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Pharmacological and Phytochemical Evaluation of a Poly-herbal Formulation for the Effective Treatment of Hepatotoxicity

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***Abstract*-- Hepatotoxicity, a major clinical concern arising from xenobiotics, drugs, and environmental toxins, necessitates effective therapeutic strategies with minimal side effects. Polyherbal formulations, rooted in traditional medicine, offer synergistic benefits through multiple bioactive compounds. The present study evaluates the pharmacological and phytochemical profile of a polyherbal formulation comprising turmeric rhizomes (Curcuma longa), seeds of Silybum marianum, fruits of Piper nigrum, and rhizomes of Zingiber officinale for hepatoprotective efficacy. Phytochemical screening revealed the presence of curcuminoids, silymarin flavonolignans, piperine, and gingerols, which collectively contribute to antioxidant, anti-inflammatory, and detoxifying activities. Pharmacological assessment demonstrated significant hepatoprotection, evidenced by normalization of liver enzymes, reduction in lipid peroxidation, and restoration of hepatic architecture in experimental models of hepatotoxicity. The inclusion of Piper nigrum enhanced bioavailability of curcumin and silymarin, thereby potentiating therapeutic outcomes. This synergistic formulation highlights the potential of combining phytochemicals to achieve superior hepatoprotective effects compared to individual extracts. The findings support the development of safe, effective, and affordable polyherbal remedies for liver disorders, warranting further clinical validation.**

***Keywords*- Hepatotoxicity, xenobiotics, antioxidant, anti-inflammatory, hepatoprotective,**

I. INTRODUCTION

The liver is a vital organ responsible for metabolism, detoxification, and homeostasis. Due to its central role in biotransformation, it is highly susceptible to damage from xenobiotics, drugs, alcohol, and environmental toxins, leading to hepatotoxicity. Hepatic injury manifests through oxidative stress, lipid peroxidation, inflammation, and apoptosis of hepatocytes, which can progress to chronic liver diseases such as fibrosis, cirrhosis, and hepatocellular carcinoma. Current pharmacological interventions for hepatotoxicity are limited and often associated with adverse effects, highlighting the need for safer and more effective alternatives.

Traditional medicine systems, particularly Ayurveda and Unani, have long advocated the use of polyherbal formulations for liver disorders. The rationale behind combining multiple herbs lies in their synergistic action, where phytochemicals from different plants complement each other to enhance therapeutic efficacy and reduce toxicity. Modern pharmacological research increasingly validates these formulations, demonstrating that multi-component herbal remedies can target diverse pathological mechanisms simultaneously.

In this context, the present study focuses on a polyherbal formulation comprising:

- *Turmeric rhizomes (Curcuma longa)* – rich in curcumin, known for its antioxidant and anti-inflammatory properties.
- *Seeds of Silybum marianum (Milk Thistle)* – containing silymarin, a flavonolignan complex with hepatoprotective and membrane-stabilizing effects.
- *Fruits of Piper nigrum (Black Pepper)* – providing piperine, which enhances the bioavailability of curcumin and silymarin.
- *Rhizomes of Zingiber officinale (Ginger)* – abundant in gingerols and shogaols, contributing anti-inflammatory and anti-apoptotic activities.

Together, these herbs offer a multifaceted hepatoprotective strategy: curcumin and silymarin mitigate oxidative stress and inflammation, ginger prevents hepatocyte apoptosis, and piperine ensures optimal absorption of bioactive compounds. This synergistic combination is hypothesized to provide superior protection against hepatotoxicity compared to individual extracts. The objective of this study is to pharmacologically and phytochemically evaluate this polyherbal formulation for its efficacy in treating hepatotoxicity. By integrating phytochemical screening with experimental pharmacological models, the research aims to establish scientific evidence supporting the traditional use of these herbs and to pave the way for developing safe, effective, and affordable hepatoprotective remedies.

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II. Materials And Methods

2.1 Plant collection and Identification

The herbal raw materials, including Ginger Officinalis (Ginger), Piper Nigrum (Black Peppar), Silybum Mariamum (Milk Thistle) and Curcuma Longa (Turmeric) were collected from nearby regions in Sangli Maharastra and Kochi Kerela, India and subsequently shade-dried under ambient conditions to preserve their phyto-constituents. All raw materials were subjected to quality and purity assessment by comparison with authenticated reference specimens. The identity of each plant was further confirmed through botanical authentication carried out by a qualified botanist at the Department of Botany, Safia College, Bhopal, MP. Voucher specimens of the collected materials were deposited in the departmental herbarium for future reference.

2.2 Preparation of Plant extract

The individual plant ingredients were standardized according to Ayurvedic, Siddha pharmacopoeias. Hydro-alcoholic extract were prepared separately by taking appropriate weight of each herbal ingredient, macerated with hydro-alcohol (7:3) for 48h and shaken vigorously in routine interval. Then sample was transferred to the round bottom flask connected to the cooling condenser and heated at 65°C for 2 h. The samples were double filtered with a muslin cloth after cooling and finally filtered through Whatman 1 filter paper. The resulting solution was dried in a vacuum dryer at the temperature less than 50°C to adjust the total solids. The resulting solution was spray dried to get dry powder.The greenish black color extract obtained was transferred to air-tight glass container and stored in a refrigerator.

2.4 Animals

The present study was conducted after obtaining approval from the Institutional Animal Ethics Committee (IAEC), Vedic Institute of Pharmaceutical Education and Research, Sagar, Madhya Pradesh and the protocol adhered to the requirements of the National Guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). (IAEC Approval No. VEDIC/CCSEA/IAEC/07, Dated 11/04/2025), Healthy Wistar rats weighing between 150–200 g were used for the study. The animals were procured and housed under standard laboratory conditions with controlled temperature, humidity, and a 12-hour light/dark cycle. They were provided with a standard pellet diet and water ad libitum.

2.5 Acute toxicity study

Acute toxicity of Methanolic leaves extract of Syzygium aqueum was evaluated according to the method described by Organization of Economic Cooperation and Development Guideline 423. The animals were kept fasting for overnight providing only water. The extract was administered orally at a dose of 5 mg/kg (suspended in 0.5% carboxymethyl cellulose (CMC)) initially to separate groups of mice and mortality was observed for 3 days. If mortality was observed in 4/6 or 6/6 animals, then the dose administered was considered as toxic dose. However, if the mortality was observed in only 1 mouse out of 6 animals, then the same dose was repeated with higher doses such as 50, 300, 500, 1000, and 2,000 mg/kg b.w. orally. The control mice were given 10 mL/kg of water. The mice were observed for behavioural changes and mortality within 24 h. After this, daily observations for toxicity and mortality were made up to the 14th day.

2.5.1 Administration of Drug Doses

The polyherbal formulation LVR05 was suspended in 0.5% carboxymethyl cellulose (CMC) and administered to groups of Wistar albino rats as a single oral dose by gavage using a feeding needle. The control group received an equal volume of the vehicle (0.5% CMC). Prior to dosing, animals were fasted for 12 hours with free access to water. Following the fasting period, each animal was weighed, and the test substance was administered at a dose of 2000 mg/kg body weight. After administration, food was withheld for an additional 3–4 hours. All procedures adhered to the principles of laboratory animal care. Observations were made systematically and continuously as per OECD guidelines following substance administration. Visual parameters included skin changes, mobility, and aggressiveness, sensitivity to sound and pain, and respiratory movements. The number of survivors was recorded after 24 hours, and surviving animals were monitored daily for a further 14 days. The toxicological effect of LVR05 was assessed primarily on the basis of mortality.

2.6 In-vitro anti-inflammatory activity

2.6.1 Inhibition of Protein Denaturation

In vitro anti-inflammatory activity of LVR05 was performed according to a previously well-established method (Chandra S et al., 2012) with slight modification.

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Diclofenac sodium was taken as a standard anti-inflammatory drug and all dilutions were made in distilled water. 1 ml of LVR05 and Diclofenac sodium (7.81–500 µg/ml) or distilled water (blank) were taken in test tubes and an equal volume of 1% aqueous solution of bovine albumin was added. The pH of the reaction mixture was adjusted to 6.3 by using 1N HCl and samples were incubated for 30 min at 37°C. Further, all the samples were subjected to a denaturation temperature of 57°C for 5 min and were immediately cooled under tap water. The turbidity of all the samples was recorded using a spectrophotometer in triplicate at a wavelength of 660 nm. Percentage inhibition of albumin denaturation by LVR05 and the standard drug was calculated using the following equation:

$$\text{Percentage inhibition} = \left(1 - \frac{\text{Abs test}}{\text{Abs control}}\right) \times 100$$

2.6.2 Membrane Stabilization Test

Membrane stabilization test is another method used for evaluating in vitro anti-inflammatory activity (Sareen R et al., 2015), and this method was used to evaluate the anti-inflammatory potential of LVR05 and Diclofenac sodium (standard). For this, a sufficient amount of blood was taken from a volunteer vein. Blood was immediately transferred into a glass tube containing 1.8 mg/ml 5% EDTA solution and was centrifuged at 3000 rpm for 15 min. The RBC pellet was washed three times with equal volume of normal saline and a 10% v/v suspension was prepared in saline. The prepared suspension was stored at 4°C and used within 6 h. The reaction mixture consisted of 1 ml phosphate buffer, 1 ml blood suspension, and 1 ml of different concentrations of LVR05, Diclofenac sodium, or distilled water (blank). The reaction mixture was incubated at 57°C for 30 min and then centrifuged at 2500 rpm for 5 min. The supernatant was collected and its absorbance was recorded at 560 nm. Entire experimentation was performed in triplicate and percentage membrane stabilization was calculated using the following equation:

$$\%\text{Membrane stabilization} = \left(1 - \frac{\text{Abs test}}{\text{Abs control}}\right) \times 100$$

2.7 Hepato-protective study

Randomization, Numbering, and Grouping of Animals A total of forty Wistar albino rats (20 males and 20 females) were selected and randomly divided into four groups, each consisting of ten animals (five males and five females).

The first group served as the control and received only the vehicle (0.5% CMC), while the remaining three groups were treated with the polyherbal formulation LVR05 at low (125 mg/kg), mid (250 mg/kg), and high (500 mg/kg) doses for 28 consecutive days. All animals were acclimatized for seven days under standard laboratory conditions prior to the initiation of treatment. The dose selection was based on the results of the acute oral toxicity study, which demonstrated that administration of LVR05 at 2000 mg/kg was safe. In accordance with OECD guideline 407, three dose levels were chosen at two-fold intervals to establish a descending gradient for toxicity evaluation. LVR05 was suspended in 0.5% carboxymethyl cellulose and freshly prepared each day before administration. The test substance was administered orally by gavage once daily for 28 days, while control animals received only the vehicle.

2.8 Blood collection, processing, and tissue analysis

2.8.1 Experimental Animals: Blood Collection and Processing

At the end of the experimental regimen (Day 50), animals were fasted overnight and anesthetized with thiopentone sodium (40 mg/kg, i.p.). Blood samples were collected into tubes with and without EDTA-K2. Samples with anticoagulant (0.3 mL) were used for hematological analysis (Hemavet Automatic Analyzer, Erba Diagnostics, Germany), while serum/plasma was separated for biochemical assays. Plasma was obtained by centrifugation at 3000 rpm for 10 min at 4°C, and serum was separated after clotting by the same procedure. Biochemical parameters including liver and kidney function, protein profile, and lipid profile were estimated using a fully automated clinical chemistry analyzer (XL-640, Erba) with standardized kits. After blood collection, animals were euthanized with CO_2, and necropsy of heart, liver, and kidney was performed. Organs were weighed, examined histological, and homogenized for biochemical assays following established methods.

2.8.2 Organ Weight and Tissue Homogenate Preparation

Body weight gain/loss and relative organ weights (liver, kidney, heart) were recorded. Organs were excised, cleaned, blotted, and weighed.

2.8.2.1 Liver homogenate: Prepared in 0.01 M phosphate buffer (pH 7.0) to yield 10% w/v suspension, centrifuged at 3000 rpm (15 min, 4°C) for TBARS assay, and further centrifuged at 12,000 g (20 min, 4°C) to obtain post-mitochondrial supernatant for glutathione and antioxidant assays.

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2.8.2.2 Kidney homogenate: Prepared as 10% w/v in ice-cold 0.1 M phosphate buffer (pH 7.8), centrifuged at 6000 rpm (15 min, 4°C). Supernatant was used for lipid peroxidation and antioxidant enzyme assays (MDA, SOD, CAT, GPx, GST, GR, glutathione, vitamins C & E).

2.8.3 Biochemical Estimations

2.8.3.1 Phospholipids: Determined by Zilversmit method (1950); phosphorus quantified by Fiske & Subbarrow (1925). Absorbance read at 680 nm.

2.8.3.2 Free Fatty Acids: Estimated by Horn & Menahan (1981) using Itaya reagent (1977); absorbance at 430 nm.

2.8.3.3 Hepatic Triglycerides: Extracted with chloroform/methanol (2:1 v/v) and measured using Sigma-Aldrich kit.

2.8.3.4 Hepatic Glycogen: Determined by Carroll method (1985) using KOH digestion, ethanol precipitation, and anthrone reagent; absorbance at 650 nm.

2.9 Data analysis

Results of the experiments and observations were expressed as mean ± standard deviation (SD). The significance of differences between groups was determined using one-way analysis of variance (ANOVA) followed by at least one of the following post hoc tests: Dunnett's multiple comparison tests $P<$ 0.05 where level of significance was considered for each test. The data is presented as mean ± S.D.

III. Result

3.1 Percentage of yield of hydro-alcoholic extract of individual raw materials

Table 1

S. No	Name of the ingredient	Qty. of extract obtained(g)	Percentage of yield
1	Turmeric Rhizomes (*Curcumalonga*)	70.8	19.43
2	Seeds of *Silybummarianum*	76.7	17.34
3	Fruits *of Pipernigrum*	75.6	14.9
4	Rhizomes of Ginger (*Zingiber officinale*)	78.4	14.52

3.2 Preparation of polyherbal formulation (LIV051)

The individual extracts were combined in different ratio to form the hydro alcoholic extract of polyherbal formulation coded as LIV 051. In the present study secondary metabolites are estimated in the polyherbal formulation using standard methods.

The total alkaloid contents is 56mg/gm, phenolics is 223 mg/gm of Gallic acid equivalents, Tannin is 115.9 mg/gm of Catechin equivalents, flavonoids is 0.655 mg/g of Quercetine equivalents, saponins 0.312 mg/g and phytosterols is 0.12 mg/g.

3.2 Acute oral toxicity studies

The acute oral toxicity of the hydroalcoholic extract was carried out as per OECD 423- guidelines (Acute toxic class method). Acute toxicity studies revealed that LD50>2000mg/kg for the extract. Hence, the biological dose was fixed at 125, 250mg and 500mg/kg body weight.

3.3 Evaluation of in-vitro anti-inflammatory activity of poly-herbal formulation

3.3.1 Inhibition of protein denaturation method

To assess the anti-inflammatory activity, albumin denaturation method is useful because it is simple and rapid. Different kinds of internal and external stressors are inducing the structural modifications, due to these, the proteins loss their functionality. Protein denaturation induces the inflammation, several researchers are cited non-steroidal anti- inflammatory drugs (NSAIDs) have ability to regulate the albumin denaturation. Based on this the study was conducted and the results are summarized and given in the table 6.35 and fig.6.52 The polyherbal formulation (LIV051) inhibits protein denaturation process in dose dependent manner. LIV 051 at the concentration of 500µg/ml showed 73.06 ± 4.11% and the same concentration standard diclofenac showed 87.43 ± 6.50% of inhibition.

Table 2
Effect of poly herbal formulation on percentage of inhibition of protein denaturation and RBC membrane stabilization

Conc. (µg/ml)	% of Inhibition of Protein Denaturation		% of HRBC Membrane Stabilization	
	LIV051	Standard	LIV 051	Standard
7.81	5.03 ± 0.50	8.13 ± 0.75	6.06 ± 0.60	10.13 ± 0.75
15.62	8.35 ± 0.75	14.63 ± 1.33	10.35 ± 0.75	16.63 ± 1.33
31.25	14.16 ± 1.10	23.16 ± 1.75	17.83 ±1.19	31.23 ± 1.26
62.5	24.33 ± 1.08	44.83 ± 4.01	32.83 ± 2.75	41.83 ± 1.01
125	42.4 ± 3.14	56.16 ± 5.10	44.43 ± 3.20	52.16 ± 2.63
250	63.16 ± 5.25	70.56 ± 4.25	57.16 ± 3.25	69.56 ± 1.75
500	73.06 ± 4.11	87.43 ± 6.50	71.73 ± 6.54	89.43 6.50

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3.3.2 Membrane stabilization test

Inflammatory response starts from oxidation of membrane phospholipids which releases more arachadonic acid which induces various pro-inflammatory and inflammatory mediaters through cyclooxygenase (COX) and lipoxygenase (LOX) pathway. Therefore, cellular membrane stabilization is an important phenomenon to reduce the inflammation and in fact most of the drugs available are capable to stabilize the membrane and suppress the inflammation. RBC membrane is considered to be suitable for in-vitro evaluation, because structurally and functionally it resembles like lysosomal membrane which is involved in inflammatory reactions within the body. NSAIDs are used to stabilize cellular membrane and thereby preventing the release of hydrolytic enzymes to red with in lysosome and they induce the inflammation process. Results of the study indicate the polyherbal formulation has significant RBC membrane stabilization effect in dose dependent manner. The polyherbal formulation showed maximum stabilization (71.73 ± 6.54%) of cellular membrane at the concentration of 500µg/ml, which was comparable to the standard drug, diclofenac sodium (89.43 ± 6.50%) (Fig. 1).

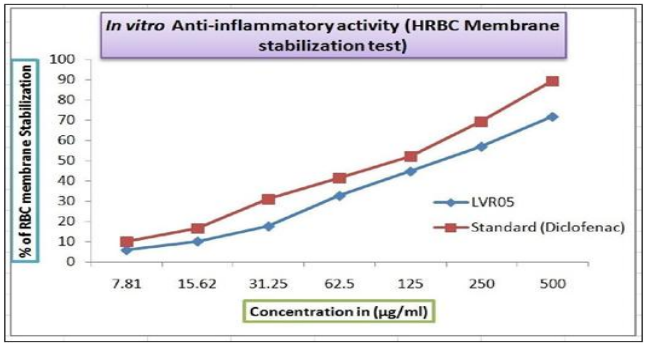

Fig. 1: In-Vitro anti-inflammatory activity (HRBC Membrane stabilization test)

3.3.3 In-vivo Hepatoprotective Activity:

After administration of different doses of polyherbal formulation to the alcohol induced hepatotoxic rat different parameters such as liver function, kidney function, protein profile, coagulation parameters profile, both circulatory and cellular level of antioxidants, lipid peroxidation marker, inflammatory markers, histopathology were observed and observation depicted in Fig 2 and 3.

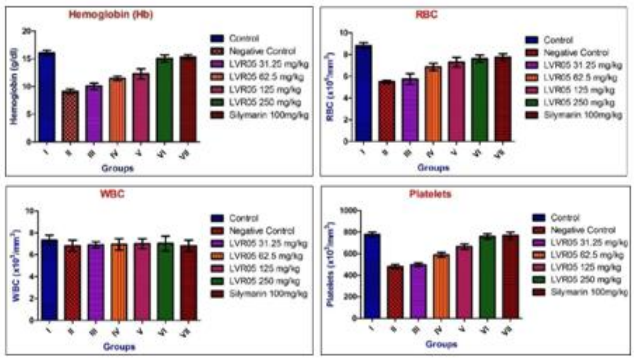

Figure 2: Effect of LIV051 administration on hematological parameters

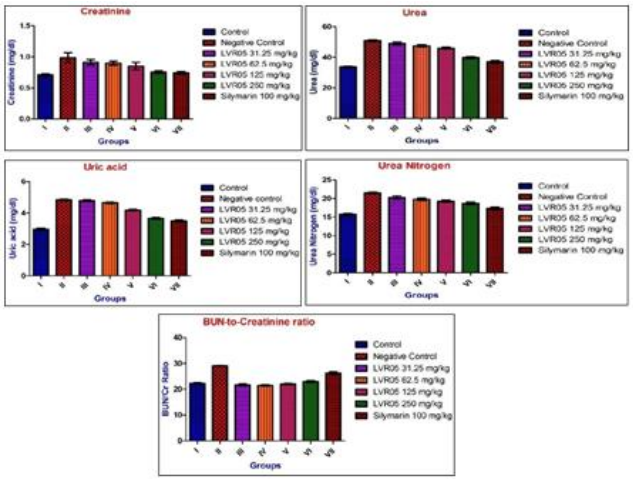

Figure 3: Effect of LIV051 administration on Kidney parameters

IV. Discussion

The present study has been undertaken to scientifically evaluate the toxicity profile and hepatoprotective activity of polyherbal formulation. The individual raw materials used for the formulation have been checked to assess the identity and purity based on ayurvedic pharmacopoeia of India and AYUSH guidelines for quality control of herbal raw materials.

The polyherbal formulation (LVR05) has been prepared by combining the individual extracts and assessed its physiochemical and safety parameters and is considered as the final product.

Quantification of marker compounds was done in poly-herbal formulation using HPLC/ UHPLC and found the following components: Picroside I - 6.291%, Picroside II - 7.457%, Picroside III - 1.134%, Cinnamic acid - 0.139%, Gallic acid - 0.43%, P- hydroxyacetophenone -0.048%, Wedelolactone- 0.439%, Demethyl wedelolactone - 0.641%, Phyllanthin - 0.03%, Hypophyllanthin - 0.01%, Nirtetralin - 0.04%, Swertiamarin - 0.16%, Mangiferin - 0.62%, Azadirachtin - 0.33% and Quercetin - 0.67%. These phytoconstituents present were responsible for antioxidant, anti- inflammatory, antifibrotic and antihepatotoxic effect.

The polyherbal formulation (LVR05) was evaluated for the antioxidant activity using different in vitro methods like DPPH, Nitric oxide scavenging, Hydroxyl radical, Superoxide radical, Hydrogen peroxide scavenging, Total antioxidant capacity, Lipid peroxidation inhibition and showed to have potent free radical scavenging activity. In all the methods the activity was compared to the standard compound ascorbic acid.

Hepatic inflammation is major concern in the alcohol induced liver damage. To prove anti-inflammatory efficacy of the polyherbal formulation, two in vitro methods were adopted and assessed for anti- inflammatory activity. The results showed that the polyherbal formulation inhibited protein denaturation up to 73.06 ± 4.11% at the dose of 500 µg/ml and standard diclofenac 87.43 ± 6.50%. In HRBC membrane assay polyherbal formulation stabilizes upto 71.73 ± 6.54%, standard 89.43 ± 6.50% at the dose of 500µg/ml. Inhibition of protein denaturation and membrane stabilization is an indication of a potent anti-inflammatory agent. Based on this result, it was confirmed that the polyherbal formulation has anti-inflammatory potential in dose dependant manner.

In-vivo toxicitystudies such as acute oral toxicity (OECD 423) and repeated oral dose for 28 days study (OECD 407) was conducted. The acute toxicity showed no behavioural changes and no mortality observed compared to control groups. The formulation was found safe up to the dose 2000mg/kg body weight orally.

Sub-acute toxicity study at the dose of 125, 250 and 500mg/kg body weight orally for a period of 28 days revealed that the formulation does not produce any alteration in the biochemical parameters and found within the reference range. The histological studies on isolated organs such as liver, kidney, heart, brain and stomach showed no abnormalities. For assessing the hepatoprotective activity, alcohol induced hepatotoxicity model was used. The polyherbal formulation was administered orally in the dose of 31.25, 62.5, 125 and 250 mg/kg body weight.

The alcohol induced hepatotoxic rats showed significant increase in the hepatic and renal parameters such as AST, ALT, ALP, total, direct and indirect bilirubin, creatinine, urea, uric acid and blood urea nitrogen. The total protein, albumin and globulin levels significantly decreased in the hepatotoxic group which further showed improvement in the drug-treated group at the dose of 62.5, 125 and 250 mg/kg as that of Silymarin group. There was an improvement in the A/G ratio of LVR05 treated group at the dose level of 250 mg/kg and it was found to be more effective in increasing protein levels compared with the negative control group. The haematological parameters were significantly increased in LVR05 treated groups.

From these studies it is concluded that the polyherbal formulation (LVR05) may be useful as a hepatoprotective agent in alcohol induced hepatotoxicity. It acts as cholagogue, potential source of antioxidants which reduces lipid peroxidation, hepatic inflammation, hepatic fibrosis, alcohol induced hepatic steatosis and helps in regeneration of hepatocytes.

The phytoconstituents present in the polyherbal formulation (LVR05) like iridoid glycosides (Picroside I, Picroside II, Picroside III), secoiridoidglycoside (Swertiamarin), xanthonoid (Mangiferin), flavonoid (Quercetin), tetranortriterpenoid (Azadirachtin), polyphenolic compounds (Gallicacid, Cinnamic acid and p-Hydroxyacetophenone), lignans (Phyllanthin, Hypophyllanthin and Nirtetralin) coumestans (Wedelolactone and Demethyl wedelolactone) could be responsible for these effects.

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