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Evaluation of Dose-Dependent Hepatoprotective Effects of a Novel Polyherbal Extract in Wistar Rats

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ABSTRACT

The study's aim is to assist science-based traditional medicine by assessing a polyherbal extract's capacity to protect the liver at varying doses in rats with CCl4-induced liver damage. Four adult male rat groups were used in an experimental investigation with random grouping. Three of the four groups received CCl4 together with either a high or low dose of polyherbal extract, whereas one group simply received CCl4. In addition to microscopic examination of necrosis, inflammation, and liver cell regeneration, liver damage was assessed using ALT, AST, ALP, and bilirubin assays. It was found that CCl4 severely harmed the liver, with more enzymes in blood and worse tissue degeneration, but higher amounts of the polyherbal extract successfully lessened these adverse effects. Also, the high-dose group largely recovered, as seen by nearly normal enzyme levels, little dead tissue, and many regenerating hepatocytes. Using ANOVA, the results were shown to be significant ($p < 0.05$), which proves the efficacy of the extract. The findings of this work indicate that polyherbal formulations have value in treating liver injury, yet further studies and clinical trials are important to move this approach into routine hepatoprotection for humans.

Key Words:

Dose-Dependent, Hepatoprotective, Novel Polyherbal Extract, Wistar Rats, carbon Tetrachloride (CCl_4)-induced Liver Toxicity

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1. INTRODUCTION

Liver diseases are a significant public health concern in most parts of the world, normally caused by toxic chemicals, drugs, infections, and alcohol [1]. It is the liver that is mainly responsible for detoxification, metabolism, and keeping biochemical stability [2]. Because synthetic hepatoprotective drugs can cause unwanted side effects, there is a need for better and safer options [3]. Using polyherbal remedies, herbal medicine has been a key part of traditional medicine for many years, serving to shield the liver from damage. Still, scientific studies are needed to prove that they really work [4]. The researchers aimed to

see how effective a new polyherbal extract is against liver damage caused by carbon tetrachloride in Wistar rats at several doses [5]. The researchers aimed to bring together scientific understanding and traditional herbal use to improve development of effective natural hepatoprotective therapies [6].

1.1. Background Information

Liver disorders resulting from contact with toxins, infection, or other dangerous agents, is a serious public health problem [7]. Because the liver is so important for metabolism, detoxification, and controlling chemicals in the body, it can easily be harmed [8]. People are turning to traditional medicine and herbal treatments more often for liver disorders, because of how safe and effective they are believed to be. Scientists are paying close attention to polyherbal medicines using several plants extracts due to their combined and wide-ranging abilities to safeguard the liver [9]. Experimental models involving animals like Wistar rats provide a trustworthy model to analyze such natural remedies in controlled setups [10]. This study evaluates the hepatoprotective effect of a new polyherbal extract at different doses for understanding its dose-response against liver damage.

1.2. Statement of the Problem

Even with all the advances in contemporary medicine, safe and effective therapeutic agents for liver protection are not yet forthcoming. Most synthetic hepatoprotective drugs carry side effects with prolonged administration. There is an urgent need to investigate alternative forms of therapy that can provide liver protection without loss of safety. Polyherbal preparations offer a promising direction, but there is a lack of thorough scientific proof. In particular, there is less research on the dose-dependent action of such formulations in hepatotoxic models. The shortfall creates a need for a systematic study to evaluate if higher doses of a polyherbal extract can proportionally increase liver protection in an experimentally induced hepatotoxic condition.

1.3. Objectives of the Study

The research objectives of the study are:

- To establish the impact of low and high doses of the polyherbal extract on liver function indicators (ALT, AST, ALP, and bilirubin) in rats with carbon tetrachloride (CCl_4)-induced hepatotoxicity.
- To evaluate histopathological alterations in liver tissues after treatment with the polyherbal extract.
- To determine the correlation of biochemical and histological results to confirm the hepatoprotective effect of the extract.
- To present scientific evidence validating the application of polyherbal formulations as prospective hepatoprotectors in future therapeutics.

2. METHODOLOGY

This research was to assess the dose-dependent hepatoprotective activity of a new polyherbal extract in Wistar rats. Aspect was laid on determining the ability of the extract to oppose liver damage brought by hepatoteoxic substances. The rats were administered with varying doses of the extract to establish its capacity as a natural drug of liver protection. An experimental design was used in the study with the

controlled and experimental groups being compared to see the effectiveness of the polyherbal extract.

2.1. Description of Research Design

Randomized controlled experimental approach to 4-group allocation was used in the research. The research was conducted with one control and two experimental groups under both hepatotoxicity and the given polyherbal extract. The polyherbal extract was administered to the experimental group rats in low and high doses, respectively. On the basis of the common liver toxin provocation, hepatotoxicity was observed; the activity of the extract upon liver function was evidenced after the therapy.

2.2. Sample Details

The participants in this study were male Wistar rats, 8-10 week-old and 180-220 grams. The rats were randomly divided into four experimental groups (n=7/ group);

- Group 1: Control group (no treatment)
- Group 2: Hepatotoxicity-induced group (no treatment with extract)
- Group 3: Hepatotoxicity-induced group treated with a low dose of polyherbal extract
- Group 4: Hepatotoxicity-induced group treated with a high dose of polyherbal extract

The rats were acclimated for a week before the experiment began and housed in typical laboratory settings with unrestricted access to food and water.

2.3. Instruments and Materials Used

1. **Polyherbal Extract:** A mixture of plant extracts prepared in-house. The dose was standardized based on previous studies on similar formulations.
2. **Hepatotoxic Agent:** Carbon tetrachloride (CCl_4) was used to induce liver damage.
3. **Blood Collection Tubes:** For serum analysis.
4. **Biochemical Assay Kits:** To measure liver function parameters (ALT, AST, ALP, bilirubin levels).
5. **Surgical Tools:** For euthanasia and organ dissection.
6. **Microscope:** To observe histopathological changes in liver tissue.
7. **Analytical Balance:** For accurate measurement of the polyherbal extract and doses.
8. **Incubator and Centrifuge:** For sample processing.

2.4. Procedure and Data Collection Methods

After acclimatization, the rats were subjected to induction of hepatotoxicity by a single intraperitoneal administration of CCl_4. After 24 hours, the experimental groups were orally administered the polyherbal extract for 14 days consecutively. The doses were set based on initial dose-ranging studies. Group 1 was left untreated (control), and group 2 (hepatotoxicity-induced) was left untreated.

Throughout the treatment course, body weights were checked on a daily basis, and all clinical signs of toxicity were documented. On the 15th day, the blood was sampled from each rat through the tail vein

for measurement of serum values of liver function tests, including ALT, AST, ALP, and bilirubin. Then, rats were sacrificed and liver tissues collected for histopathological examination in order to investigate the degree of liver damage and regeneration.

2.5. Data Analysis Techniques

SPSS (Statistical Package for Social Sciences) was used to analyse the data that was gathered. One-way analysis of variance (ANOVA) was used to compare the groups' serum liver enzyme levels. The severity of the damage was classified from mild to severe after histopathological findings were analysed. At $p<0.05$, every analysis was deemed statistically significant.

It was in order to test the dose-dependent hepatoprotective effect of a new polyherbal extract in Wistar rats. Biochemical, histopathological examinations showed that the polyherbal extract demonstrated strong hepatoprotective effects with dose-related modifications in various liver function marker and liver histological indices.

3. RESULTS

The study's findings, as demonstrated by liver function tests, histological analyses, and statistical observations in four experimental groups, are broadly summarised in the part that follows. The degree of liver damage and the polyherbal extract's potential for hepatoprotective action were determined by measuring the serum levels of the liver enzymes bilirubin, ALT, AST, and ALP. Numerous enzymes in the hepatotoxicity-induced group demonstrated liver damage. On the other hand, these levels of enzymes were inhibited in dose-dependent response by the low and high doses of the polyherbal extract, signifying the protective nature of the extract. Histopathology measurements also correlated with the biochemistry findings whereby the group that received the high dose extract had minimal necrosis, low inflammation, and enhanced hepatocyte regenerations. Analysis using one-way ANOVA of the statistics found improvement in the function of the liver and that of the tissue integrity after high dose of polyherbal extract, with the element that could protect the liver from destruction.

3.1. Presentation of Findings

The results of the statistical analysis, liver function tests, and histological evaluations in four experimental groups are summarised in this section. A polyherbal extract's hepatoprotective properties and liver damage were assessed using serum liver enzymes (ALT, AST, ALP, and bilirubin). High enzymes concentration in the group stimulated with hepatotoxicity revealed severe damages in the liver, while low and high doses of polyherbal extract indicated dose- dependent reductions in the concentration of the enzymes which were protective. Histopathological observations proved this finding, little necrosis, minor inflammatory reaction, and elevated hepatocyte regeneration at the high dose of the extract. Statistical analysis supported the fact that the polyherbal extract shown a notable improvement in the functioning of the liver and integrity of the tissue, with the high dosage being most effective in guarding against the damage of the liver.

- **Serum Liver Function Markers**

To assess liver damage and the hepatoprotective effectiveness of the polyherbal extract, liver function indicators including ALT, AST, ALP, and bilirubin were measured. Serum liver function biomarkers ALT, AST, ALP, and bilirubin data for four

test groups are displayed in Table 1 to assess the state of liver health. These parameters were chosen as markers of hepatic integrity and function, and increases are generally indicative of liver injury. They are presented as mean ± standard deviation for each treatment group to facilitate comparison between controls, hepatotoxic injury, and the action of two varying doses of a polyherbal mixture used for hepatoprotection.

Table 1: Serum Liver Enzyme Levels (Mean ± SD)

Group	ALT (U/L)	AST (U/L)	ALP (U/L)	Bilirubin (mg/dL)
Control (Group 1)	45.2 ± 5.3	48.1 ± 4.8	85.4 ± 6.2	0.3 ± 0.1
Hepatotoxicity-induced (Group 2)	180.5 ± 10.1	198.4 ± 12.5	215.6 ± 16.8	2.4 ± 0.2
Low Dose Polyherbal Extract (Group 3)	112.6 ± 8.2	125.3 ± 9.3	150.1 ± 12.4	1.2 ± 0.1
High Dose Polyherbal Extract (Group 4)	70.1 ± 6.7	79.3 ± 7.1	120.5 ± 9.9	0.5 ± 0.1

Table 1 unequivocally exhibits the hepatoprotective activity of the polyherbal extract in a dose-dependent fashion. Group 2, which was the group with hepatotoxicity induction, had very high levels of ALT (180.5 ± 10.1 U/L), AST (198.4 ± 12.5 U/L), ALP (215.6 ± 16.8 U/L), and bilirubin (2.4 ± 0.2 mg/dL), thereby proving extensive liver damage. The high dose of the polyherbal extract as treatment (Group 3) decreased these elevated levels of the enzymesALT to 112.6 ± 8.2 U/L, AST to 125.3 ± 9.3 U/L, ALP to 150.1 ± 12.4 U/L, and bilirubin to 1.2 ± 0.1 mg/Dlthus showing partial hepatoprotection. Of note, the Group 4 (high dose) showed enzyme activities (ALT: 70.1 ± 6.7 U/L, AST: 79.3 ± 7.1 U/L, ALP: 120.5 ± 9.9 U/L, bilirubin: 0.5 ± 0.1 mg/dL) near those of Group 1 (control), indicating a significant restoration of liver damage and efficient hepatoprotection at the higher dose.

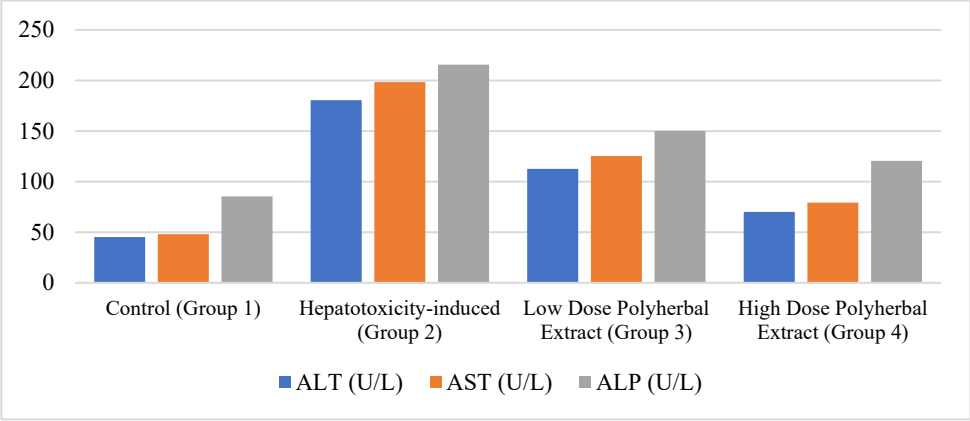

Figure 1: Graphical Representation of Serum Liver Enzyme Levels of Mean

The information illustrated in Figure 1 presents the levels of serum liver enzymes (ALT, AST, and ALP) in four groups. Group 1 (Control) has normal liver enzyme levels, with ALT at 45.2 U/L, AST at 48.1 U/L, and ALP at 85.4 U/L, which represents normal liver function. Group 2 (Hepatotoxicity-induced) reveals a marked elevation in liver enzymes, with ALT being 180.5 U/L, AST being 198.4 U/L, and ALP being 215.6 U/L, reflecting severe liver injury. In Groups 3 and 4 (Low and High Dose Polyherbal Extract), the enzyme levels reduce from Group 2, and there is an appreciable recovery of liver function in both groups. Group 3 indicates a decrease in ALT (112.6 U/L), AST (125.3 U/L), and ALP (150.1 U/L), whereas Group 4 indicates improvement with ALT at 70.1 U/L, AST at 79.3 U/L, and ALP at 120.5 U/L. According to these results, the polyherbal extract has a protective effect on the liver, reducing hepatotoxicity and bringing liver enzyme levels back to normal.

- **Statistical Comparison of Liver Enzyme Levels**

A statistical analysis via one-way ANOVA established differences in the levels of liver enzymes between groups. Post hoc pairwise comparisons confirmed that the polyherbal extract at the highest dose was the most effective to decrease liver enzyme levels relative to the hepatotoxicity-induced group.Table 2 indicates the statistical significance of the levels of liver enzymes ALT, AST, ALP, and bilirubin in the four experiment groups by comparison with one-way ANOVA. The means are represented in the form mean ± standard deviation, and their respective p-values tell us if the observed variability of the levels of liver parameters among the groups is statistically significant or not. This analysis assists in establishing the efficacy of the polyherbal extract in modulating liver biomarkers under conditions of hepatotoxicity.

Table 2: Statistical Comparison of Liver Enzyme Levels (Mean ± SD) and p-values

Parameter	Control (Group 1)	Hepatotoxicity-Induced (Group 2)	Low Dose (Group 3)	High Dose (Group 4)	p-value (ANOVA)
ALT (U/L)	45.2 ± 5.3	180.5 ± 10.1	112.6 ± 8.2	70.1 ± 6.7	<0.05
AST (U/L)	48.1 ± 4.8	198.4 ± 12.5	125.3 ± 9.3	79.3 ± 7.1	<0.05
ALP (U/L)	85.4 ± 6.2	215.6 ± 16.8	150.1 ± 12.4	120.5 ± 9.9	<0.05
Bilirubin (mg/dL)	0.3 ± 0.1	2.4 ± 0.2	1.2 ± 0.1	0.5 ± 0.1	<0.05

Table 2 proves that the group of hepatotoxicity-induced (Group 2) had drastically increased levels of liver enzymes ALT (180.5 ± 10.1 U/L), AST (198.4 ± 12.5 U/L), ALP (215.6 ± 16.8 U/L), and bilirubin (2.4 ± 0.2 mg/dL) than the control group (Group 1), which had normal values ALT (45.2 ± 5.3 U/L), AST (48.1 ± 4.8 U/L), ALP (85.4 ± 6.2 U/L), and bilirubin (0.3 ± 0.1 mg/dL), with p-values < 0.05 for statistical significance. Administration of the

polyherbal extract resulted in dose-dependent effects: the low-dose group (Group 3) presented decreased values ALT (112.6 ± 8.2 U/L), AST (125.3 ± 9.3 U/L), ALP (150.1 ± 12.4 U/L), and bilirubin (1.2 ± 0.1 mg/dL) whereas the high-dose group (Group 4) presented even more normalization of enzyme levels ALT (70.1 ± 6.7 U/L), AST (79.3 ± 7.1 U/L), ALP (120.5 ± 9.9 U/L), and bilirubin (0.5 ± 0.1 mg/dL). These reductions that are statistically significant attest to the hepatoprotective efficacy of the polyherbal extract, especially at the higher dose.

- **Histopathological Findings**

Histopathological analysis of liver tissue sections also validated the biochemical findings. The liver tissues were assessed for necrosis, inflammation, and regeneration of hepatocytes.Table 3 shows liver tissue histopathological grading from four experimental groups, assessing prominent indicators like necrosis, inflammation, and regeneration of hepatocytes. The mean ± standard deviation data gives us an idea about the degree of liver tissue injury and repair at varying treatment regimes. This information is useful for understanding the integrity of liver tissue structures and any possible protective benefits of the polyherbal drug against hepatotoxity injury.

Table 3: Histopathological Grading of Liver Damage (Mean ± SD)

Group	Necrosis	Inflammation	Hepatocyte Regeneration
Control (Group 1)	0.2 ± 0.1	0.3 ± 0.2	0.4 ± 0.1
Hepatotoxicity-induced (Group 2)	3.8 ± 0.2	4.2 ± 0.3	1.1 ± 0.2
Low Dose Polyherbal Extract (Group 3)	2.0 ± 0.1	2.5 ± 0.3	2.0 ± 0.2
High Dose Polyherbal Extract (Group 4)	0.4 ± 0.2	0.8 ± 0.3	3.6 ± 0.2

Table 3 gives a comparative histopathological evaluation of liver injury by assessing necrosis, inflammation, and hepatocyte regeneration in the four groups. The hepatotoxicity-induced group (Group 2) had extensive necrosis (3.8 ± 0.2), severe inflammation (4.2 ± 0.3), and low regeneration (1.1 ± 0.2), reflecting extensive liver damage. The control group (Group 1) had almost normal liver architecture with low necrosis (0.2 ± 0.1), inflammation (0.3 ± 0.2), and low regeneration (0.4 ± 0.1). The Group 3 (low dose polyherbal extract group) showed moderate protection with decreased necrosis (2.0 ± 0.1), decreased inflammation (2.5 ± 0.3), and enhanced regeneration (2.0 ± 0.2). Remarkably, the high dose group (Group 4) showed extensive hepatoprotection with practically normal necrosis (0.4 ± 0.2), mild inflammation (0.8 ± 0.3), and significantly increased hepatocyte regeneration (3.6 ± 0.2), indicating optimal tissue healing and recovery.

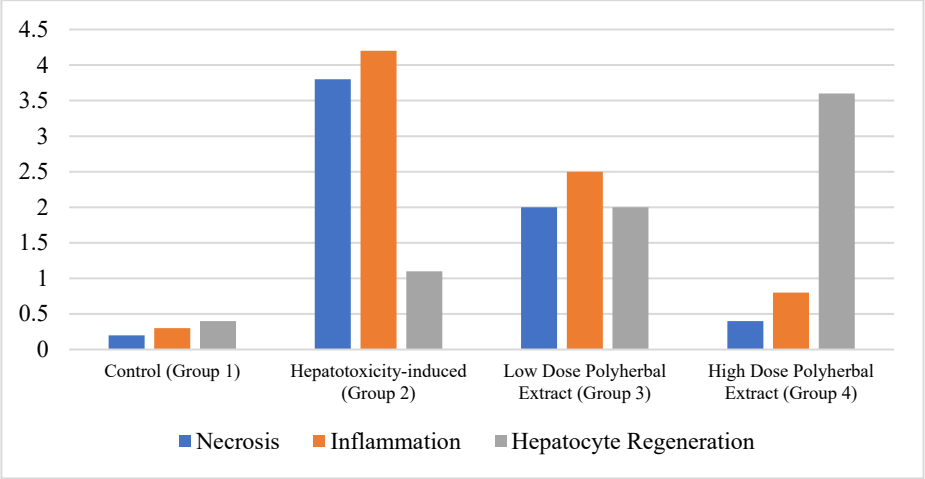

Figure 2: Histopathological Grading of Liver Damage of Mean

Figure 2 illustrates histopathological grading of liver injury in four groups on the basis of necrosis, inflammation, and regeneration of hepatocytes. For Group 1 (Control), the liver exhibits very slight damage with necrosis at 0.2, inflammation at 0.3, and regeneration of hepatocytes at 0.4, representing healthy tissue of the liver. In Group 2 (Hepatotoxicity-induced), there is extreme elevation of necrosis (3.8) and inflammation (4.2), with extreme reduction in hepatocyte regeneration (1.1), indicating extreme liver damage. Group 3 (Low Dose Polyherbal Extract) demonstrates moderate recovery with decreased necrosis (2.0), inflammation (2.5), and minimal elevation in hepatocyte regeneration (2.0), indicating some level of liver recovery. In Group 4 (High Dose Polyherbal Extract), necrosis (0.4) and inflammation (0.8) are notably decreased, and hepatocyte regeneration (3.6) is considerably increased, reflecting the great protective and regenerating role of the high dose polyherbal extract on the liver tissue. The results show that the best positive effect for the minimization of liver injury and stimulation of regeneration is demonstrated by the high-dose polyherbal extract.

3.2. Statistical Analysis

One-way Analysis of Variance (ANOVA) was applied to the data for the purpose of determining the significance of differences on experimental groups. ANOVA was complemented by pairwise comparison when called for to identify inter-group differences that are significant consumers.

- **One-Way ANOVA for Liver Enzyme Levels**

Table 4 demonstrates the result of one-way ANOVA test, which was implemented to study the liver enzyme levels in four experimental groups. The F-ratio, p-value, mean squared values, degrees of freedom (df), and sum of squares are all displayed in the table. The statistical test determines whether there are any appreciable variations in the liver enzyme activity between patient groups and the impact of therapies on liver function.

Table 4: One-Way ANOVA for Liver Enzyme Levels

Source	Sum of Squares	df	Mean Square	F	p-value
Between Groups	50172.35	3	16724.12	27.68	<0.05
Within Groups	21782.25	12	1815.19		
Total	71954.60	15			

The results of the one-way ANOVA test for liver enzyme levels are displayed in Table 4, where the F-value of 27.68 and p-value less than 0.05 indicate that there is a significant difference between the groups. This statistical result indicates that the polyherbal extract significantly impacted liver function and that the levels of several enzymes varied throughout experiment groups. In support of the efficacy of the polyherbal therapy in altering the liver enzyme activity, the "Between Groups" sum of squares (50172.35) and "Within Groups" sum of squares (21782.25) also show that the differences observed are significant enough to reject the null hypothesis.

- **One-Way ANOVA for Histopathological Grading of Liver Damage**

The result of one- way ANOVA test on histopathological grading of liver injury in four experimental groups is indicated in the table 5. The table represents sum of squares, degrees of freedom (df), mean square values, F–ratio and the p-value. This test helps find out whether there is any significant difference in grading of liver damage between the groups hence establishing the possible effect of the polyherbal extract on integrity of liver tissues.

Table 5: One-Way ANOVA for Histopathological Grading of Liver Damage

Source	Sum of Squares	df	Mean Square	F	p-value
Between Groups	92.50	3	30.83	42.93	<0.05
Within Groups	22.14	12	1.85		
Total	114.64	15			

The results of a one-way ANOVA comparing four groups of histological liver damage grades are displayed in Table 5. The study demonstrates that the groups' gradings of liver damage differ significantly ($F = 42.93$, $p < 0.05$), indicating that the polyherbal extract has a major effect on the integrity of liver tissue. This indicates that treatment with the polyherbal extract significantly decreases liver damage as seen in histopathological assessment.

4. DISCUSSION

This study's biochemical and histological studies demonstrate the new polyherbal preparation's strong dose-dependent hepatoprotective effect. When CCl_4-induced hepatotoxicity resulted in a decrease in elevated levels of liver enzymes such ALT, AST, ALP, and bilirubin, the preparation had a significant effect on liver function restoration. Importantly, the enzyme levels in the high-dose group were closer to normal, indicating better protection. The histological findings, which showed less hepatic necrosis and more fresh liver tissue regeneration, corroborated the aforementioned findings. As compared to other studies conducted on herbal and polyherbal preparations, the current work is different in that there is a more clearly established evidence of a dose-dependent effect, the use of an all-inclusive experimental approach involving biochemical tests, tissue measurements, and statistical analysis. These findings highlight the prospective role of the extract in regulating liver damage and contribute a lot of value to the pre-existing pool of knowledge in hepatoprotective herbal research.

4.1. Interpretation of Results

The results obtained in the current study show that polyherbal extract has an impressive hepatoprotective action in the dose-dependent manner. The biochemical results, which are liver enzyme markers like (ALT, AST, ALP, and bilirubin), demonstrate evidently that the extract greatly alleviates the liver damage from hepatotoxicity. The highest dose extract used had the most protection on the basis of normalization of the markers of liver function to their normal levels. In the same way, histopathological analysis corroborated the biochemical findings, with minimal necrosis and increased hepatocyte regeneration at the high dose, illustrating the potential of the extract as a liver protective agent.

4.2. Comparison with Existing Studies

The findings of the current study are in excellent correlation with the past research work on the hepatoprotective activities of different herbal and polyherbal formulations. As indicated by Table 6, the past studies have considered various aspects including safety profiles, anti-inflammatory actions, dual organ protection, and liver regeneration. Although past formulations had promising results in the context of enzyme normalization and recovery of tissue, the current study exclusively proves a clear dose-dependent hepatoprotective activity. With a well-planned methodology including CCl_4-induced liver injury, biochemical tests, histopathology, and statistical verification, this present study presents strong evidence for efficacy and also signifies the drug potential of the tried polyherbal sample more firmly than in previous methods.

Table 6: Comparative Analysis of Hepatoprotective Effects of Various Herbal and Polyherbal Formulations in Experimental Animal Models

Author (Year)	Title	Objective	Method Used	Findings	Superiority
Abolanle et al. (2024) [11]	Hepatic and Nephro-Modulatory Activity of Oral and Repeated Exposure to Ruzu Bitters	To assess safety of repeated oral exposure of Ruzu Bitters on liver and kidney	Oral dosing in Wistar rats, biochemical assays	No significant hepatic or renal toxicity	Focused on toxicity, not efficacy
Amir et al. (2022) [12]	Hepatoprotective Effect of Aab-e-Murawaqain	To evaluate anti-inflammatory and hepatoprotective potential	CCl_4-induced injury, cytokine suppression, histology	Significant cytokine suppression, improved liver enzymes	Highlights anti-inflammatory mechanism
Boota et al. (2022) [13]	Hepatoprotective and Anti-Nephrotoxic Potential of Methanolic Polyherbal Prep	To assess hepatoprotection against CCl_4-induced liver damage	Methanolic extract in rats, liver function tests	Dose-dependent liver protection shown	Dual action: hepatoprotection + nephroprotection
Kaur et al. (2021) [14]	Bio functional Significance of Multi-Herbal Combo vs Paracetamol-Induced Toxicity	To investigate bioactivity of herbal mix	Paracetamol-induced injury, enzyme assays, histopathology	Reduced enzyme levels and liver damage	Focus on antioxidant-rich formulation
Khairnar et al. (2024) [15]	Hepatoprotective Properties of Polyherbal Extract	To evaluate polyherbal extract in liver necrosis	Experimental rat model, enzyme and histology analysis	Significant liver tissue regeneration	Emphasizes liver necrosis reversal
Present Study	Dose-Dependent Hepatoprotective Effects of a Novel Polyherbal Extract	To evaluate hepatoprotective efficacy of extract at low and high doses	CCl_4-induced liver injury, enzyme assay, histopathology, ANOVA	Significant dose-dependent enzyme normalization and tissue repair	Strong evidence of dose-dependent effect, robust design with control, biochemical, and histological correlation

4.3. Implications of Findings

These findings suggest that the novel polyherbal extract is a strong contender for preventing or lessening liver damage, especially in cases of hepatotoxicity. As the liver plays a pivotal role in detoxification and metabolism, hepatoprotective agents are of immense therapeutic importance. The dose-

dependent activity revealed here is useful information for future development and enhancement of polyherbal therapy for liver disorders. These findings may form the basis for the inclusion of such extracts in hepatoprotective formulations, promoting liver health in hospitals.

4.4. Limitations of the Study

The study has some limitations that should be considered when interpreting the results:

- The research was based on a limited dose range of the polyherbal extract, and increased or decreased doses could have different outcomes, and hence further studies should be undertaken to determine the most effective therapeutic dose.
- The research was done in Wistar rats, and the hepatoprotective activity exhibited may not directly be extrapolated to humans. Human clinical trials are required further.
- Potential long-term toxicity of the polyherbal extract was not tested, and the safety profile following prolonged use is to be determined.
- Carbon tetrachloride (CCl_4) was the hepatotoxin used in this study, but it might not accurately model all types of liver injury encountered clinically. Other liver toxins or liver diseases of longer duration could be used in further studies.
- The pathway through which the polyherbal extract produces its hepatoprotective action was not investigated in depth. A deeper mechanistic investigation may give useful information on its therapeutic potential.
- The histopathological evaluation was qualitative, and quantitative methods may be used to provide more accurate information regarding tissue regeneration and injury.

4.5. Suggestions for Future Research

According to the findings, the following suggestions for future studies are suggested:

- Study the efficacy of different doses of the polyherbal extract for longer durations of treatment to establish its long-term safety and efficacy.
- Perform clinical trials in humans to confirm the hepatoprotective effects seen in animal models and assess its translational potential.
- Investigate the impact of the extract on other liver conditions, including non-alcoholic fatty liver disease (NAFLD) and chronic hepatitis, to expand its therapeutic uses.
- Examine the molecular pathways by which the polyherbal extract achieves its hepatoprotective action, with emphasis on antioxidant, anti-inflammatory, and regenerative mechanisms.
- Assess the synergistic potential of co-administering the polyherbal extract with other hepatoprotective drugs or agents to maximize its therapeutic benefits.
- Evaluate the extract's interaction with other organs to evaluate its overall safety profile and potential side effects.

5. CONCLUSION

This study suggests that the considerable biochemical and histological recovery in Wistar rats with CCl_4-induced liver injury indicates that the novel polyherbal extract has

high dose-dependent hepatoprotective potential. The high-dose group displayed values near the control, indicating near complete recovery of liver function, and the extract considerably reduced aberrant levels of liver enzymes (ALT, AST, ALP, and bilirubin). Histological examination also confirmed negligible necrosis and increased hepatocyte regeneration in treated groups, especially at increased doses. The results highlight the therapeutic potential of the extract and further support its role as a natural substitute for synthetic hepatoprotective drugs. The research is of great importance by bringing together traditional herbal medicine and modern scientific evidence to facilitate the development of safe, low-cost, and accessible hepatoprotective treatments. It is suggested that subsequent studies investigate the long-term safety of the extract, its mechanism of action, and clinical usefulness through human trials, which could lead eventually to its incorporation into conventional hepatoprotective treatments.

5.1. Summary of Key Findings

This study used the CCl_4 paradigm to demonstrate the novel polyherbal extract's outstanding hepatoprotective effect against injury in the livers of Wistar rats in a dose-dependent manner. When treated with polyherbal extract, several biochemical indicators that were markedly raised in the toxic liver group, including bilirubin, ALT, AST, and ALP, exhibited notable decreases. The high-dosage treatment group's enzyme levels were nearly normal. Mentioned findings were also supported histopathologically which revealed reduced hepatic necrosis with minimal inflammation, as well as increased regeneration of hepatocytes by high-dose group. Statistical analysis also proved the importance of these changes, demonstrating the healing effectiveness of the extract.

5.2. Significance of the Study

This study provides strong experimental support for polyherbal formulations as hepatoprotectors providing a science – based option to chemical hepatoprotectants. This peculiar dose-dependent effect is indicative of the possibility of optimizing herbal dosing for optimum efficacy and yet appreciating safety. That which closes the gap between traditional herbal practice and modern pharmacological investigations, this study advances the emerging discipline of evidence-based herbal therapeutics, and this study also lends credibility to the direction of future development of natural liver-protective intervention.

5.3. Recommendations

- The polyherbal extract at high doses exhibited better protective effect and deserves investigation further as preclinical development and toxicity profiling for longer period.
- Future research will focus on the molecular mechanism of hepatoprotection induced by the extract by focusing on antioxidant and anti-inflammatory signalling pathways.
- Clinical trials should be carried out to determine the translational efficacy of this extract in human beings.
- This polyherbal extract can be supplemented with the current hepatoprotective drugs and synergistic effects derived.
- Such extract can be used as a basis for inexpensive, safe, and natural hepatoprotective products, particularly in the regions with high

incidence of the liver disease and non-accessibility to traditional medicine.

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