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Original Article

Hepatoprotective Properties of *Polyherbal* Extract: A Promising Approach for Liver Necrosis Management

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ABSTRACT

Aim/Background: Alcohol-induced liver necrosis requires effective treatment. This study tests Polyherbal Extract's hepatoprotective effects on alcohol-induced liver necrosis in rats. The extract's impact on inflammatory pathways, serum liver enzymes (AST, ALT and ALP), bilirubin and histopathological changes are examined to determine mechanisms. **Materials and Methods:** We tested Polyherbal Extract's hepatoprotective effect in rats with ethanol-induced liver injury, which mimics human liver necrosis. To assess liver health, multiple Polyherbal Extract dosage groups were carefully established. In conventional and untreated groups, liver damage progression was monitored. Polyherbal Extract's liver and immune effects were assessed by inflammatory pathways. Bilirubin, AST, ALT and ALP showed hepatoprotection. The entire histology showed liver architecture changes. This integrated experimental design examines Polyherbal Extract's potential hepatoprotective mechanisms in alcohol-induced liver necrosis using ethanol-induced liver damage, diverse treatment groups, phytochemical analysis, toxicity assessment and parameter measurements. **Results:** This study found Polyherbal Extract's hepatoprotective effects promising. Its therapeutic potential was confirmed by phytochemical analysis. The acute toxicity study confirmed the extract's safety at specified dosages, supporting its therapeutic use. In liver function, Polyherbal Extract was effective. Compared to toxins, treated groups had significantly lower AST, ALT and ALP levels. Bilirubin levels decreased dose-dependently, indicating the extract's liver health benefits. Histopathological findings were useful at higher extract doses. Hepatocellular necrosis and inflammatory cell infiltration decreased in treated groups, suggesting the extract may reduce liver tissue structural damage. **Conclusion:** The Polyherbal Extract demonstrated promising hepatoprotective effects against alcohol-induced liver necrosis in the rat model. The reduction in liver enzymes, bilirubin levels and histopathological improvements, along with its safety profile, suggest its potential as an effective hepatoprotective agent. Further investigations into its mechanisms and clinical applications are warranted.

Keywords: Polyherbal Extract, Hepatoprotective, Liver Necrosis, Alcohol-induced, Rat Model, Serum Enzymes, Histopathological, Bioactive Compounds.

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INTRODUCTION

The prevalence and detrimental impact of alcohol-induced liver damage on public health provides a significant challenge in combating liver diseases, which affect a substantial proportion of the global population. Liver necrosis is a prevalent pathological disease that may occur in the liver due to alcohol use.[1,2]

There is an urgent need to find safe and effective hepatoprotective medicines, even if medical progress has led to several treatment strategies. In this context, natural products have come to light as a possible source of relief from liver damage due to the wide variety of bioactive components they contain[3,4]

A thorough comprehension of Polyherbal extract's effectiveness in controlling alcohol-induced liver necrosis is currently missing, while there is mounting evidence indicating its helpful involvement in liver illnesses. There is a lack of effective treatments for alcohol-induced liver impairment and those that are available often come with unwanted side effects. Consequently, there is a dearth of research examining novel, secure and efficacious therapeutic medications for the treatment of liver necrosis resulting from chronic alcohol use.[5,6]

In order to address this information gap, we want to investigate the hepatoprotective properties of Polyherbal extract in a rat

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model of alcohol-induced liver necrosis. This research aims to further our knowledge of the extract's action mechanisms by investigating its effects on alcohol-induced liver damage-related inflammatory pathways, serum liver enzymes, histological abnormalities and oxidative stress indicators. This research intends to clarify the potential of Polyherbal extract in reducing alcohol-induced liver necrosis. In doing so, it aims to contribute to the expanding corpus of information indicating that natural compounds with therapeutic potential might be beneficial in managing alcohol-related liver diseases.[7,8]

We hope that this study will shed light on the medicinal possibilities of Polyherbal extract and lead to its future examination as a possible treatment for liver necrosis. To add to the rising burden of alcohol-induced liver impairment, filling this research gap may provide fresh insights into alternative and adjunct treatment options.

Animals

The rats used were albinos, with a weight range of 150 to 200 grams, purchased from reliable suppliers. The animals were given an unlimited supply of food and water and fed a standard pellet diet. The animals were housed in an acceptable manner by the K.B.H.S.S.T's Institute of Pharmacy's Institutional Animal Ethics committee (1566/PO/a/11/CPCSEA) Bhaygaon Road, Malegaon.

The chemicals and standard medicine utilized in this study were Silymarin (manufactured by Merck Pharmaceuticals Ltd.) dissolved in ethanol and normal saline solution.

Preparation of extract

A substantial quantity of *Mentha piperita, Curcuma longa, Zingiber officinale* and *Glycyrrhiza glabra* were gathered and subsequently identified. After thorough drying, these botanicals were pulverized using a mortar and pestle. The resulting dried powder samples were utilized for diverse analyses. The Polyherbal extraction process involved using 20g of the ground calyx sample in a Soxhlet extractor with various solvents, namely distilled water, ethanol, methanol, ethyl acetate and petroleum ether.[9]

Preliminary phytochemical evaluation

Polyherbal extract underwent a battery of tests to ascertain the presence of various components as part of its preliminary phytochemical assessment. Shaking the extract vigorously with water was the foam test for saponins; the development of persistent foam indicated the presence of the compounds. If the extract caused a haemolytic zone to form when added to a blood drop on a glass slide, it meant that saponins were present, according to the hemolytic test. Each of the four alkaloid tests, namely Dragendorff's, Mayer's, Wagner's and Hager's, confirmed the presence of alkaloids in the extract by generating distinct precipitates upon treatment with the relevant reagents. After subjecting the extract to certain agents, flavonoid assays such the Zinchydrochloride test, alkaline reagent test and Shinoda test might detect the presence of flavonoids by observing color changes or the production of precipitates. By observing the development of a red ring in the Libermann-Burchard test and the sinking of sulfur powder in the sulfur powder test, triterpenoid presence was determined, respectively. Results showed that lignin was present when treated with solutions of strong HCl and phloroglucinol and thionine, respectively, producing pink and bluish-violet colors.[10-12]

Determination of Acute toxicity (LD_{50})

Administered a dose of 2000 mg/kg of body weight to the animal by dissolving the Polyherbal extract in Tween-80. I then gave the animal 1 mL/100 g b.w. of the solution. We used albino male rats to conduct acute toxicology research on the extract by the acute toxic classic approach (OECD guideline 425, 2006).[13]

The subjects in the acute toxicity study were provided with water and fasted overnight. During a 14-day timeframe, a solitary animal was given a solution of the extract in Tween-80 orally, at a dosage of 2000 mg/kg. Close observation was maintained, with special care given to the first 4 hr after administration and then continuous monitoring thereafter. The test had five animals overall; if one of them survived, the next four would sequentially receive dosages. Using a battery of tests measuring mental state, motor skills, pain threshold, reflexes and autonomic processes including writhing, faeces, urine and piloerection, researchers analyzed the subjects' neurological, behavioural and autonomic characteristics. The objective of this method was to assess if the extract caused any pain or negative side effects.[14-17]

Experimental Design

Animals

Overnight fasting with unrestricted access to water will be practiced on male Wistar rats (200-250 g) for the research. There was a total of sixty-six animals used in the studies, with six animals in each group.[18]

Treatment Protocol

The experimental treatment protocol involved six distinct groups of animals: a normal control group receiving 10 mL/kg of saline via intraperitoneal injection daily; a toxic control group administered with 5 mL/kg of ethanol via oral gavage daily; the control group received a conventional dosage of 100 mg/kg of silymarin by intravenous injection daily. The experimental groups were divided into three test groups, each receiving various dosages (100 mg/kg, 200 mg/kg and 400 mg/kg) of a polyherbal extract through oral administration daily. Each group comprised six animals and the specified treatments were administered consistently over a designated duration, respecting ethical guidelines for animal experimentation.[19,20]

Blood Collection and Biochemical Analysis

Six groups of animals were used in the experiment: one group received 10 mL/kg of saline intraperitoneally every day, another group received 5 mL/kg of ethanol orally gavage every day, the control group received a conventional dosage of 100 mg/kg of silymarin by intravenous injection daily. The experimental groups were divided into three test groups, each receiving various dosages (100 mg/kg, 200 mg/kg and 400 mg/kg) of a polyherbal extract through oral administration daily. We followed all applicable ethical protocols for testing on animals; each group included six animals and we gave each of them the same therapy at the same time for a certain amount of time.[21,22]

Clinical Parameter Evaluation

Serum liver enzymes including Aspartate Transaminase (AST), Alanine Transaminase (ALT) and Alkaline Phosphatase (ALP) are important indicators for assessing liver disease and damage. While an increase in which is mostly present in the liver indicates destruction to liver cells, an increase in AST might indicate acute damage to other organs as well. Extensive liver damage reduces total protein levels, which include albumin, fibrinogen and other plasma proteins produced by liver cells. This may cause hypoalbuminemia and, in certain chronic inflammatory diseases, hyperglobulinemia. Jaundice may be caused by an excess of bilirubin, poor hepatic processing, or inadequate excretion; understanding bilirubin metabolism, which is essential for bile formation, helps identify these causes and sheds insight into biliary disease.[23,24]

Statistical analysis

A one-way Analysis of Variance (ANOVA) using Dunnett's *t*-test. With a sample size of 6, the data is presented as Mean±SEM. A notable discovery is the existence of a $p<0.001$ in comparison to the control group that received the vehicle and toxic control treatments. In comparison to the toxic control, there are additional noteworthy results such as $p>0.05$, $^{**}p<0.01$ and $^{***}p<0.001$.

RESULTS

Phytochemical investigation

Sample phytochemical analysis identified many bioactive substances. The sample included a variety of compounds, including tannins, anthocyanins, terpenoids, saponins and flavonoids. There is a wide range of biological activity and possible health advantages associated with these chemicals.

Results from the Acute Oral Toxicity Study

Using an oral dose of 2000 mg/kg and following the guidelines laid forth in OECD guideline Annexure 425, the acute toxicity of the Polyherbal extract was investigated. There was no discernible adverse effect from oral dosing up to 2000 mg/kg body weight. There were no fatalities or signs of toxicity, therefore the extracts are safe to use. As part of this study, researchers settled on two dose levels: 200 mg/kg, or 10% of body weight and 400 mg/kg, or 15% of body weight. Substance exposure in acute toxicity research resulted in a thorough evaluation of many physiological parameters in the individuals. As far as anybody could tell, there were no negative side effects on breathing, movement, seizures, reflexes, eyes, mouth, pain, gastrointestinal symptoms, or skin. Specifically, no respiratory blockage, dyspnea, or other respiratory abnormalities were noted. Motor activities remained normal, with no signs of somnolence, catalepsy, or convulsions observed. Reflexes such as corneal, eyelid closure and light reflexes were normal, while ocular signs like lacrimation, meiosis and conjunctivitis were absent. Furthermore, no signs of salivation, piloerection, altered response to induced pain, gastrointestinal alterations, or skin reactions such as oedema or erythema were detected. Overall, the study outcomes suggest a lack of acute toxic effects based on the observed parameters in the tested subjects following exposure to the substance under investigation.

Pharmacological Effects

The impact of a Polyherbal extract on liver necrosis produced by alcohol in rats

In this investigation, hepatotoxicity was induced by the injection of ethanol. The dosage of 0.5 mL/100 g was administered orally every other day. We measured the blood levels of bilirubin, AST, ALT and ALP to determine the hepatoprotective activity of HI. AST stands for aspartate transaminase in serum.

Influence of Polyherbal Extract on AST Levels in Serum

Research found that ethanol significantly raised serum AST levels compared to the control group. A very substantial ($p<0.001$) decrease in serum AST level was the outcome of administering both the conventional medication (Silymarin) and the treatment with 200 mg/kg and 400mg/kg of Polyherbal extract. The results are presented in Table 2 and Figure 1.

Assessment of Polyherbal Extract on Alanine Transaminase (ALT) Level in Serum

The investigation aimed to assess the impact of Polyherbal Extract on serum Alanine Aminotransferase (ALT) levels in rats subjected to Ethanol-induced liver damage.

The findings demonstrated that the groups given varying doses of Polyherbal Extract had ALT level reductions that were dose-dependent. Toxic control subjects exposed to ethanol, on the other hand, had significantly elevated ALT levels.

In particular, 100 mg/kg, 200 mg/kg and 400 mg/kg doses of Polyherbal Extract considerably mitigated the ethanol-induced rise in ALT levels, suggesting potential protective effects on the liver. Based on these results, Polyherbal Extract may

have hepatoprotective properties and be useful in treating ethanol-induced liver damage. Further investigations focusing on elucidating the precise mechanisms underlying the observed hepatoprotective effects are essential to validate and comprehend the therapeutic significance of Polyherbal Extract in liver-related disorders. The results are presented in Table 3 and Figure 2.

Serum Alkaline Phosphatase (ALP)

Impact of Polyherbal Extract on Serum Alkaline Phosphatase (ALP) Levels

The investigation focused on evaluating the influence of Polyherbal Extract on serum Alkaline Phosphatase (ALP) levels in rats subjected to Ethanol-induced liver damage. Top of Form

The findings demonstrated that the toxic control group administered ethanol had significantly elevated ALP levels. There was a dose-dependent reduction in ALP levels in the treatment groups that received varying quantities of Polyherbal Extract, in contrast to the toxic control group. To be more specific, Polyherbal Extract was able to protect the liver against Ethanol damage when dosed at 100 mg/kg, 200 mg/kg and 400 mg/kg, as evidenced by the substantial decreases in ALP levels. Results showing a significant reduction in ALP levels in the treated groups compared to the toxic control group indicate that the Polyherbal Extract may have hepatoprotective effects. These findings suggest the potential therapeutic application of Polyherbal Extract in ameliorating Ethanol-induced liver damage and signify its role as a possible hepatoprotective agent. However, further exploration elucidating its underlying mechanisms and long-term effects is imperative to validate its efficacy and safety in liver-related disorders. The results are presented in Table 4 and Figure 3.

Bilurubin

Effect of Polyherbal Extract on Serum Level of Bilirubin

The investigation scrutinized the impact of Polyherbal Extract on serum Bilirubin levels in rats subjected to Ethanol-induced liver damage. The results are presented in Table 5 and Figure 4.

Examinations of liver tissue at a histopathological study

We wanted to know how Polyherbal Extract affected ethanol-induced liver damage, thus we did a histopathology analysis of liver tissue. Varied experimental groups showed varied changes in liver architecture according to the histological study. Damage to the liver, including fatty alterations, inflammatory cell infiltration and hepatocellular necrosis, was substantial in the ethanol-treated toxic control group. Liver histology improved in a dose-dependent manner in contrast to groups treated with different

Table 1: Experimental Design.[19]

Group	Name	Treatment	No. of animals
G1	Normal control group.	10 mL/kg Saline.	6
G2	Toxic control.	Ethanol 5 mL/kg.	6
G3	Standard	Silymarin 100 mg/kg.	6
G4	Test low dose.	*Polyherbal* extract 100 mg/kg.	6
G5	Test medium dose.	*Polyherbal* extract 200 mg/kg.	6
G6	Test High dose	*Polyherbal* extract 400 mg/kg	6

Table 2: Aspartate Aminotransferase (AST) levels in their serum.

Group	Treatment	AST (IU/L)
Group I	Control administration (10 mL/kg of normal saline).	93.06±1.67
Group II	Administration of toxic control solution at a dosage of 5 mL/kg of body weight, consisting of ethanol.	345.28±0.45
Group III	The toxic control solution, including ethanol, is administered at a dose of 5 mL/kg of body weight.	103.28±0.17***
Group IV	Treatment (100 mg/kg Polyherbal extract).	131.07±0.91***
Group V	Treatment (200 mg/kg Polyherbal extract).	117.16±2.87***
Group VI	Treatment (400 mg/kg Polyherbal extract).	114.72±1.52**

Table 3: Assessment of Polyherbal Extract on Alanine Transaminase (ALT) Level in Serum.

Group	Treatment	ALT (IU/L)
Group I	Control administration (10 mL/kg of normal saline).	45.98±1.38
Group II	Administration of toxic control solution at a dosage of 5 mL/kg of body weight, consisting of ethanol.	265.18±1.5
Group III	When administering the toxic control solution, which contains ethanol, 5 mL/kg of body weight is the recommended dosage.	57.02±2.09**
Group IV	Treatment (100 mg/kg Polyherbal extract).	202.04±1.11***
Group V	Treatment (200 mg/kg Polyherbal extract).	77.98±1.76***
Group VI	Treatment (400 mg/kg Polyherbal extract).	75.28±1.35**

Table 4: The serum levels of Alkaline Phosphatase (ALP) were measured in six rats from each group and the mean values were calculated.

Group	Treatment	ALP (IU/L)
Group I	Control administration (10 mL/kg of normal saline).	120.52±1.20
Group II	Administration of toxic control solution at a dosage of 5 mL/kg of body weight, consisting of ethanol.	391.42±0.24
Group III	When administering the toxic control solution, which contains ethanol, 5 mL/kg of body weight is the recommended dosage.	132.82±0.75***
Group IV	Treatment (100 mg/kg Polyherbal extract).	253.24±0.87***
Group V	Treatment (200 mg/kg Polyherbal extract).	139.65±2.98***
Group VI	Treatment (400 mg/kg Polyherbal extract).	137.34±1.25***

Table 5: The serum levels of bilirubin.

Group	Treatment	Bilirubin (mg/dL)
Group I	Control administration (10 mL/kg of normal saline).	1.33±0.42
Group II	Administration of toxic control solution at a dosage of 5 mL/kg of body weight, consisting of ethanol.	4.23±1.20
Group III	Toxic control solution, contains ethanol, 5 mL/kg of body weight is the recommended dosage.	1.73±1.80***
Group IV	Treatment (100 mg/kg Polyherbal extract).	2.90±0.65**
Group V	Treatment (200 mg/kg Polyherbal extract).	2.35±1.2**
Group VI	Treatment (400 mg/kg Polyherbal extract).	2.18±0.62**

amounts of Polyherbal Extract. In particular, as compared with the harmful control group, the groups administered Polyherbal Extract showed less hepatocellular necrosis, less fatty alterations and less inflammatory cell infiltration. The liver histology of the groups given the highest dosage of Polyherbal Extract showed the greatest improvement, with almost normal architecture and very little inflammation and necrosis. Polyherbal Extract may have hepatoprotective properties against Ethanol-induced liver injury, according to these histopathological results. This suggests that it may have a role in reducing hepatic damage and that it might be worth investigating further as a possible treatment for liver diseases. Confirming the histological data and fully comprehending the therapeutic potential of Polyherbal Extract in liver-related illnesses requires more investigations that include precise processes and long-term consequences. The results are presented in Figure 5.

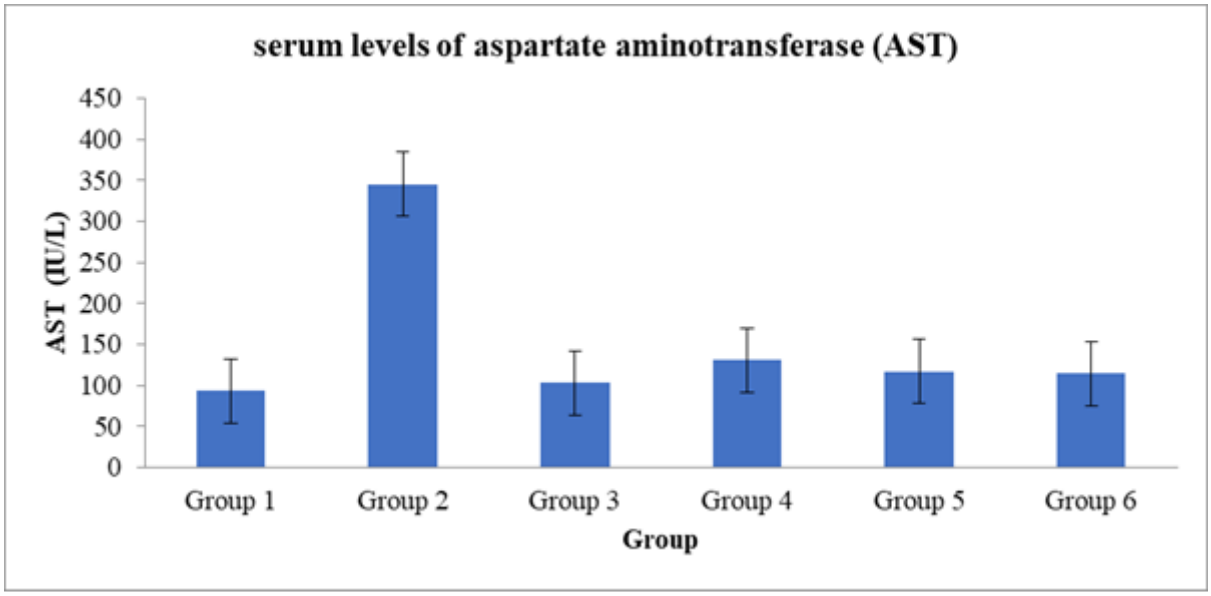

Figure 1: Graph depicting the serum levels of Aspartate Aminotransferase (AST) values for the mean of 6 rats in each group. A one-way Analysis of Variance (ANOVA) using Dunnett's *t*-test. With a sample size of 6, the data is presented as Mean±SEM. A notable discovery is the existence of a $p<0.001$ in comparison to the control group that received the vehicle and toxic control treatments. In comparison to the toxic control, there are additional noteworthy results such as $p>0.05$, $^{**}p<0.01$ and $^{***}p<0.001$.

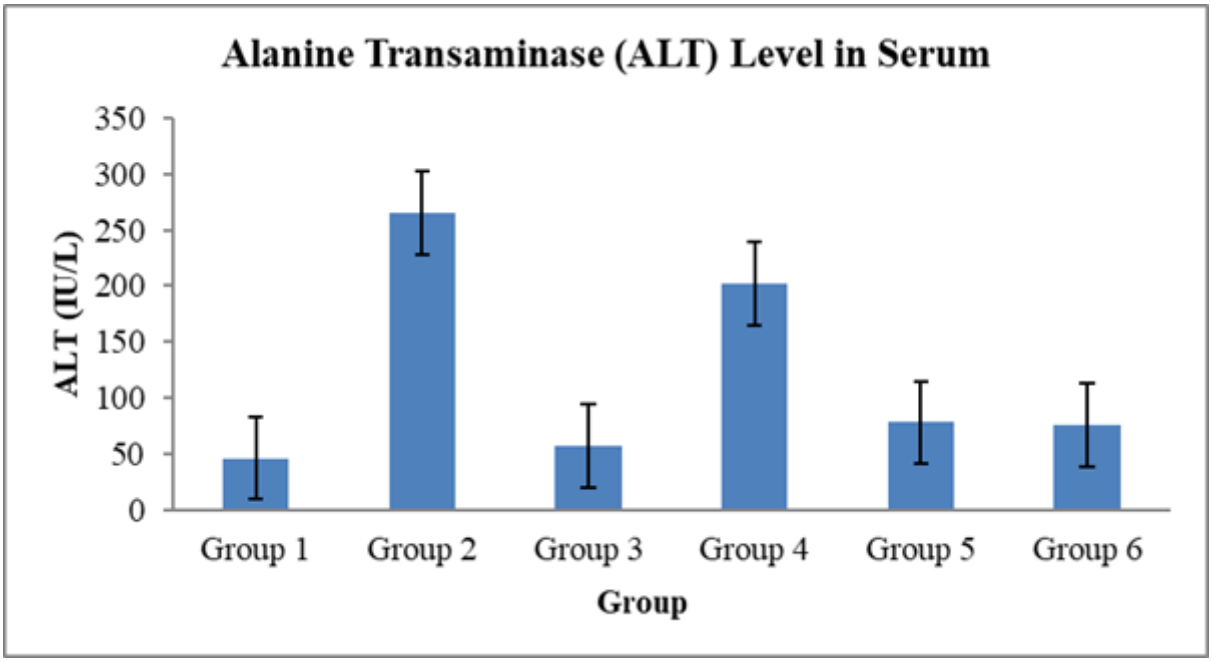

Figure 2: Assessment of Polyherbal Extract on Alanine Transaminase (ALT) Level in Serum. A one-way Analysis of Variance (ANOVA) using Dunnett's t-test. With a sample size of 6, the A one-way Analysis of Variance (ANOVA) using Dunnett's *t*-test. With a sample size of 6, the data is presented as Mean± SEM. A notable discovery is the existence of a $p<0.001$ in comparison to the control group that received the vehicle and toxic control treatments. In comparison to the toxic control, there are additional noteworthy results such as $p>0.05$, $^{**}p<0.01$ and $^{***}p<0.001$.

DISCUSSION

The phytochemical analysis of the Polyherbal Extract revealed the presence of various bioactive substances, including tannins, anthocyanins, terpenoids, saponins and flavonoids. These compounds are associated with a diverse range of biological activities and potential health benefits. The acute oral toxicity study, conducted according to OECD guidelines, demonstrated the safety of the extract up to 2000 mg/kg body weight, indicating its suitability for therapeutic use. Two selected dosage levels, 200 mg/kg and 400 mg/kg, were chosen for further investigations.

In the pharmacological evaluation, the Polyherbal Extract exhibited hepatoprotective effects against ethanol-induced

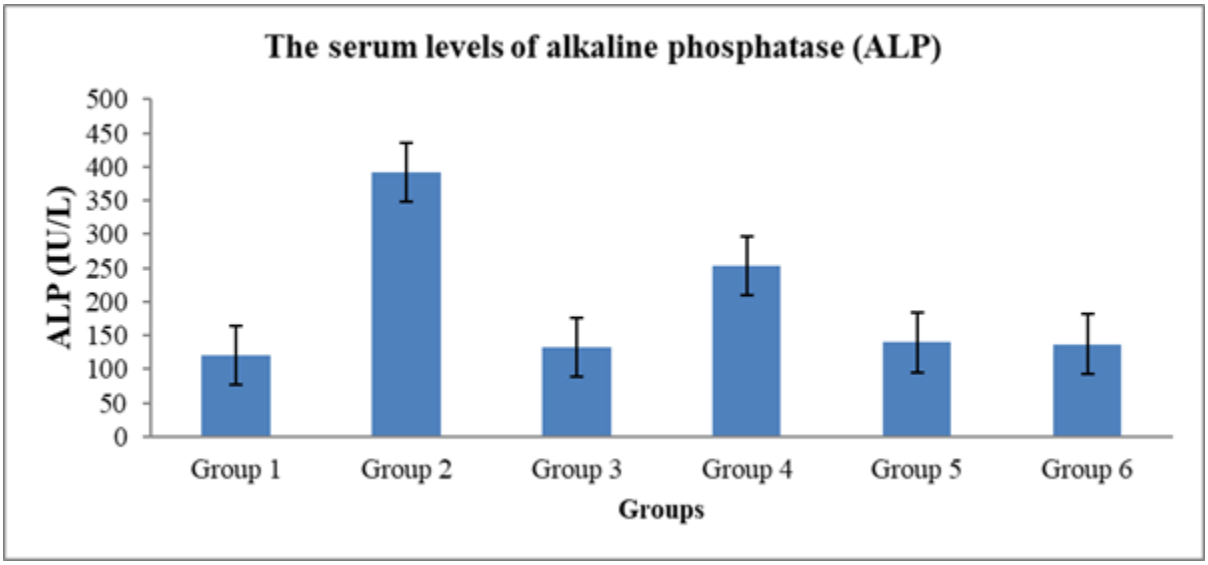

Figure 3: The serum levels of Alkaline Phosphatase (ALP) were measured in six rats from each group and the mean values were calculated. A one-way Analysis of Variance (ANOVA) using Dunnett's *t*-test. With a sample size of 6, the data is presented as Mean±SEM. A notable discovery is the existence of a $p<0.001$ in comparison to the control group that received the vehicle and toxic control treatments. In comparison to the toxic control, there are additional noteworthy results such as $p>0.05$, $^{**}p<0.01$ and $^{***}p<0.001$.

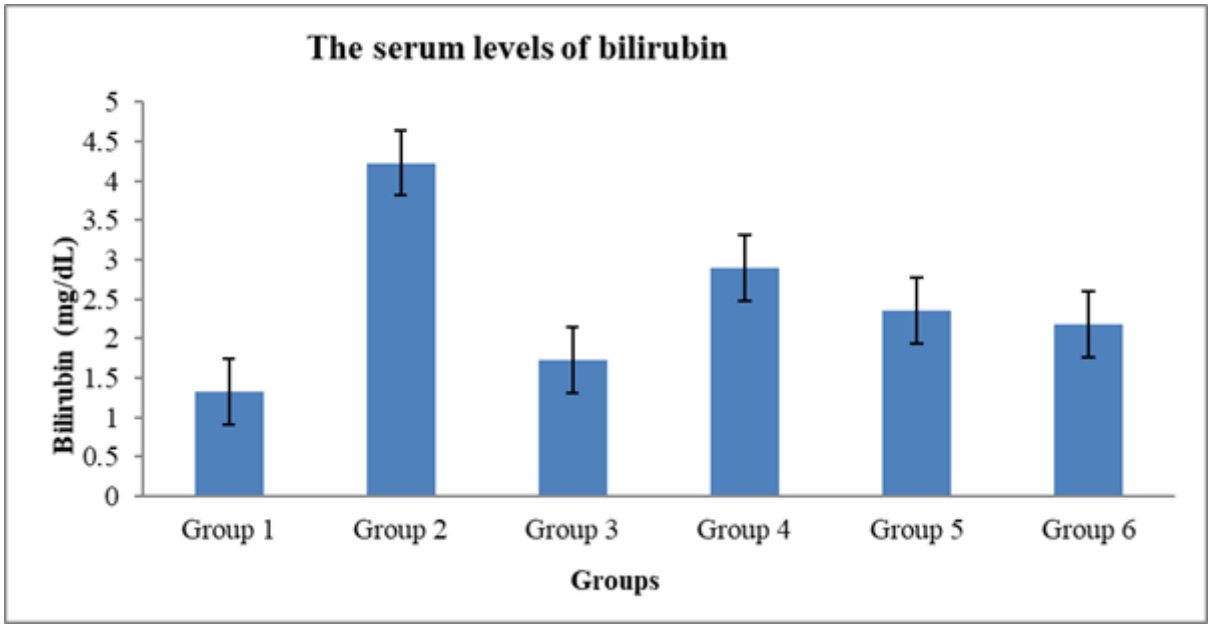

Figure 4: Serum levels of BILURUBIN values. A one-way Analysis of Variance (ANOVA) using Dunnett's *t*-test. With a sample size of 6, the data is presented as Mean±SEM. A notable discovery is the existence of a $p<0.001$ in comparison to the control group that received the vehicle and toxic control treatments. In comparison to the toxic control, there are additional noteworthy results such as $p>0.05$, $^{**}p<0.01$ and $^{***}p<0.001$.

liver damage in rats. Serum levels of liver enzymes (AST, ALT and ALP) significantly decreased in groups treated with the extract, indicating its potential to mitigate liver injury. The dose-dependent reduction in ALT levels in the treated groups further supported the extract's hepatoprotective properties.

The impact of the Polyherbal Extract on serum Alkaline Phosphatase (ALP) levels revealed a dose-dependent reduction compared to the toxic control group, suggesting its potential to protect the liver against ethanol-induced damage. The examination of bilirubin levels supported the hepatoprotective effects of the extract.

Histopathological analysis provided visual evidence of the Polyherbal Extract's protective effects, showing less hepatocellular necrosis, fatty alterations and inflammatory cell infiltration in treated groups compared to the toxic control group. The highest dosage of the extract exhibited almost normal liver architecture with minimal inflammation and necrosis.

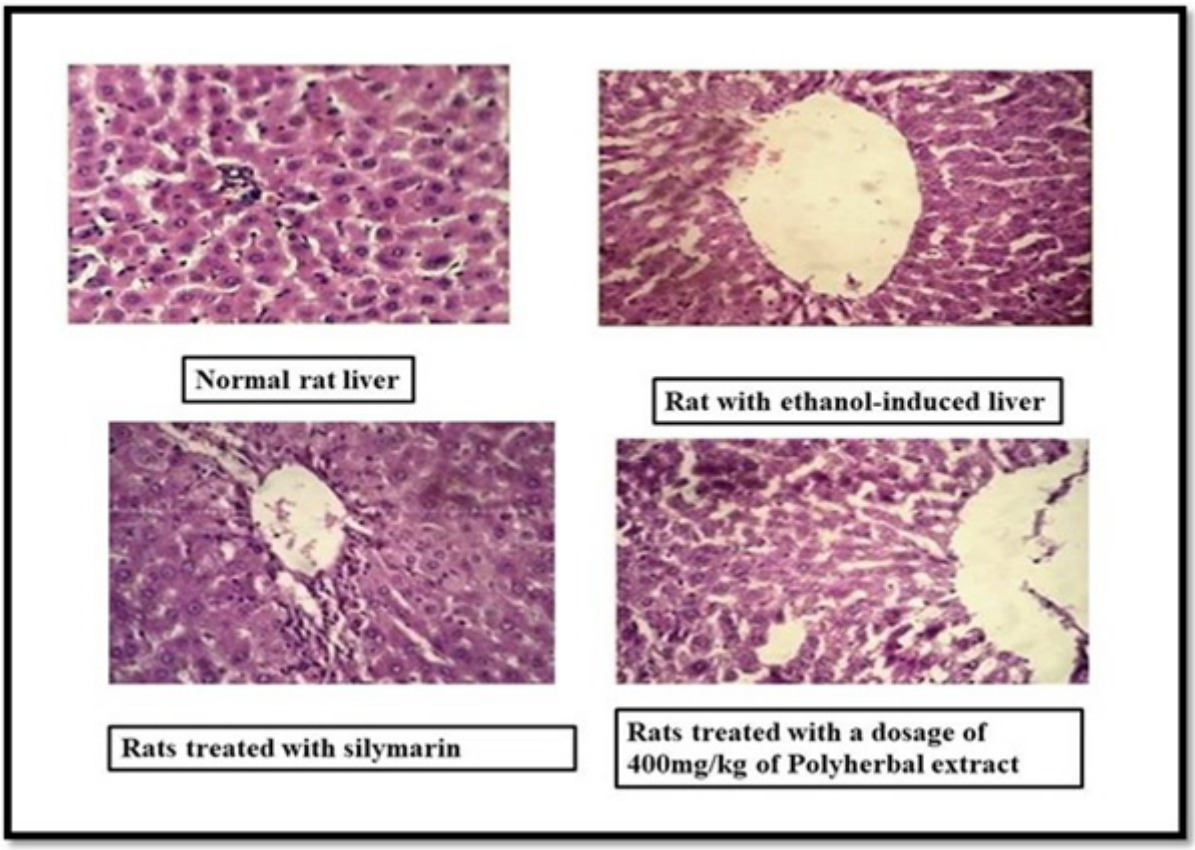

Figure 5: The impact of polyherbal extract and silymarin on histopathology of the liver in cases with ethanol-induced liver damage.

Overall, the comprehensive findings suggest that the Polyherbal Extract has hepatoprotective properties against ethanol-induced liver damage. Further investigations into the underlying mechanisms and long-term effects are crucial for validating its therapeutic potential and safety in liver-related disorders. The extract holds promise as a potential treatment for liver diseases, warranting further exploration and research.

CONCLUSION

The investigation into the hepatoprotective properties of Polyherbal Extract against alcohol-induced liver necrosis has yielded promising outcomes. The study encompassed phytochemical analysis, acute toxicity evaluation and an ethanol-induced liver damage rat model. The Phytochemical Evaluation showcased a rich composition of bioactive compounds within the Polyherbal Extract, including tannins, anthocyanins, terpenoids, saponins and flavonoids, hinting at its diverse potential health benefits. Results from the Acute Toxicity Study indicated the safety of the Polyherbal Extract at oral doses up to 2000 mg/kg body weight, demonstrating its potential for safe usage without adverse effects. The Experimental Study Design involving diverse treatment groups displayed the significant hepatoprotective effects of the Polyherbal Extract. It notably reduced serum levels of liver enzymes (AST, ALT and ALP) and bilirubin compared to the toxic control group, exhibiting its efficacy in mitigating alcohol-induced liver damage in a dose-dependent manner. Histopathological Evaluation further supported these findings, revealing improved liver architecture, diminished hepatocellular necrosis, fatty alterations and reduced inflammatory cell infiltration in groups treated with Polyherbal Extract, especially at higher dosages. This suggests a protective effect against ethanol-induced liver injury. Further research is essential to uncover the precise mechanisms behind its effects, aiding in targeted treatment development. Translation of these findings into clinical trials is crucial to assess safety and efficacy in humans with alcohol-induced liver diseases. Long-term studies are necessary to ensure sustained efficacy and safety, while formulation standardization and exploring combination therapies could enhance its therapeutic outcomes. Additional investigations and clinical studies are imperative to validate its efficacy, safety and therapeutic application in liver-related disorders.

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ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The animals were housed acceptably under the oversight of the K.B.H.S.S.T's Institute of Pharmacy's Institutional Animal Ethics Committee (Approval No: 1566/PO/a/11/CPCSEA) on Bhaygaon Road, Malegaon. The study received ethical approval and consent was obtained for participation.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

AST: Aspartate Transaminase; **ALT:** Alanine Aminotransferase; **ALP:** Alkaline Phosphatase.

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