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EVALUATION AND PHYTOCHEMICAL SCREENING OF ANTIHYPERLIPIDEMIC ACTIVITY OF TERMINALIA ARJUNA

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Abstract:

The present study was undertaken to evaluate the hepatoprotective activity of Terminalia arjuna extract against paracetamol-induced hepatotoxicity in experimental animals. The liver plays a central role in metabolism and detoxification, making it highly susceptible to injury caused by excessive exposure to hepatotoxic agents. Paracetamol-induced hepatic injury is associated with the formation of the reactive metabolite N-acetyl-p-benzoquinone imine (NAPQI), depletion of hepatic glutathione, oxidative stress, and subsequent hepatocellular damage. Therefore, the present investigation was designed to assess whether T. arjuna extract could attenuate the biochemical alterations produced by paracetamol administration. Experimental animals were divided into five groups, with six animals in each group. Group I served as the normal control and received vehicle treatment, while Group II served as the toxic control and received paracetamol. Group III received paracetamol along with the standard hepatoprotective drug silymarin. Groups IV and V received paracetamol along with T. arjuna extract at doses of 200 and 400 mg/kg, respectively. Following the experimental treatment, serum biochemical parameters were evaluated to determine the extent of hepatic injury and the protective effect of the extract. The parameters assessed included serum glutamic oxaloacetic transaminase (SGOT/AST), serum glutamic pyruvic transaminase (SGPT/ALT), alkaline phosphatase (ALP), total bilirubin, and total protein.

Paracetamol administration produced marked alterations in the hepatic biochemical profile. The toxic control group showed substantial increases in SGOT/AST, SGPT/ALT, ALP, and total bilirubin levels, together with a reduction in total serum protein compared with the normal control group. These changes indicated significant hepatocellular injury and impairment of hepatic functional activity. Treatment with silymarin substantially reversed the paracetamol-induced biochemical changes, confirming its hepatoprotective effect in the experimental model.

Administration of T. arjuna extract at 200 mg/kg produced significant improvement in the altered biochemical parameters compared with the toxic control group. A greater degree of protection was observed following administration of the 400 mg/kg dose. The higher dose reduced the elevated serum SGOT/AST, SGPT/ALT, ALP, and total bilirubin levels and improved the decreased total protein concentration toward normal values. The dose-dependent improvement suggests that T. arjuna extract may exert a protective effect against paracetamol-induced hepatic damage.

The hepatoprotective activity of T. arjuna may be associated with its diverse phytochemical constituents, particularly phenolic compounds, flavonoids, tannins, and triterpenoid constituents, which may contribute to antioxidant and hepatocellular protective effects. These constituents may help reduce oxidative damage, preserve cellular membrane integrity, and support normal hepatic function. However, the precise molecular mechanisms responsible for the observed hepatoprotective effect require further investigation.

In conclusion, the present study demonstrates that Terminalia arjuna extract possesses promising hepatoprotective activity against paracetamol-induced hepatic injury in experimental animals. The 400 mg/kg dose exhibited greater biochemical protection than the 200 mg/kg dose and showed an overall improvement in the evaluated liver function parameters. Further studies involving hepatic histopathology, oxidative stress markers, inflammatory mediators, quantitative phytochemical characterization, and isolation of active constituents are warranted to establish the mechanisms underlying the hepatoprotective activity of T. arjuna.

Keywords: *Terminalia arjuna, paracetamol, hepatotoxicity, hepatoprotective activity, SGOT, SGPT, ALP, bilirubin, total protein, silymarin.*

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INTRODUCTION:

Hyperlipidemia is a metabolic disorder characterized by elevated levels of plasma lipids, including total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), and very low-density lipoprotein (VLDL), along with decreased levels of high-density lipoprotein cholesterol (HDL-C). It is one of the most significant risk factors for the development of cardiovascular diseases such as atherosclerosis, coronary artery disease, myocardial infarction, and stroke. According to the World Health Organization, cardiovascular diseases account for the highest number of deaths globally, and dyslipidemia plays a central role in their pathogenesis.

Lipids are essential for various physiological functions including energy storage, hormone synthesis, and maintenance of cellular membrane integrity. However, abnormalities in lipid metabolism result in excessive accumulation of cholesterol and triglycerides in the bloodstream. Elevated LDL-C promotes deposition of cholesterol in arterial walls, initiating atherosclerotic plaque formation. Progressive plaque buildup leads to narrowing of blood vessels, impaired circulation, and increased risk of thrombotic events.

Hyperlipidemia may be classified as primary (genetic) or secondary (acquired). Secondary causes include sedentary lifestyle, high-fat diet, obesity, diabetes mellitus, hypothyroidism, nephrotic syndrome, and excessive alcohol consumption. Oxidative stress and chronic inflammation further contribute to lipid peroxidation and vascular damage, accelerating the progression of cardiovascular complications.

Conventional antihyperlipidemic drugs such as statins, fibrates, niacin, and bile acid sequestrants are widely used to manage abnormal lipid levels. These agents primarily act by inhibiting cholesterol biosynthesis, enhancing lipid metabolism, reducing intestinal lipid absorption, or increasing bile acid excretion. Although effective, long-term use of synthetic drugs may produce adverse effects including hepatotoxicity, myopathy, gastrointestinal disturbances, and drug interactions. These limitations have prompted the search for safer and cost-effective alternatives.

Medicinal plants have been used since ancient times for the treatment of various metabolic disorders. Traditional systems of medicine such as Ayurveda, Siddha, and Unani describe numerous plants with lipid-lowering and cardioprotective properties. Many plant-derived compounds including flavonoids, saponins, alkaloids, tannins, phenolic acids, sterols, and glycosides have demonstrated significant antihyperlipidemic activity in experimental studies. These phytoconstituents exert their effects through multiple mechanisms such as inhibition of HMG-CoA reductase, enhancement of lipoprotein lipase activity, antioxidant action, prevention of lipid peroxidation, and improvement of hepatic lipid metabolism.

Several medicinal plants like Allium sativum, Curcuma longa, Trigonella foenum-graecum, Emblica officinalis, and Glycyrrhiza glabra have shown promising lipid-lowering effects in both preclinical and clinical investigations. Their therapeutic potential is often attributed to their antioxidant and anti-inflammatory properties, which play a vital role in preventing atherosclerotic changes.

Experimental evaluation of antihyperlipidemic activity is commonly performed using animal models such as high-fat diet-induced hyperlipidemia, triton-induced hyperlipidemia, and genetically modified models. Assessment parameters include serum lipid profile, atherogenic index, hepatic enzyme levels, and histopathological studies of liver and aorta.

Although synthetic antihyperlipidemic agents such as statins and fibrates are effective, their long-term use is associated with adverse effects including hepatotoxicity, myopathy, and gastrointestinal disturbances. Therefore, there is increasing interest in medicinal plants as safer and cost-effective alternatives. Traditional systems of medicine such as Ayurveda and Unani describe numerous herbs with lipid-lowering properties. Scientific validation has confirmed that many plant-derived phytoconstituents exhibit antihyperlipidemic activity through multiple mechanisms including inhibition of cholesterol synthesis, antioxidant action, enhancement of lipid metabolism, and prevention of lipid peroxidation [1-3].

Hyperlipidemic

Hyperlipidemia is a metabolic disorder characterized by abnormal elevation of lipids and lipoproteins in the blood. It includes increased levels of total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), and very low-density lipoprotein (VLDL), along with decreased high-density lipoprotein cholesterol (HDL-C). Hyperlipidemia is one of the most important modifiable risk factors for cardiovascular diseases such as atherosclerosis, coronary artery disease (CAD), myocardial infarction, and stroke.

According to the World Health Organization, cardiovascular diseases remain the leading cause of mortality worldwide, accounting for millions of deaths annually. Dyslipidemia significantly contributes to this global health burden. Elevated LDL and triglycerides accelerate the development of atherosclerotic plaques, leading to narrowing and hardening of arteries.

Lipids are essential for normal physiological functions including energy storage, synthesis of steroid hormones, bile acid formation, and maintenance of cell membrane integrity. However, disturbances in lipid metabolism result in hyperlipidemic activity, which may eventually lead to severe cardiovascular complications [4].

Classification of Hyperlipidemia

Hyperlipidemia is broadly classified based on its cause, pattern of lipid abnormality, and type of lipoprotein involved. Proper classification is important for understanding the underlying mechanism, assessing cardiovascular risk, and selecting appropriate treatment strategies.

Hyperlipidemia can first be classified into primary (familial) and secondary (acquired) types based on etiology. Primary hyperlipidemia results from inherited genetic defects that affect lipid metabolism. These genetic abnormalities may involve defective LDL receptors, apolipoproteins, or enzymes responsible for lipid transport and clearance. Conditions such as familial hypercholesterolemia and familial combined hyperlipidemia fall under this category. Primary hyperlipidemia often presents early in life and is associated with a higher risk of premature cardiovascular disease.

Secondary hyperlipidemia develops due to external factors, underlying diseases, or certain medications. Common causes include diabetes mellitus, hypothyroidism, obesity, nephrotic syndrome, chronic kidney disease, excessive alcohol consumption, and a high-fat diet. Certain drugs such as corticosteroids, oral contraceptives, and beta-blockers may also contribute to lipid abnormalities. In secondary hyperlipidemia, management focuses on treating the underlying cause along with lipid-lowering therapy.

Hyperlipidemia can also be classified according to the specific lipid abnormality present in the blood. Hypercholesterolemia refers to elevated total cholesterol and LDL cholesterol levels and is strongly associated with atherosclerosis. Hypertriglyceridemia involves increased triglyceride levels and may increase the risk of acute pancreatitis when levels are markedly elevated. Mixed or combined hyperlipidemia is characterized by elevation of both cholesterol and triglycerides and is commonly seen in metabolic syndrome. Another pattern includes isolated low HDL cholesterol, which increases cardiovascular risk because HDL plays a protective role in reverse cholesterol transport.

Another widely accepted classification system is the Fredrickson classification, which was later adopted by the World Health Organization. This system categorizes hyperlipidemia into Types I to V based on the pattern of lipoprotein elevation identified through electrophoresis. Type I involves elevated chylomicrons and primarily increases triglycerides. Type IIa is characterized by elevated LDL and increased cholesterol levels and is strongly associated with coronary artery disease. Type IIb shows elevation of both LDL and VLDL, resulting in increased cholesterol and triglycerides. Type III involves intermediate-density lipoproteins (IDL) and is linked with premature cardiovascular disease. Type IV is marked by elevated VLDL and increased triglycerides, commonly seen in obesity and diabetes. Type V involves elevation of both chylomicrons and VLDL, leading to very high triglyceride levels and risk of pancreatitis.

Hyperlipidemia may also be categorized based on severity. Mild hyperlipidemia involves slight elevation of lipid levels and is often managed through lifestyle modifications such as diet and exercise. Moderate hyperlipidemia typically requires pharmacological intervention in addition to lifestyle changes. Severe hyperlipidemia, particularly when triglyceride levels exceed 500 mg/dL, increases the risk of acute pancreatitis and requires urgent medical management.

Thus, hyperlipidemia can be classified in multiple ways depending on etiology, lipid pattern, lipoprotein involvement, and severity. Understanding these classifications helps in accurate diagnosis, effective treatment planning, and prevention of cardiovascular complications [5-6].

MATERIALS AND METHODS:

Collection and authentication.

Terminalia arjuna are collected and identified was purchased from Tirupati Andra Pradesh , India, and authenticated by Dr. K. Madhava Chetty, Assistant Professor, Department of Botany, S.V University, Tirupati.

Extraction

The shade-dried of Terminalia arjuna. Were reduced to coarse powder, and around 200 g of powdered seed material was subjected to (Soxhlet extractor) with methanol. Finally, the extract was then filtered to dryness under reduced pressure using a Rota evaporator. The extract obtained with solvent was weighed and its percentage in terms of the air-dried weight of the seed material was calculated. Also, the color and consistency of the extract were noted. The results of the successive solvent extraction of air-dried material of the Terminalia arjuna.

Acute toxicity [53]

Acute toxicity studies are conducted to evaluate the safety profile of a drug or plant extract after a single dose administration, primarily to determine the LD_{50} value and identify any immediate toxic effects. These studies are commonly performed

according to the guidelines of the Organisation for Economic Co-operation and Development (OECD), particularly OECD 423 or 425. In this method, healthy adult rats or mice (usually females) are fasted overnight and administered a single oral dose of the test substance (commonly 300 mg/kg or 2000 mg/kg). The animals are observed continuously for the first 4 hours and then daily for 14 days for signs of toxicity such as behavioral changes, tremors, convulsions, salivation, respiratory distress, changes in food and water intake, body weight variations, and mortality. If no mortality is observed at 2000 mg/kg, the substance is considered relatively safe and categorized under low toxicity. Acute toxicity studies are essential before pharmacological evaluation, such as antihyperlipidemic activity testing, to ensure the safe dose range of the test compound.

Induction of hepatotoxicity [6]

Paracetamol-induced hepatotoxicity is a widely used experimental model for evaluating hepatoprotective activity in laboratory animals. Paracetamol, when administered in high doses, is metabolized in the liver by cytochrome P450 enzymes to form a highly reactive toxic metabolite known as N-acetyl-p-benzoquinone imine (NAPQI). Under normal therapeutic conditions, NAPQI is detoxified by conjugation with glutathione (GSH). However, in overdose conditions, excessive NAPQI formation depletes hepatic glutathione stores, leading to oxidative stress, mitochondrial dysfunction, and hepatocellular necrosis. In experimental studies, hepatotoxicity is typically induced in rats by administering paracetamol orally at a dose of 500–1000 mg/kg body weight. After 24–48 hours, liver injury is confirmed by significant elevation of serum biomarkers such as SGOT (AST), SGPT (ALT), ALP, and total bilirubin, along with histopathological changes including centrilobular necrosis and inflammatory cell infiltration. This model is simple, reproducible, and closely mimics human drug-induced liver injury, making it suitable for screening hepatoprotective agents.

Experimental Animals (Rats)

Healthy adult Wistar albino rats weighing between 150–250 g is commonly used for induction of hepatotoxicity and evaluation of hepatoprotective activity. The animals are obtained from a registered animal house and housed in clean polypropylene cages under standard laboratory conditions, maintained at a temperature of 22 ± 2°C, relative humidity of 55–65%, and a 12-hour light/dark cycle. They are fed with standard pellet diet and provided water ad libitum. Prior to experimentation, the rats are acclimatized to laboratory conditions for at least 7 days. Before induction of hepatotoxicity (e.g., paracetamol-induced model), the animals are fasted overnight with free access to water. All experimental procedures are conducted in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) to ensure ethical handling and care of laboratory animals.

Pre-treatment

Administration of the Terminalia arjuna before and during the induction of hyperlipidemia to assess its protective effect. Healthy Wistar albino rats (150–200 g) are randomly divided into different groups. The test groups receive bark extract of Terminalia arjuna at selected doses (e.g., 200 mg/kg and 400 mg/kg body weight, p.o.) once daily for a total period of 28 days. The standard group receives atorvastatin (10 mg/kg, p.o.), while the hyperlipidemic control group receives only the high-fat diet (HFD). In HFD models, the extract and standard drug are administered simultaneously with the high-fat diet throughout the study period. At the end of the treatment, animals are fasted overnight, blood samples are collected for estimation of serum lipid profile (total cholesterol, triglycerides, LDL, VLDL, HDL), and the antihyperlipidemic effect is assessed. A significant reduction in elevated lipid levels in pre-treated groups compared to the hyperlipidemic control group indicates protective activity.

Selection of Doses for Animal Study

Selection of doses for animal studies is a crucial step in pharmacological evaluation to ensure safety and therapeutic effectiveness of the test compound. The dose for experimental animals is generally selected based on results obtained from acute toxicity studies conducted according to the guidelines of the Organisation for Economic Co-operation and Development (OECD 423 or 425). After performing acute oral toxicity testing, if the test extract shows no mortality or toxic signs up to 2000 mg/kg body weight, it is considered relatively safe. Based on this, experimental doses are commonly selected as a fraction of the maximum safe dose. Typically, 1/10th and 1/5th of the maximum non-lethal dose are chosen for pharmacological studies. For example, if no toxicity is observed at 2000 mg/kg, doses such as 200 mg/kg and 400 mg/kg body weight are selected for evaluation. In studies involving Terminalia arjuna, similar dose selection procedures are followed. The extract is administered orally once

daily during the experimental period. The selected doses should be sufficient to produce therapeutic effects without causing toxicity.

ANIMAL GROUPING [7]

Group I: Normal control (vehicle only)

Group II: Toxic control (Paracetamol only)

Group III: Standard (Paracetamol + Silymarin 25–50 mg/kg)

Group IV: Paracetamol + T. arjuna extract (200 mg/kg)

Group V: Paracetamol + T. arjuna extract (400 mg/kg)

PARAMETERS

Estimation of Total Cholesterol [8]

Total cholesterol in serum is commonly estimated using the enzymatic CHOD–PAP (Cholesterol Oxidase–Peroxidase) method. In this method, cholesterol esters in the serum are first hydrolyzed by cholesterol esterase to yield free cholesterol and fatty acids. The free cholesterol is then oxidized by cholesterol oxidase to produce cholest-4-en-3-one and hydrogen peroxide. In the presence of peroxidase, hydrogen peroxide reacts with phenol and 4-aminoantipyrine to form a red-colored quinoneimine complex, the intensity of which is directly proportional to the cholesterol concentration. Briefly, serum is separated from collected blood samples by centrifugation. A measured volume of serum is mixed with the enzymatic reagent, and the mixture is incubated at 37°C for 10 minutes. The absorbance of the resulting color is then measured spectrophotometrically at 505 nm against a reagent blank. Total cholesterol concentration is calculated by comparing the absorbance of the test sample with that of a cholesterol standard. This method is widely used due to its specificity, simplicity, and reproducibility.

Estimation of Triglycerides (TG) [9]

Serum triglycerides are commonly estimated using the enzymatic GPO–PAP (Glycerol-3-Phosphate Oxidase–Peroxidase) method. In this method, triglycerides present in the serum are first hydrolyzed by lipase to produce glycerol and free fatty acids. Glycerol is then phosphorylated by glycerol kinase to form glycerol-3-phosphate, which is subsequently oxidized by glycerol-3-phosphate oxidase to generate dihydroxyacetone phosphate and hydrogen peroxide. The hydrogen peroxide reacts with 4-aminoantipyrine and p-chlorophenol in the presence of peroxidase to form a red-colored quinoneimine complex. The intensity of the color produced is directly proportional to the triglyceride concentration in the sample. Briefly, serum is separated from the blood by centrifugation, and a fixed volume is mixed with the working enzymatic reagent. The mixture is incubated at 37°C for 10–15 minutes, and the absorbance is measured spectrophotometrically at 505 nm against a reagent blank. The triglyceride concentration is calculated by comparing the absorbance of the sample with that of a standard solution. This method is preferred for its specificity, sensitivity, and reproducibility.

Estimation of Low-Density Lipoprotein (LDL) [10]

Low-density lipoprotein cholesterol (LDL-C) is commonly estimated using the Friedwald formula when triglyceride levels are below 400 mg/dL, or by direct enzymatic methods in clinical and experimental studies. In the Friedwald method, LDL is calculated indirectly from measured serum total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and triglycerides (TG) using the formula:

$$\text{LDL-C (mg/dL)} = \text{TC} - \text{HDL-C} - \frac{\text{TG}}{5}$$

Here, TG/5 represents the very-low-density lipoprotein cholesterol (VLDL-C) fraction. Blood samples are collected, and serum is separated by centrifugation. Total cholesterol, HDL, and triglycerides are determined using standard enzymatic methods (CHOD–PAP for TC, GPO–PAP for TG, and precipitation or direct HDL assay). LDL-C is then calculated using the formula. In direct enzymatic methods, LDL is selectively solubilized and measured using cholesterol esterase and cholesterol oxidase reactions, producing hydrogen peroxide that reacts with 4-aminoantipyrine and phenol in the presence of peroxidase to form a colored complex, measured spectrophotometrically at 505 nm.

Estimation of Very Low-Density Lipoprotein (VLDL) [11]

Very low-density lipoprotein cholesterol (VLDL-C) is usually calculated indirectly in experimental and clinical studies using serum triglyceride levels. Since VLDL carries most of the circulating triglycerides, it is commonly estimated by the formula:

$$\text{VLDL-C (mg/dL)} = \frac{\text{Triglycerides (TG)}}{5}$$

This calculation assumes that the ratio of triglycerides to cholesterol in VLDL is approximately 5:1 when TG levels are below 400 mg/dL. Blood is collected from the experimental animals, and serum is separated by centrifugation. Triglyceride concentration is determined enzymatically using the GPO–PAP method. VLDL-C is then calculated using the above formula. A reduction in VLDL-C levels in treated groups compared to hyperlipidemic control indicates the antihyperlipidemic activity of the test compound.

HDL

High-density lipoprotein cholesterol (HDL-C) is commonly estimated using enzymatic or precipitation methods. In the precipitation method, non-HDL lipoproteins, such as LDL and VLDL, are selectively precipitated by reagents containing heparin–magnesium chloride or phosphotungstate–magnesium ions. Blood samples are collected, and serum is separated by centrifugation. The serum is treated with the precipitating reagent and centrifuged again to remove LDL and VLDL. The cholesterol remaining in the supernatant represents HDL-C, which is then measured using the CHOD–PAP (Cholesterol Oxidase–Peroxidase) enzymatic method. In this method, cholesterol esters in HDL are hydrolyzed to free cholesterol by cholesterol esterase, which is oxidized by cholesterol oxidase to produce hydrogen peroxide. Hydrogen peroxide reacts with phenol and 4-aminoantipyrine in the presence of peroxidase to form a red-colored quinoneimine complex, and the intensity of the color is measured spectrophotometrically at 505 nm. An increase in HDL-C levels in treated groups compared to the hyperlipidemic control indicates the beneficial antihyperlipidemic effect of the test compound.

Biochemical Parameters

Serum Glutamic Oxaloacetic Transaminase (SGOT/AST) [12]

Serum Glutamic Oxaloacetic Transaminase (SGOT), also known as Aspartate Aminotransferase (AST), is a liver enzyme commonly measured to assess hepatocellular damage. The enzyme catalyzes the reversible transfer of an amino group from L-aspartate to α-ketoglutarate, forming oxaloacetate and L-glutamate. In liver injury, SGOT/AST is released into the bloodstream, resulting in elevated serum levels. The assay is typically performed using a UV kinetic method based on the Reitman and Frankel procedure. Briefly, serum is collected from blood samples after centrifugation. The serum sample is mixed with the substrate solution containing L-aspartate and α-ketoglutarate and incubated at 37°C for a specified time. Oxaloacetate produced reacts with 2,4-dinitrophenylhydrazine (DNPH) to form a colored hydrazone complex. After addition of sodium hydroxide to stabilize the color, the intensity is measured spectrophotometrically at 505 nm. The increase in absorbance is proportional to the SGOT activity and is expressed in international units per liter (IU/L).

Serum Glutamic Pyruvic Transaminase (SGPT/ALT) [13]

Serum Glutamic Pyruvic Transaminase (SGPT), also known as Alanine Aminotransferase (ALT), is an important liver enzyme used to assess hepatocellular injury. ALT catalyzes the reversible transfer of an amino group from L-alanine to α-ketoglutarate, producing pyruvate and L-glutamate. In liver damage, ALT is released into the bloodstream, and elevated serum levels indicate hepatocellular injury. The assay is commonly performed using the UV kinetic method based on the Reitman and Frankel procedure. Serum is separated from collected blood samples and mixed with a substrate solution containing L-alanine and α-ketoglutarate. The reaction mixture is incubated at 37°C, allowing the formation of pyruvate. Pyruvate reacts with 2,4-dinitrophenylhydrazine (DNPH) to form a colored hydrazone complex. After the addition of sodium hydroxide to stabilize the color, the absorbance is measured spectrophotometrically at 505 nm. The increase in absorbance is proportional to ALT activity and is expressed in international units per liter (IU/L).

Alkaline Phosphatase (ALP) [14]

Alkaline Phosphatase (ALP) is an important enzyme found in the liver, bone, and other tissues, and elevated serum levels are indicative of hepatobiliary disorders or bile duct obstruction. ALP catalyzes the hydrolysis of phosphate esters under alkaline conditions, releasing inorganic phosphate and an alcohol. The assay is commonly performed using the p-nitrophenyl phosphate (pNPP) method. In this method, serum is separated from collected blood samples by centrifugation. A measured volume of serum is added to a buffered substrate solution containing p-nitrophenyl phosphate at alkaline pH (usually pH 10). ALP in the serum hydrolyzes the substrate to release p-nitrophenol, which forms a yellow-colored complex under alkaline conditions. The intensity of

the color produced is directly proportional to the enzyme activity and is measured spectrophotometrically at 405 nm. ALP activity is calculated in international units per liter (IU/L).

Estimation of Total Bilirubin [15]

Total bilirubin in serum is measured to evaluate liver function and to detect hepatocellular damage or cholestasis. Bilirubin is formed from the breakdown of hemoglobin and exists in serum as unconjugated (indirect) and conjugated (direct) forms. The total bilirubin is the sum of these fractions.

The Jendrassik–Grof method is commonly used for total bilirubin estimation. In this method, serum is mixed with an alkaline solution of caffeine or sodium benzoate to accelerate the reaction of bilirubin with diazotized sulfanilic acid, forming azobilirubin, a colored complex. The intensity of the color is measured spectrophotometrically at 540 nm. The concentration of total bilirubin is calculated by comparing the absorbance of the sample with that of a bilirubin standard. An increase in serum bilirubin levels indicates impaired liver function or bile duct obstruction.

Estimation of Total Protein [16]

Total protein in serum is an important biochemical parameter used to assess liver function and overall nutritional status. Serum proteins mainly consist of albumin and globulins. Changes in total protein levels may indicate liver disease, kidney disorders, or malnutrition. The Biuret method is commonly used for the estimation of total protein. In this method, proteins in the serum react with copper ions in an alkaline solution to form a violet-colored complex (cupric-protein complex). The intensity of the color produced is directly proportional to the protein concentration and is measured spectrophotometrically at 540 nm. Briefly, serum is separated from collected blood by centrifugation. A measured volume of serum is mixed with Biuret reagent and incubated for a few minutes. The absorbance of the resulting color is read against a reagent blank, and total protein concentration is calculated using a standard protein solution (e.g., bovine serum albumin) for comparison.

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.

RESULTS:

Percentage of yield

$$\text{Percentage of yield} = \frac{\text{Weight of crude extract}}{\text{Weight of dried material}} \times 100$$

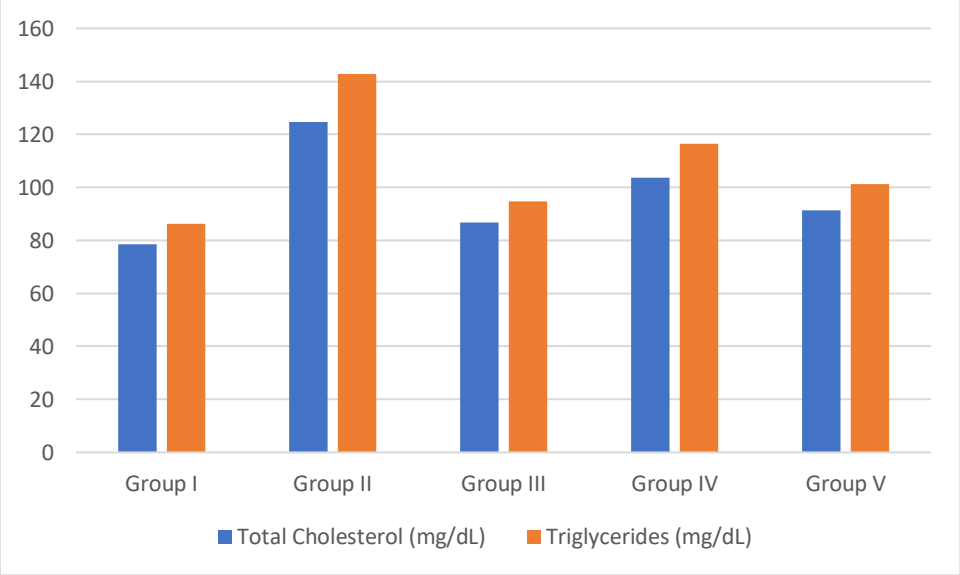
$$\text{Percentage of yield} = \frac{20\text{g}}{50\text{g}} \times 100 = 40\%$$

50gms of drug dissolved in 200ml of solvent in 1:4 Ratio

Estimation of Total Cholesterol and Triglycerides

Group	Treatment	Total Cholesterol (mg/dL)	Triglycerides (mg/dL)
Group I	Normal control (Vehicle only)	78.42 ± 2.16	86.35 ± 2.48
Group II	Toxic control (Paracetamol only)	124.68 ± 3.52***	142.76 ± 4.16***
Group III	Standard (Paracetamol + Silymarin 50 mg/kg)	86.74 ± 2.38###	94.62 ± 2.74###
Group IV	Paracetamol + T. arjuna extract 200 mg/kg	103.56 ± 2.86##	116.48 ± 3.28##
Group V	Paracetamol + T. arjuna extract 400 mg/kg	91.38 ± 2.54###	101.24 ± 2.92###

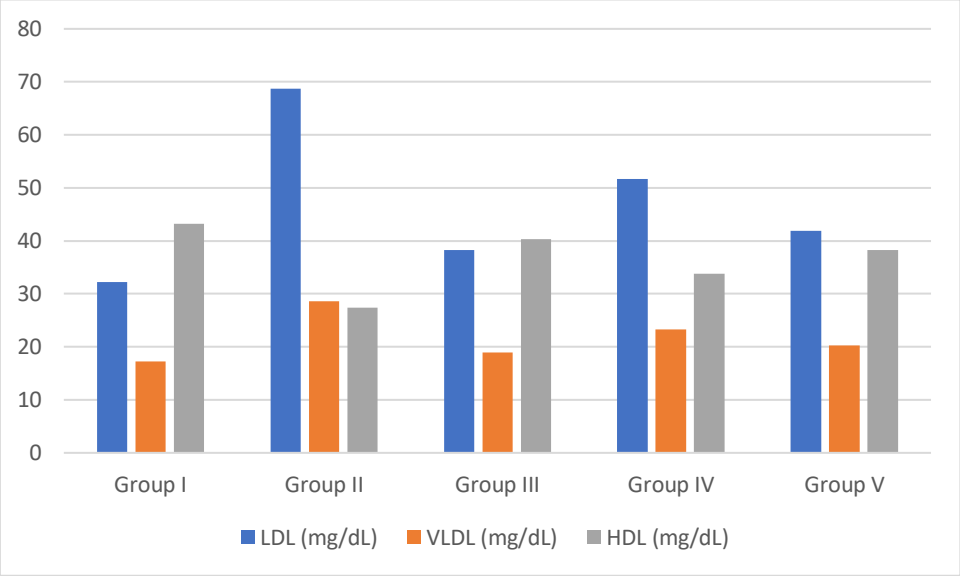
Values are expressed as Mean ± SEM (n=6); *p<0.001 vs. Group I (normal control), ##p<0.01 and ###p<0.001 vs. Group II (toxic control), analyzed by one-way ANOVA followed by Dunnett's multiple comparison test.



Estimation of LDL, VLDL and HDL

Group	Treatment	LDL (mg/dL)	VLDL (mg/dL)	HDL (mg/dL)
Group I	Normal control (Vehicle only)	32.18 ± 1.42	17.27 ± 0.50	43.16 ± 1.24
Group II	Toxic control (Paracetamol only)	68.74 ± 2.36***	28.55 ± 0.83***	27.42 ± 1.08***
Group III	Standard (Paracetamol + Silymarin 50 mg/kg)	38.26 ± 1.56###	18.92 ± 0.55###	40.38 ± 1.18###
Group IV	Paracetamol + T. arjuna extract 200 mg/kg	51.62 ± 1.94##	23.30 ± 0.68##	33.84 ± 1.12##
Group V	Paracetamol + T. arjuna extract 400 mg/kg	41.86 ± 1.68###	20.25 ± 0.59###	38.26 ± 1.16###

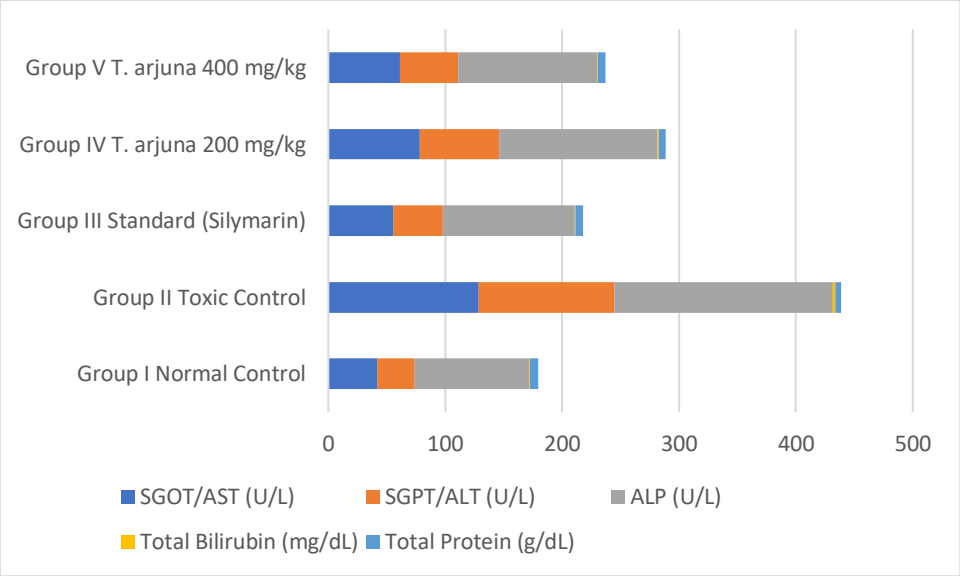
Values are expressed as Mean ± SEM (n=6); ***p<0.001 vs. Group I (normal control), ##p<0.01 and ###p<0.001 vs. Group II (toxic control), analyzed by one-way ANOVA followed by Dunnett's multiple comparison test.



Biochemical Parameters

Parameter	Group I Normal Control	Group II Toxic Control	Group III Standard (Silymarin)	Group IV T. arjuna 200 mg/kg	Group V T. arjuna 400 mg/kg
SGOT/AST (U/L)	42.18 ± 1.52	128.64 ± 3.86***	55.72 ± 1.84###	78.46 ± 2.64##	61.38 ± 2.12###
SGPT/ALT (U/L)	31.46 ± 1.28	116.28 ± 3.42***	42.36 ± 1.56###	67.82 ± 2.38##	49.64 ± 1.76###
ALP (U/L)	98.32 ± 2.64	186.54 ± 4.72***	112.46 ± 3.18###	135.28 ± 3.64##	118.72 ± 3.26###
Total Bilirubin (mg/dL)	0.62 ± 0.03	2.18 ± 0.08***	0.86 ± 0.04###	1.24 ± 0.05##	0.94 ± 0.04###
Total Protein (g/dL)	7.12 ± 0.18	4.86 ± 0.16***	6.64 ± 0.15###	5.78 ± 0.14##	6.38 ± 0.16###

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:

The liver is one of the major organs responsible for the metabolism, detoxification, biotransformation, and elimination of endogenous and exogenous substances. Because of its extensive metabolic activity, the liver is particularly susceptible to injury caused by drugs and toxic chemicals. Paracetamol-induced hepatotoxicity is a widely used experimental model for evaluating the hepatoprotective potential of medicinal plants and their phytoconstituents. In the present study, the hepatoprotective activity of *Terminalia arjuna* extract was evaluated against paracetamol-induced liver injury in experimental animals using biochemical parameters including SGOT/AST, SGPT/ALT, ALP, total bilirubin, and total protein.

Administration of paracetamol to the toxic control group produced a pronounced elevation of serum SGOT/AST and SGPT/ALT compared with the normal control group. The SGOT and SGPT values increased from 42.18 ± 1.52 U/L and 31.46 ± 1.28 U/L in the normal control group to 128.64 ± 3.86 U/L and 116.28 ± 3.42 U/L, respectively, in the toxic control group. The marked elevation of these transaminases indicates hepatocellular damage and leakage of intracellular enzymes into the circulation. SGPT/ALT is considered relatively more associated with hepatocellular injury, whereas SGOT/AST may also increase following injury to other tissues. Therefore, the increased transaminase activities observed following paracetamol administration are consistent with substantial hepatic cellular damage.

The hepatotoxic effect of paracetamol is primarily associated with the formation of its reactive metabolite, N-acetyl-p-benzoquinone imine (NAPQI), particularly when the normal conjugation pathways become overwhelmed. Under normal conditions, most paracetamol undergoes glucuronidation and sulfation, while a smaller fraction is metabolized by cytochrome P450 enzymes to NAPQI. NAPQI is normally detoxified by conjugation with reduced glutathione (GSH). During excessive paracetamol exposure, hepatic glutathione stores become depleted, allowing the reactive metabolite to interact with cellular proteins

and other macromolecules. This process can result in oxidative stress, mitochondrial dysfunction, membrane damage, and hepatocellular necrosis, ultimately contributing to the release of intracellular enzymes into the blood.

In the present investigation, a significant increase in ALP was also observed in the paracetamol-treated toxic control group. ALP increased from 98.32 ± 2.64 U/L in the normal control group to 186.54 ± 4.72 U/L in the toxic control group. ALP is associated mainly with the hepatobiliary system and is present in the canalicular and sinusoidal membranes of hepatocytes, although it is also found in several extrahepatic tissues. An increase in ALP may reflect impairment of normal hepatic and biliary function. The elevated ALP observed in the toxic control animals therefore supports the occurrence of hepatic dysfunction following paracetamol administration.

Total bilirubin was also markedly increased in the toxic control group. The total bilirubin concentration increased from 0.62 ± 0.03 mg/dL in the normal control group to 2.18 ± 0.08 mg/dL following paracetamol administration. Bilirubin is produced during the degradation of haemoglobin and is subsequently transported to the liver, conjugated, and excreted through bile. Hepatocellular injury can interfere with bilirubin uptake, conjugation, and excretion, resulting in increased circulating bilirubin concentrations. Thus, the elevation of total bilirubin in the toxic control group provides additional evidence of impaired hepatic functional capacity.

A reduction in total serum protein was observed in the paracetamol-treated toxic control group. Total protein decreased from 7.12 ± 0.18 g/dL in the normal control group to 4.86 ± 0.16 g/dL in the toxic control group. The liver plays an essential role in the synthesis of many plasma proteins, including albumin and several other proteins involved in transport and homeostasis. Severe hepatic injury can therefore be associated with impaired protein synthetic function. The reduction in total protein observed in the present study, together with the increased hepatic enzymes and bilirubin, indicates that paracetamol exposure produced both hepatocellular injury and impairment of normal hepatic function.

Treatment with silymarin, used as the standard hepatoprotective drug, markedly attenuated the biochemical alterations produced by paracetamol. In the standard-treated group, SGOT/AST, SGPT/ALT, ALP, and total bilirubin were reduced toward normal values, while total protein was restored. The protective effect observed with silymarin supports the validity of the experimental model and provides a pharmacological reference for evaluating the activity of the *T. arjuna* extract. Silymarin is widely investigated for its antioxidant and hepatoprotective properties, and its activity has been associated with protection of hepatocyte membranes and attenuation of oxidative damage.

Treatment with *T. arjuna* extract at 200 mg/kg produced a significant improvement in the biochemical parameters compared with the toxic control group. SGOT/AST decreased from 128.64 ± 3.86 U/L in the toxic control group to 78.46 ± 2.64 U/L, while SGPT/ALT decreased from 116.28 ± 3.42 U/L to 67.82 ± 2.38 U/L. Similarly, ALP decreased from 186.54 ± 4.72 U/L to 135.28 ± 3.64 U/L and total bilirubin decreased from 2.18 ± 0.08 mg/dL to 1.24 ± 0.05 mg/dL. Total protein showed improvement from 4.86 ± 0.16 g/dL in the toxic control group to 5.78 ± 0.14 g/dL. These findings suggest that the lower dose of *T. arjuna* extract was capable of attenuating the biochemical manifestations of paracetamol-induced hepatic injury.

A more pronounced protective effect was observed with *T. arjuna* extract at 400 mg/kg. At this dose, SGOT/AST, SGPT/ALT, and ALP were reduced to 61.38 ± 2.12, 49.64 ± 1.76, and 118.72 ± 3.26 U/L, respectively. Total bilirubin was reduced to 0.94 ± 0.04 mg/dL, while total protein increased to 6.38 ± 0.16 g/dL. The greater restoration observed with the 400 mg/kg dose compared with the 200 mg/kg dose indicates a dose-related improvement in the measured biochemical parameters. Although the values did not necessarily become identical to those of the normal control group, the overall direction of change indicates attenuation of paracetamol-induced hepatic dysfunction.

The hepatoprotective effect of *T. arjuna* may be related, at least in part, to the phytochemicals present in the extract. *T. arjuna* is known to contain several classes of secondary metabolites, including phenolic compounds, flavonoids, tannins, triterpenoids, and other bioactive constituents. Phenolic and flavonoid compounds are of particular interest because of their ability to participate in antioxidant mechanisms and reduce oxidative damage. However, the present biochemical findings alone cannot establish the precise molecular mechanism responsible for the hepatoprotective effect. The observed protection may involve multiple complementary mechanisms, including reduction of oxidative stress, preservation of hepatocyte membrane integrity, and maintenance of hepatic functional activity.

The improvement in total protein following *T. arjuna* treatment further supports the possibility of preservation or restoration of hepatic functional capacity. The increase from 4.86 ± 0.16 g/dL in the toxic control group to 5.78 ± 0.14 g/dL and 6.38 ± 0.16 g/dL following treatment with 200 and 400 mg/kg, respectively, demonstrates progressive improvement. This finding is particularly relevant when interpreted together with the reduction in serum transaminases, ALP, and bilirubin, because assessment of several biochemical parameters

provides a more comprehensive indication of hepatic protection than relying on a single marker.

Overall, the biochemical findings demonstrate that paracetamol administration produced significant hepatic injury, as evidenced by increased SGOT/AST, SGPT/ALT, ALP, and total bilirubin together with decreased total protein. Treatment with *T. arjuna* extract substantially reversed these alterations, with the 400 mg/kg dose producing greater improvement than the 200 mg/kg dose. The pattern of biochemical recovery was comparable in direction to that observed with the standard hepatoprotective treatment. These findings suggest that *T. arjuna* possesses hepatoprotective potential against paracetamol-induced hepatic injury, possibly through antioxidant and membrane-stabilizing effects.

The present biochemical observations provide supportive evidence for the hepatoprotective potential of *T. arjuna*, but further investigations would be useful to establish the underlying mechanism more conclusively. Assessment of hepatic oxidative stress markers such as MDA, GSH, SOD, CAT, and GPx, together with inflammatory markers and histopathological examination of liver tissue, would provide additional evidence regarding the protective action of the extract. Quantitative phytochemical analysis and identification of the active constituents could further clarify which compounds contribute to the observed activity. Nevertheless, the present results indicate that *T. arjuna* extract, particularly at 400 mg/kg, attenuated the biochemical alterations associated with paracetamol-induced hepatotoxicity in experimental animals.

CONCLUSION:

The present study was undertaken to evaluate the hepatoprotective potential of *Terminalia arjuna* extract against paracetamol-induced hepatotoxicity in experimental animals. Paracetamol administration produced marked biochemical alterations, confirming the successful induction of hepatic injury. The toxic control group showed significant elevations in SGOT/AST, SGPT/ALT, ALP, and total bilirubin, accompanied by a reduction in total serum protein when compared with the normal control group. These alterations indicate hepatocellular damage and impairment of normal hepatic function.Treatment with the standard drug silymarin significantly attenuated the paracetamol-induced biochemical changes and restored the evaluated parameters toward normal values. This response confirms the protective effect of the standard treatment and provides a suitable reference for evaluating the hepatoprotective activity of the test extract. Administration of *T. arjuna* extract at doses of 200 and 400 mg/kg produced significant improvement in the biochemical parameters when compared with the paracetamol-treated toxic control group. The extract reduced the elevated SGOT/AST, SGPT/ALT, ALP, and total bilirubin levels and simultaneously improved the reduced total protein concentration. The protective effect was more pronounced at 400 mg/kg than at 200 mg/kg, indicating a dose-related improvement in the hepatic biochemical profile.

The observed hepatoprotective activity may be associated with the phytoconstituents present in *T. arjuna*, particularly phenolic compounds, flavonoids, tannins, and triterpenoid constituents, which may contribute to antioxidant and cellular protective effects. These constituents may help limit oxidative damage and preserve hepatocyte membrane integrity. However, the exact molecular mechanisms responsible for the observed protection require further experimental confirmation. Taken together, the findings indicate that *Terminalia arjuna* extract attenuated the biochemical manifestations of paracetamol-induced hepatic injury and demonstrated promising hepatoprotective potential. Among the tested doses, 400 mg/kg showed greater protection than 200 mg/kg, with improvement across the major liver function parameters. The overall response supports the potential of *T. arjuna* as a source of hepatoprotective phytoconstituents.

Further studies involving liver histopathology, detailed oxidative stress assessment, inflammatory and apoptotic markers, quantitative phytochemical analysis, and isolation of active constituents are recommended to establish the precise mechanism of action and identify the compounds responsible for the hepatoprotective activity. Thus, the present study provides experimental evidence supporting further investigation of *T. arjuna* extract as a potential natural hepatoprotective agent.

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