion or reduced activity of antioxidant enzymes such as catalase. As a result, CAT activity significantly decreases in the toxic control group. Treatment with a standard drug like Silymarin or a plant extract restores catalase activity toward normal levels, indicating antioxidant and hepatoprotective potential. Therefore, estimation of catalase activity is an important parameter in evaluating protection against oxidative liver damage. Catalase activity is commonly measured by monitoring the decomposition of hydrogen peroxide spectrophotometrically at 240 nm.

Glutathione Peroxidase (GPx) [28-29]

Glutathione Peroxidase (GPx) is an important antioxidant enzyme that protects cells from oxidative damage by reducing hydrogen peroxide (H_2O_2) and lipid hydroperoxides into water and corresponding alcohols. It uses reduced glutathione (GSH) as a substrate and converts it into oxidized glutathione (GSSG) during the reaction. In paracetamol-induced hepatotoxicity, excessive production of reactive oxygen species leads to depletion of GSH and reduced GPx activity, resulting in accumulation of peroxides and increased lipid peroxidation. Therefore, GPx activity significantly decreases in the toxic control group. Restoration of GPx levels in treatment groups (such as those receiving Silymarin or plant extracts) indicates antioxidant and hepatoprotective potential. Hence, GPx estimation is an important parameter in assessing protection against oxidative liver injury. GPx activity is commonly measured by monitoring the oxidation of NADPH to $NADP^+$ in the presence of glutathione reductase, and the decrease in absorbance is recorded at 340 nm.

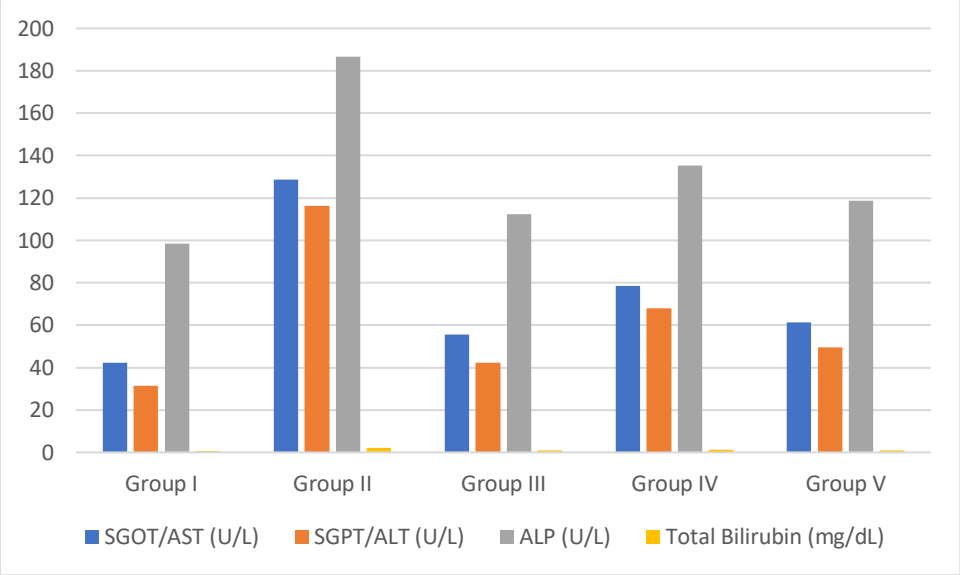
STATISTICAL ANALYSIS

The data obtained from animal experiments are expressed as mean ± SEM (standard error of mean). For statistical analysis data were subjected to analysis of variance (ANOVA) followed by Student's t-test. Values are considered statistically significant from ANOVA by $P < 0.05$ for t-test.

Serum Biochemical Parameters

Group	Treatment	SGOT/AST (U/L)	SGPT/ALT (U/L)	ALP (U/L)	Total Bilirubin (mg/dL)
I	Normal control	42.18 ± 1.52	31.46 ± 1.28	98.32 ± 2.64	0.62 ± 0.03
II	Paracetamol 2 g/kg	128.64 ± 3.86*	116.28 ± 3.42*	186.54 ± 4.72*	2.18 ± 0.08*
III	Silymarin 50 mg/kg	55.72 ± 1.84###	42.36 ± 1.56###	112.46 ± 3.18###	0.86 ± 0.04###
IV	Extract 200 mg/kg	78.46 ± 2.64##	67.82 ± 2.38##	135.28 ± 3.64##	1.24 ± 0.05##
V	Extract 400 mg/kg	61.38 ± 2.12###	49.64 ± 1.76###	118.72 ± 3.26###	0.94 ± 0.04###

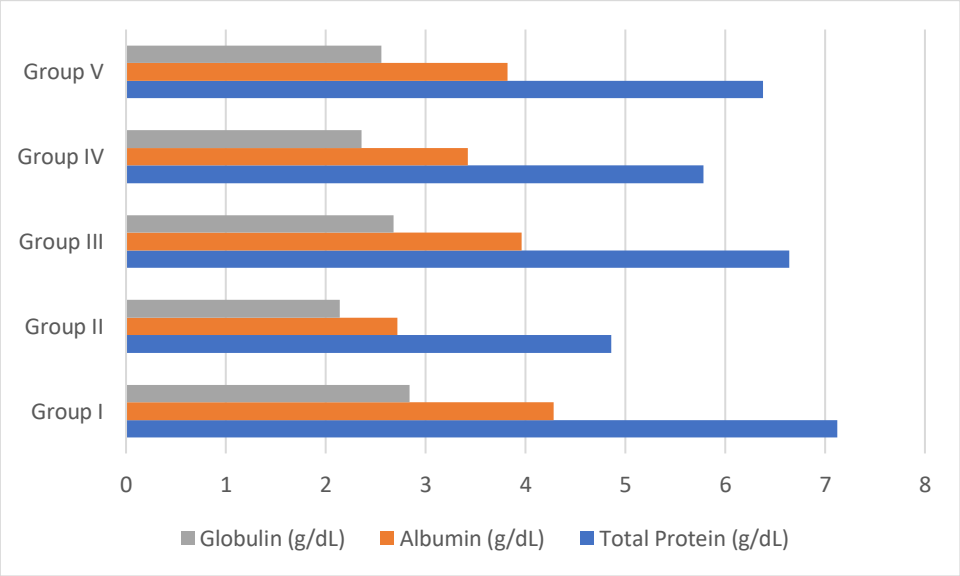
Values are expressed as Mean ± SEM, n = 6. Statistical analysis was performed by one-way ANOVA followed by Dunnett's multiple comparison test. $p < 0.05$ was considered statistically significant.



Serum Protein, Albumin and Globulin

Group	Treatment	Total Protein (g/dL)	Albumin (g/dL)	Globulin (g/dL)
Group I	Normal control	7.12 ± 0.18	4.28 ± 0.12	2.84 ± 0.10
Group II	Paracetamol 2 g/kg	4.86 ± 0.16***	2.72 ± 0.11***	2.14 ± 0.08***
Group III	Silymarin 50 mg/kg	6.64 ± 0.15###	3.96 ± 0.10###	2.68 ± 0.07###
Group IV	Methanolic extract 200 mg/kg	5.78 ± 0.14##	3.42 ± 0.09##	2.36 ± 0.07##
Group V	Methanolic extract 400 mg/kg	6.38 ± 0.16###	3.82 ± 0.11###	2.56 ± 0.08###

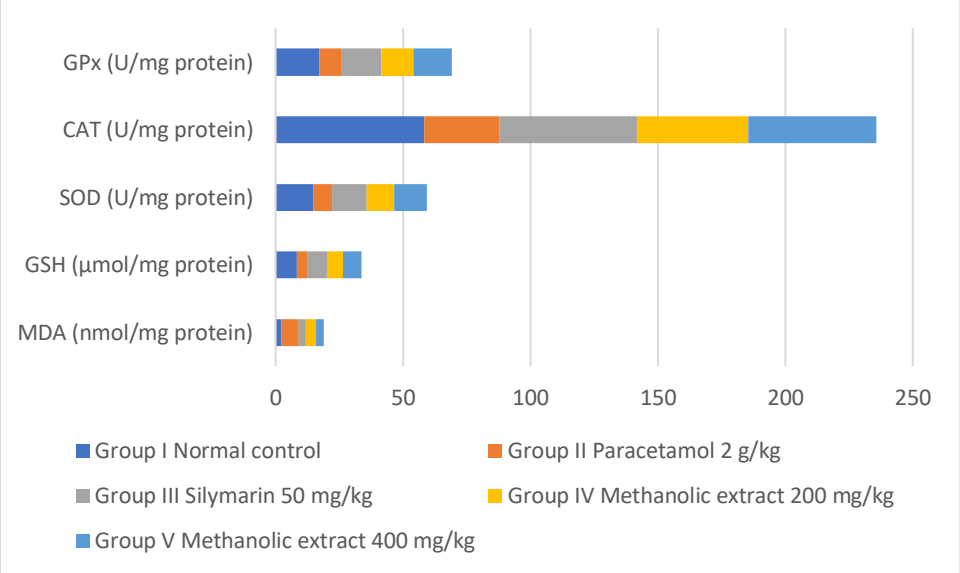
Values are expressed as Mean ± SEM (n=6); ***p<0.001 vs. Group I (normal control), ##p<0.01 and ###p<0.001 vs. Group II (toxic control), one-way ANOVA followed by Dunnett's multiple comparison test.



Oxidative Stress and Antioxidant Parameters

Group	Treatment	MDA (nmol/mg protein)	GSH (µmol/mg protein)	SOD (U/mg protein)	CAT (U/mg protein)	GPx (U/mg protein)
Group I	Normal control	2.18 ± 0.09	8.42 ± 0.24	14.86 ± 0.42	58.32 ± 1.64	17.24 ± 0.48
Group II	Paracetamol 2 g/kg	6.84 ± 0.21***	4.18 ± 0.16***	7.26 ± 0.28***	29.46 ± 1.12***	8.36 ± 0.32***
Group III	Silymarin 50 mg/kg	2.72 ± 0.11###	7.64 ± 0.22###	13.52 ± 0.38###	53.84 ± 1.48###	15.86 ± 0.44###
Group IV	Methanolic extract 200 mg/kg	4.16 ± 0.15##	6.18 ± 0.19##	10.86 ± 0.34##	43.72 ± 1.36##	12.64 ± 0.38##
Group V	Methanolic extract 400 mg/kg	3.02 ± 0.12###	7.18 ± 0.21###	12.76 ± 0.36###	50.28 ± 1.42###	14.92 ± 0.42###

Values are expressed as Mean ± SEM (n=6); *p<0.001 vs. Group I (normal control), ##p<0.01 and ###p<0.001 vs. Group II (toxic control), one-way ANOVA followed by Dunnett's multiple comparison test.



DISCUSSION:

Paracetamol-induced hepatotoxicity is a widely accepted experimental model for investigating the hepatoprotective efficacy of medicinal plants, phytoconstituents, and pharmacological agents. The toxic effects of paracetamol at an excessive dose are primarily associated with the formation of the reactive metabolite N-acetyl-p-benzoquinone imine (NAPQI), which is generated predominantly through cytochrome P450-mediated metabolism. Under normal conditions, the small amount of NAPQI formed is efficiently detoxified by conjugation with reduced glutathione (GSH). However, following administration of an excessive dose of paracetamol, the normal glucuronidation and sulfation pathways become relatively saturated, resulting in increased formation of NAPQI. When hepatic GSH reserves become depleted, NAPQI can bind to cellular proteins and initiate oxidative and mitochondrial injury, ultimately leading to hepatocellular damage.

In the present investigation, administration of paracetamol at a dose of 2 g/kg, p.o., produced a pronounced alteration in the oxidative stress and antioxidant parameters compared with the normal control group. The toxic control animals demonstrated a significant increase in malondialdehyde (MDA), accompanied by

significant reductions in GSH, SOD, CAT, and GPx. This pattern of alteration indicates the development of substantial oxidative stress in hepatic tissue following paracetamol exposure. The observed changes are consistent with the ability of excessive paracetamol metabolism to increase reactive oxygen species (ROS) generation and overwhelm the normal antioxidant defense system.

The significant elevation of MDA in the toxic control group provides evidence of increased lipid peroxidation. MDA is generated as a secondary product during the oxidative degradation of polyunsaturated fatty acids present in biological membranes and is therefore commonly used as an indicator of oxidative damage. Increased ROS production can attack membrane lipids and initiate a chain reaction of lipid peroxidation, resulting in progressive alteration of membrane structure and function. Excessive lipid peroxidation may compromise the integrity of hepatocyte plasma membranes and intracellular organelles, including mitochondria and endoplasmic reticulum. Consequently, the increased MDA observed in the paracetamol-treated animals may reflect the increased oxidative damage associated with hepatocellular injury.

In contrast to MDA, GSH levels were significantly decreased in the toxic control group. GSH is one of the major non-enzymatic antioxidants present in the liver and plays an essential role in maintaining cellular redox balance. It participates directly in the detoxification of electrophilic compounds and reactive oxygen species and serves as a substrate for glutathione-dependent antioxidant enzymes. During excessive paracetamol exposure, hepatic GSH is consumed in the detoxification of NAPQI. Continued oxidative stress may further increase GSH utilization, resulting in depletion of the intracellular antioxidant reserve. The marked reduction in GSH observed in the present study therefore suggests that the hepatic antioxidant capacity was substantially compromised by paracetamol administration.

The toxic control group also showed a significant reduction in SOD activity. SOD constitutes an important first-line enzymatic antioxidant defense mechanism and catalyzes the dismutation of superoxide anions into hydrogen peroxide and molecular oxygen. Under conditions of excessive ROS generation, the antioxidant system may become overwhelmed, resulting in depletion or reduced functional activity of antioxidant enzymes. Decreased SOD activity may consequently allow greater accumulation of superoxide radicals, which can interact with other reactive species and contribute to oxidative modification of cellular lipids and proteins. Restoration of SOD activity following treatment can therefore be considered an important indicator of improvement in the hepatic antioxidant defense system.

A marked reduction in catalase (CAT) activity was also observed following paracetamol administration. Catalase plays a major role in maintaining cellular hydrogen peroxide homeostasis by converting hydrogen peroxide into water and molecular oxygen. When ROS production is increased, excessive hydrogen peroxide may accumulate if the enzymatic antioxidant defense system is inadequate. Reduced CAT activity can therefore contribute to further oxidative injury by allowing hydrogen peroxide and related reactive species to persist within the hepatic cells. The decreased CAT activity in the toxic control animals, together with reduced SOD and GSH, indicates substantial impairment of the enzymatic and non-enzymatic antioxidant defenses.

Similarly, glutathione peroxidase (GPx) activity was significantly reduced in the paracetamol-treated group. GPx is an important antioxidant enzyme that utilizes GSH to reduce hydrogen peroxide and lipid hydroperoxides. Through this mechanism, GPx helps prevent accumulation of reactive oxygen species and limits lipid membrane damage. Depletion of GSH can directly interfere with GPx-dependent antioxidant activity because GSH serves as an essential reducing substrate. Therefore, the simultaneous reduction in GSH and GPx observed in the toxic control group may indicate a compromised glutathione-dependent antioxidant system. The combined decrease in SOD, CAT, GPx, and GSH provides further evidence of severe oxidative imbalance following paracetamol administration.

Treatment with silymarin at 50 mg/kg significantly reversed the alterations induced by paracetamol. Silymarin-treated animals exhibited a marked reduction in MDA and significant restoration of GSH, SOD, CAT, and GPx compared with the toxic control group. The reduction in MDA suggests attenuation of lipid peroxidation, whereas restoration of the antioxidant parameters indicates improvement of endogenous antioxidant defense. The findings are consistent with the established hepatoprotective and antioxidant properties of silymarin and demonstrate the effectiveness of the standard drug in limiting oxidative damage in this experimental model.

Administration of the methanolic extract at 200 mg/kg also produced a significant improvement in the oxidative stress profile. The extract reduced the elevated MDA level and increased the levels or activities of GSH, SOD, CAT, and GPx compared with the paracetamol toxic control. This indicates that the extract was capable of counteracting the oxidative imbalance produced by paracetamol. The reduction in lipid peroxidation together with restoration of endogenous antioxidants suggests that the protective action of the extract may involve both suppression of oxidative damage and

preservation of the intrinsic antioxidant defense mechanisms.

The 400 mg/kg dose of the methanolic extract produced a more pronounced protective effect than the 200 mg/kg dose. At this dose, MDA levels were reduced substantially, while GSH, SOD, CAT, and GPx showed greater restoration toward normal values. This dose-dependent response suggests that increasing the concentration of bioactive constituents may enhance the ability of the extract to neutralize reactive species and preserve antioxidant defenses. The closer approximation of the 400 mg/kg values to those of the normal control further supports the presence of significant antioxidant potential in the methanolic extract.

The antioxidant activity of the methanolic extract may be related to its phenolic, flavonoid, tannin, and other bioactive phytochemical constituents. Phenolic compounds can contribute to antioxidant activity through their ability to donate hydrogen atoms or electrons to reactive free radicals, thereby terminating oxidative chain reactions. Flavonoids may additionally interact with reactive oxygen species and may help reduce oxidative damage to cellular membranes. These phytochemicals may also contribute indirectly to preservation of endogenous antioxidant systems by reducing the overall oxidative burden. However, the exact contribution of individual phytoconstituents cannot be established from the present study alone and requires further isolation and characterization studies.

The reduction in MDA observed following treatment is particularly important because lipid peroxidation can amplify cellular injury. Once initiated, lipid peroxidation may generate additional reactive aldehydes and lipid-derived radicals, thereby extending oxidative injury beyond the initial site of ROS generation. By reducing MDA formation, the methanolic extract may help preserve the structural and functional integrity of hepatocyte membranes. Maintenance of membrane integrity can subsequently reduce leakage of intracellular hepatic enzymes into the circulation, which may explain the parallel improvement observed in serum SGOT, SGPT, and ALP levels.

The improvement in antioxidant parameters also corresponds with the observed restoration of total protein, albumin, and globulin levels. Hepatocytes are responsible for the synthesis of several important plasma proteins, particularly albumin. Severe oxidative and structural injury to hepatic cells can impair synthetic function and contribute to reductions in circulating protein levels. By reducing oxidative injury and supporting hepatocellular integrity, the methanolic extract may help preserve hepatic synthetic activity. Thus, the improvement in antioxidant status may be functionally associated with the normalization of both liver enzyme markers and serum protein parameters.

The findings collectively indicate that paracetamol produced a characteristic oxidative stress profile, represented by increased MDA and decreased GSH, SOD, CAT, and GPx, whereas treatment with the methanolic extract substantially reversed these alterations. The effects observed at 400 mg/kg were more pronounced than those produced by 200 mg/kg, indicating a dose-related response. The overall pattern was comparable to the protective effect produced by silymarin, although the magnitude of protection should be interpreted using the actual statistical analysis and experimental observations.

The present findings therefore suggest that the hepatoprotective activity of the methanolic extract may involve an important antioxidant mechanism. The extract appears to reduce lipid peroxidation while maintaining or restoring endogenous enzymatic and non-enzymatic antioxidant defenses. This antioxidant action may subsequently contribute to preservation of hepatocellular membrane integrity, reduction of enzyme leakage, maintenance of hepatic protein synthesis, and improvement of overall liver function.

In conclusion, the oxidative stress findings provide supportive biochemical evidence for the hepatoprotective potential of the methanolic extract against paracetamol-induced hepatic injury. The significant reduction in MDA and restoration of GSH, SOD, CAT, and GPx demonstrate attenuation of oxidative damage and enhancement of antioxidant defense. When considered together with the normalization of SGOT, SGPT, ALP, total bilirubin, total protein, albumin, and globulin, these findings suggest that the methanolic extract exerts a multifaceted protective effect on hepatic tissue. Further investigations involving histopathological assessment, quantitative phytochemical analysis, antioxidant gene-expression studies, and isolation of individual active constituents would help establish the precise molecular mechanisms underlying the observed hepatoprotective activity.

CONCLUSION:

The present investigation was undertaken to evaluate the hepatoprotective and antioxidant potential of the methanolic extract against paracetamol-induced hepatic injury in experimental animals. Paracetamol-induced hepatotoxicity is associated with excessive generation of reactive metabolites, oxidative stress, depletion of endogenous antioxidants, lipid peroxidation, and impairment of normal hepatic functions. Therefore, evaluation of serum biochemical markers and hepatic antioxidant parameters provides useful evidence for assessing the protective effects of the investigated extract.

Administration of paracetamol at a dose of 2 g/kg, orally, produced a pronounced alteration in the biochemical and antioxidant parameters when compared with the normal control group. The toxic control animals showed significant elevations in hepatic marker enzymes, including SGOT (AST), SGPT (ALT), and ALP, together with an increase in total bilirubin. These changes indicate disruption of hepatocellular integrity and leakage of intracellular enzymes into the circulation. A reduction in total protein, albumin, and globulin was also observed following paracetamol administration, suggesting impairment of normal hepatic synthetic function.

Paracetamol administration additionally produced substantial oxidative imbalance in the hepatic tissue. A marked increase in MDA, an important indicator of lipid peroxidation, was observed in the toxic control group. At the same time, endogenous antioxidant defenses were considerably reduced, as evidenced by decreased GSH, SOD, CAT, and GPx levels. Depletion of GSH may facilitate accumulation of reactive metabolites and free radicals, while reduced SOD, CAT, and GPx activities may further compromise the ability of hepatic tissue to neutralize reactive oxygen species. Collectively, these findings confirm the development of significant oxidative stress and hepatic injury in the paracetamol-treated animals.

Treatment with the standard hepatoprotective drug silymarin at 50 mg/kg significantly attenuated the biochemical and antioxidant alterations produced by paracetamol. Silymarin reduced the elevated SGOT, SGPT, ALP, and total bilirubin levels and improved the decreased total protein, albumin, and globulin levels. It also significantly reduced MDA and restored GSH, SOD, CAT, and GPx toward normal values. These findings demonstrate the suitability of silymarin as a standard reference drug in the present experimental model.

Administration of the methanolic extract at doses of 200 and 400 mg/kg produced significant protection against paracetamol-induced hepatic alterations. The extract treatment reduced the elevated hepatic enzyme and bilirubin levels and improved the altered protein parameters. The antioxidant profile was also substantially improved, as demonstrated by reduction of MDA and restoration of GSH, SOD, CAT, and GPx. These findings indicate that the extract can counteract the oxidative imbalance produced by excessive paracetamol exposure.

The hepatoprotective activity was more pronounced at the 400 mg/kg dose than at 200 mg/kg, indicating a dose-related protective response under the conditions of the present study. The improvement in antioxidant parameters suggests that attenuation of oxidative stress may represent an important mechanism contributing to the observed hepatoprotective effect. Reduction in lipid peroxidation together with preservation of endogenous antioxidant defenses may help maintain cellular membrane integrity and reduce leakage of hepatic enzymes into the circulation.

The observed activity may be related to the presence of biologically active phytoconstituents in the methanolic extract, particularly phenolic and flavonoid compounds. Such constituents may contribute to free-radical scavenging, inhibition of lipid peroxidation, and preservation of endogenous antioxidant systems. However, the precise constituents responsible for the observed activity cannot be established solely from the present biochemical findings and require further phytochemical isolation and mechanistic investigation.

Taken together, the present findings demonstrate that the methanolic extract exhibited significant protective effects against paracetamol-induced hepatic injury, as reflected by improvement in serum liver function markers, protein parameters, and hepatic antioxidant status. The findings suggest that the extract possesses promising hepatoprotective and antioxidant activity, particularly at the 400 mg/kg dose.

Therefore, the methanolic extract may serve as a potential source of hepatoprotective phytoconstituents. Further investigations involving histopathological examination, quantitative phytochemical analysis, isolation and characterization of active compounds, and molecular studies are recommended to establish the precise mechanism of action and to validate the hepatoprotective potential of the extract.

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