Vol. 5 No.1 (2026)

Pakistan Journal of Medical & Cardiological Review

https://pakjmcr.com/index.php/1/about

Online ISSN 3007-2387

Print ISSN 3007-2379

Hepatoprotective Potential of Polyherbal Formulation in Acetaminophen-induced Toxicity in Rabbit

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Received on **01** March, 2026
Accepted on **20 Mar** 2026
Published on **30 Mar** 2026

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Abstract

Introduction: Individually, *Cymbopogon citratus, Commiphora mukul, Berberis aristate,* and *Terbulus terrestris* single plant extract have been reported to possess hepatoprotective effects. However, the literature survey shows that no scientific data has been published on the pharmacological evaluation of these plants in combined form. **Objective of the Study:** To evaluate the hepatoprotective activity of aqueous extract of polyherbal

against paracetamol-induced hepatic inflammation in experimental rabbits. **Materials and Method:** Animals were divided into different groups. Group I was considered as Control Aqueous-extract of Polyherbal at doses 4ml /kg body weight, p.o., was administered for 15 days, then paracetamol (1.5gm/kg p.o) to induce toxicity in, and again administered polyherbal formulation for 08 days. Group II was given silymarin (100 mg/kg, p.o.) for 28 days along with induction of Acetaminophen toxicity after 15 days of administration of Standard. Biochemical parameters, alkaline phosphatase (ALP), serum glutamic oxalo-acetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), and total bilirubin (TB) levels were recorded to investigate the degree of improvement in the conditions of the rabbits. Group III is considered as standard. The animal of all groups was dissected for liver biopsy for histopathological studies. Phytochemical screening of the extract was also carried out. **Results:** The levels of biochemical parameters were increased in paracetamol-intoxicated rabbits when compared with the normal group. The extract, at doses of 950 mg/kg, exhibited a significant ($p < 0.05$) reduction in biochemical parameters (ALP, SGOT, SGPT, and TB). Hepatoprotective activity was also confirmed by histopathological findings. Furthermore, the phytochemical profile of the extract revealed the presence of tannins, alkaloids, saponins, and flavonoids. **Conclusions:** These results suggest that the aqueous extract of the polyherbal formulation possesses a hepatoprotective effect against paracetamol-induced hepatotoxicity. This observed effect may be attributed to the presence of bioactive constituents within the formulation. Further investigations into the specific compounds responsible for the hepatoprotective properties could provide valuable insights for potential therapeutic applications.

Keywords: Hepatoprotective activity, Acetaminophen, liver biomarkers, Poly-herbal Formulation.

Introduction

The liver is one of the vital aerobic organs that produce oxidative byproducts that damage the tissues. Reactive oxidative byproducts induce lipid peroxidation that results in cell membrane inflammation and resulting clinical manifestations such as Diabetes Mellitus, gout, ischemic heart disease, and malignancies [1, 2]. Almost 5% of hospital in-patient and 50% of acute liver failures are associated with drug-induced hepatotoxicity since many drugs have been withdrawn from the market due to hepatotoxicity [3]. Several polyherbal formulations are reported to have hepatocurative and hepatoprotective activity, which are available for the treatment of hepatic disorders [4]. But to cure liver ailments by using a single herbal drug is still a compelling problem [5, 6].

A major defense mechanism is the antioxidant enzyme, which converts active oxygen molecules into non-toxic compounds [6, 7]. Acetaminophen is considered safe at therapeutic doses, whereas at higher doses, it produces centrilobular hepatic necrosis that can be lethal [8]. Recently, it has been reported that Acetaminophen toxicity is responsible for approximately one-half of all cases of acute liver failure in the United States and Great Britain [9]. The current data supports the release of inflammatory mediators in the initiation of regenerative processes leading to the restoration of liver function [10]. *Cymbopogon citratus* is responsible for anti-inflammatory activity; either it is induced by chemicals present within tissue or by physical stress [9]. Mechanistically, macrophages and lipopolysaccharides (LPS) are responsible for the secretion of nitric oxide (NO) and prostaglandin E2 (PGE2) [11]. The flavonoids, alkaloids, tannins, saponins, phlobo-tannins, C-glycosylflavones, orientin, cardiac glycoside, chlorogenic acid, iso-orie,ntin, and citral oil possess numerous pharmacological activities [12]. *Commiphora mukul* is used traditionally as an anti-inflammatory, antispasmodic, carminative, hypoglycemic, alterative, antiseptic, astringent, thyroid stimulant, anthelmintic, antihyperlipidemic, antidiabetic, and antioxidant properties [13]. The most valuable constituents responsible for therapeutic potential are E- and Z-guggulsterone [13, 14]. the pharmacological actions of *Tribulus Terrestris* have been reported for the herbal extracts and constituents [15]. The dry fruit extract of *Tribulus terrestris* exhibited free radical antioxidant activity [14, 16]. *Berberis aristate* contains several types of alkaloids such as berberine. It is also responsible for multiple pharmacological activities like anti-microbial, anti-viral, anti-inflammatory, and anti-hyperlipidemic [17, 18].

The purpose of the study is to evaluate hepatoprotective indigenous polyherbal formulation (Aqueous extract) against Acetaminophen-induced hepatotoxicity in animal models. To perform phytochemical and physicochemical screening of individual plant extracts. To assess the effect of *Cymbopogon citratus*, *Commiphora mukul*, *Tribulus terrestris*, and *Berberis aristata* aqueous extract on the biochemical (hepatic) and histopathological parameters.

Methodology

Plant Material Collection

The plant materials were procured from the herbal market given voucher no. 99. The plant materials were identified by the herbal expert at the Pharmacognosy Department of Jinnah University for Women. The plant materials were brought in single batches and a lot of herbarium numbers JUW-H1 003 (*Cymbopogon citratus),* JUW-H1 004 *(Commiphora mukul),* JUW-H1 005 *(Tribulus terristris*) and JUW- H1 006 (*Berberis*

aristata). Then it was further dried under the shade and ground into fine powder in an electrical grinder (Braun company), and stored in a well-closed container till the preparation of the extract.

Preparation of Aqueous Extract

The 200gm powder of *Cymbopogon citratus* was prepared by decoction. The 200gm dried ground powder of *Commiphora mukul, Berberis aristate,* and *Tribulus terristris* were soaked separately in a conical flask with distilled water, the mixture was covered with foil for 72 hours at room temperature (RT) with continuous mixing. The soaked materials were filtered separately by using several layers of muslin cloth. The maximum concentrated residues were obtained by maximum evaporation of filtrate in a water bath. The residues were weighed and the percentage yield was calculated. The fresh solution was prepared from the residue according to the calculated dose [18]. The stock solution was prepared individually at known doses by evaporating maximum water in a water bath.

Polyherbal Formulation

This formulation was prepared by calculating the dose of each herbal extract according to the required dose. The dose of *Commiphora mukul* is 200 mg/kg, *Cymbopogon citratus* is 200 mg/kg, *Tribulus terrestris* is 50 mg/kg and *Berberis aristata* is 500 mg/kg. Polyherbal formulation was prepared by mixing 1ml of each herbal extract and then 4ml /kg of body weight. The formulation was mixed geometrically and prepared daily [19].

Drug and Chemicals

Silymarin and Paracetamol (Acetaminophen) were procured from the pharmacy. The Batch # and Expiry date of Silymarin was 60223XV and June 2017 respectively; manufactured by Abbott Laboratories. Paracetamol (Panadol) was manufactured by Glaxo Smith Kline having batch # 103PK and an expiry date of 2021. The chemicals utilized in phytochemical screening were of analytical grade and procured from Merck Pharma Karachi.

Physicochemical Properties

Organoleptic and Physico-chemical studies like, total ash, moisture content at 105°C, and solid content were carried out for individual herbs [19, 20]

Phytochemical Screening

The physicochemical and qualitative phytochemical screening of each plant extract was performed to identify the constituents, using standard phytochemical procedures [20, 21]. The phytochemical screening reveals the presence of carbohydrates, proteins, alkaloids, flavonoids, saponins, tannins, and glycosides.

Animal grouping and drug dosing for Hepatoprotective Study

The experimental animals were divided into three groups each group having 10 rabbits (n = 10). In the present study, Group III (Treated -1) was first administered a polyherbal formulation of 4ml/kg orally for 15 days. Administration of acetaminophen (1500 mg/kg body weight in 10 ml of distilled water) was started on the 16th day of the study, for six days. On the 21st day, the extract was again administered from 21st to 28th days. The blood sample was analyzed on 0, 15th, 21^{st,} and 28th day, for serum enzymes including SGPT, SGOT, ALP, GT, DB, and TB [22]. After completion of the study, animals were sacrificed for liver biopsy.

Group –I (Negative control): Administered distilled water for 15 days and on the 16th day start dosing of Paracetamol 1500 mg/kg for 6 days. And then again administered distilled water for 28 days.

Group-II (Treated group): Hepatoprotective Studies = administered polyherbal formulation for 15 days (4ml/kg); after that 6-day dosing of Paracetamol (1500 mg/kg) and then again start dosing 4ml/kg polyherbal extract till 28 days.

Group-III (Standard group): Hepatoprotective effect = administered Silymarin (50mg/kg) for 15 days; after that 6-day dosing of Paracetamol and again silymarin till 28 days.

Biochemical Parameters

The blood samples were drawn by cardiac puncture using 5ml of sterile syringe. The blood samples for biochemical assay were placed into test tubes without disturbance. The serum was separated by centrifugation for 15-20 minutes at 5000 rpm. The blood serums of each group were analyzed for SGOT, SGPT, ALP, GT, Total bilirubin, and Direct bilirubin.

Histopathology

The liver sections were examined at different magnifications by using a microscope. Photomicrographs of the selected liver samples were taken with a Nikon Eclipse Ci microscopic camera of Model No. DS-L 3[23].

Statistical Analysis

The data was expressed as mean ± SD analyzed by one-way ANOVA followed by Tukey's post hoc test for individual herbal effects while for hepatoprotective and hepatocurative studies analyzed by two-way ANOVA followed by Tukey's using SPSS software version 19.0 program, p values $\leqslant$ 0.05 were considered to be statistically significant.

Result

Phytochemical Screening

The phytochemical results of CC, CM, BA, and TT aqueous extract are shown in Table 1 and Physicochemical Parameters of Crude Herbs are mentioned in Table 2.

Table 1: *Phytochemical Screening of Herbal Aqueous extract*

TEST	CC	CM	BA	TT
Carbohydrate:				
Molish Test	+	+	+	+
Benedict Test	+	+	+	+
Protein:				
Biuretic Test	-	-	-	-
Tannins	-	-	-	-
Alkaloids	-	+	-	-
Saponins	-	+	+	+
Cardiac Glycoside:				
Kill killer Test	-	-	-	-
Steroidal Test:				
Salwoski	-	-	-	-
Flavanoids	+	+	+	+

(+) = presence, (-) = absence.

CC = Cymbopogon citratus, CM = Commiphora mukul, BA=Berberis aristata and TT=Tribulus terrestris

Table 2: *Physicochemical Parameters of Crude Herbs*

Parameters	CC	CM	BA	TT
Moisture content	8.22%	1.55%	8.03%	7.18%
Solid content	91.78%	98.45%	91.97%	92.82%

Ash content	5.405%	4.207%	6.51%	7.506%

CC = Cymbopogon citratus, *BA=Berberis aristata and*
CM = Commiphora mukul, *TT=Tribulus terrestris*

Variation in Hepatic Parameters of Hepatoprotective Treatment

The enzyme levels were monitored over a 28-day period in negative control, herbal-treated, and standard groups. Results showed that SGPT levels decreased significantly at day 21 in the herbal-treated group compared to the negative control and standard groups, but decreased by day 28. SGOT levels decreased notably at day 15 and 21 in the herbal-treated group compared to controls, while ALP levels were slightly elevated at day 21 and 28. GGT levels decreased significantly at day 21 and 28 in the herbal-treated group. Total bilirubin levels decreased markedly at day 15 in the herbal-treated group, while direct bilirubin levels remained relatively stable throughout the study. Overall, herbal treatment demonstrated varying effects on enzyme levels over the observed period, suggesting potential therapeutic implications. Variation in Hepatic Parameters of Hepatoprotective Treatment are mentioned in Table 3 and Hepatoprotective activity of Polyherbal Formulation are shown in figure 1. While figure 2 shows the Estimation of Bilirubin level of polyherbal Formulation.

Table 3: *Variation in Hepatic Parameters of Hepatoprotective Treatment*

Enzyme Level	Negative Control				Herbal Treated				Standard			
DAYS	0	15	21	28	0	15	21	28	0	15	21	28
SGPT	101±5.5	105±3.1	195±2.4	164±2.6	110±2.2	95±3.4$^{***\$\$\$}$	105±1.8$^{**\$\$\$}$	89±2.5$^{**\$\$}$	95±2.4	71±5.5***	150±7.8***	75±11.2***
SGOT	66±3.4	65±4.1	70±5.48	68±4.7	65±0.8	46±5.2$^{**\$\$\$}$	54±2.7$^{***\$\$\$}$	49±6.7$^{**\$\$\$}$	62±1.5	53±2.5***	95±4.2**	75±2.4
ALP	34±2.1	36±2.2	69±5.2	61±2.8	36±.1.4	28±3.7$^{*\$\$}$	41±11.2$^{**\$\$\$}$	40±1.7$^{**\$\$\$}$	40±5.2	49±3.4	76±5.7***	160±1.5***
GGT	8±.01	08±0.55	10±1.2	10±1.7	9±1.7	10±1.5$^{**\$\$\$}$	6±1.3$^{**\$\$\$}$	5±1.3$^{***\$\$\$}$	8±.8	8±2.6	18.0±1.0**	20±1.2***
TB	0.06±.01	0.06±1.2	0.06±.01	0.06±.5	.06±.4	.03±.02$^{**\$\$\$}$	0.18±0.2$^{\$\$\$}$	0.15±1.2$^{\$\$\$}$	.06±.02	0.08±1.6	0.23±2.2***	0.8±0.5***
DB	0.04±.5	0.04±1.7	0.05±.2	0.05±.4	.04±.2	0.02±0.5**	0.04±0.1	0.04±.5$^{\$\$\$}$	.04±.02	0.04±.8	0.01±.5	0.32±0.5***

Data express Mean± SD; n= 10 in each group: followed by two Way ANOVA , Tukey's Pairwise comparison test; $^{*}p\leqslant0.05$ is considered as significant, $^{**}p\leqslant0.01$ is considered as moderately significant,$^{***}p\leqslant0.001$ is considered as highly significant in comparison of control with treated and standard ; $^{\$\$}$ $p\leqslant0.01$ is considered as moderately significant; $^{\$\$\$}$ $p\leqslant0.001$ is considered as highly significant when treated compared with standard.

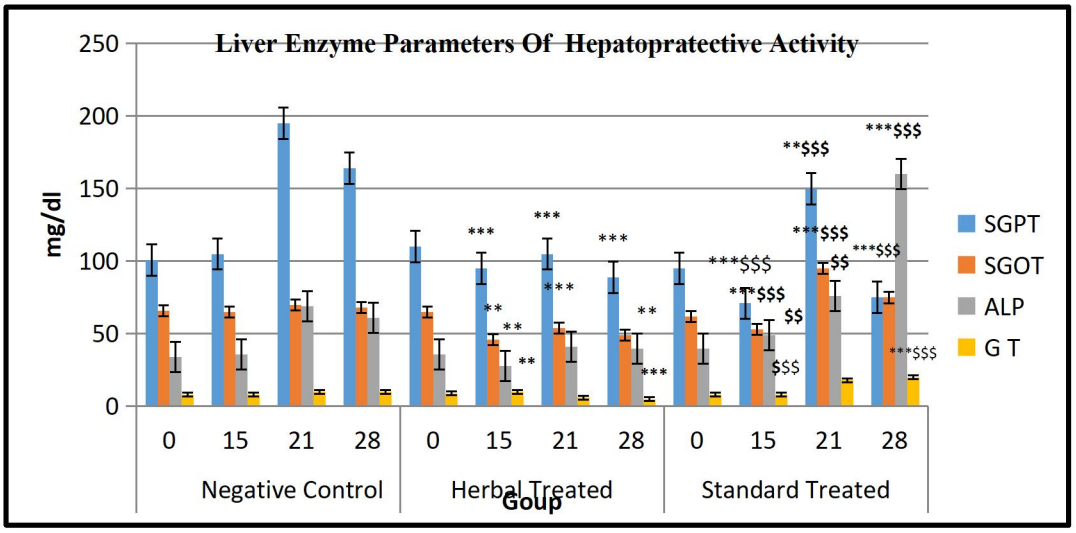


Figure 1: Hepatoprotective activity of Polyherbal Formulation

$^{**}p\leqslant 0.01$ is considered as moderately significant, $^{***}p\leqslant 0.001$ is considered as highly significant in comparison of control with treated and standard; $^{\$\$}$ $p\leqslant 0.01$ is considered as moderately significant; $^{\$\$\$}$ $p\leqslant 0.001$ is considered as highly significant when treated compared with standard.

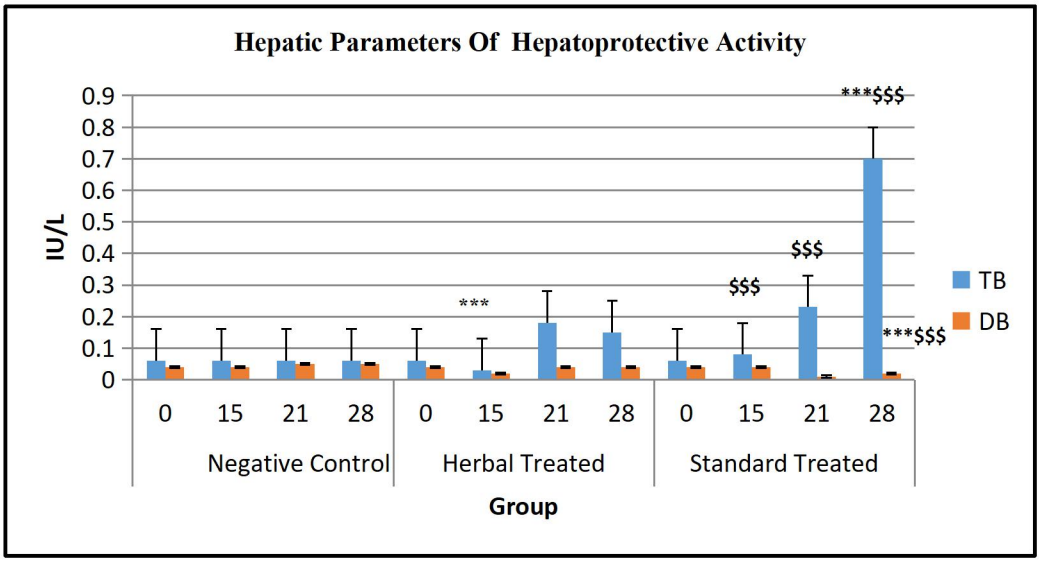


Figure 2: Estimation of Bilirubin level of polyherbal Formulation

$^{***}p\leqslant 0.001$ is considered highly significant in comparison of control with treated and standard;

$^{\$\$\$}$ $p\leqslant 0.001$ is considered highly significant when treated compared with standard.

Pakistan Journal of Medical & Cardiological Review
https://pakjmcr.com/index.php/1/about
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Histopathological Examination

Histopathological evaluation was carried out to find out histological changes in different groups. The result reveals no pathological changes in the tissue of Group I (Control). Group II was (negative control) administered with acetaminophen 1500 mg/kg to induce hepatotoxicity, it shows scattered lymphocyte infiltration, Scanty hepatocyte necrosis was seen along with suspected cholestasis, and mild lobules. Group III treated with polyherbal formulation also did not show any pathological changes. Group IV is standard and Liver tissue shows focal scanty mild lobulitis with focal mild portal tract inflammation which are shown in figure 3 to 6.

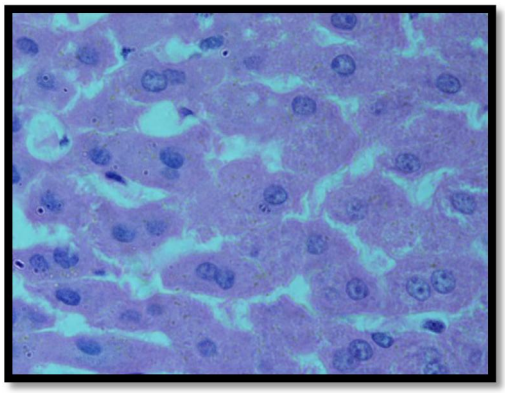


Figure 1: Gp I: no pathological changes

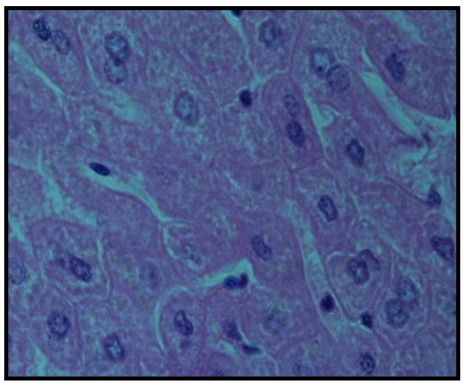


Figure 2:Gp II Scanty Hepatocytes with necrosis

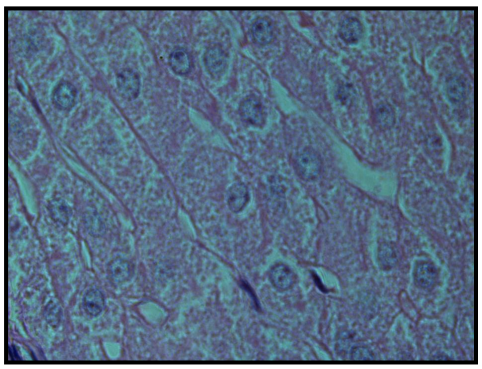


Figure 3: Group III . not showing Pathological changes

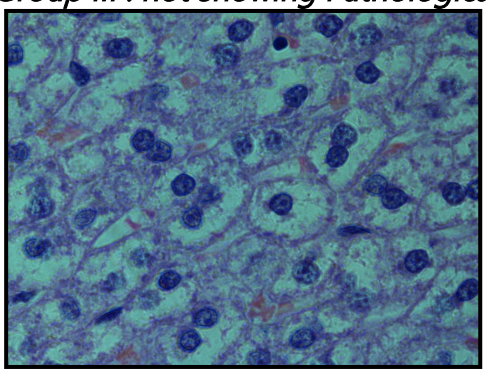


Figure 4: Group IV: Mild Inflammation

Discussion

Herbal and alternative therapy has been recommended as beneficial and curative remedies for the treatment and prevention of multiple health problems with no harmful effects for centuries. In recent year's alternative medicine used extensively to resolve healthcare problems in developing countries. For many years, in the US the use of herbs has continued to cure different ailments [24] after conducting the first national study by Eisenberg et al. about the medicinal use of alternative and complementary medicine [25]. Alternative medicines are highly effective in curing chronic illness, especially hepatic diseases; as compared to allopathic treatment. This is because some Ayurvedic medicines include some effective ingredients that can rejuvenate our liver [25, 26]. Although several allopathic medicines are prepared by using natural ingredients, these are synthesized in laboratories for the cure of various diseases [15].

During the hepato-protective experimental model, blood samples were collected and analyzed at 15 days for all groups included in the model. The result shows that there is a significant decrease in enzyme levels in treated and standard groups as compared to controls. Paracetamol toxicity results in zonal necrosis, this type of injury particularly confined to a zone of the liver lobule. It may result in elevated levels of

alanine aminotransferase and severe liver dysfunction leading to ALF (acute liver failure). SGPT is specific for hepatotoxicity, and enzyme elevation is helpful in diagnosis of hepatocellular necrosis [27]. SGOT is a liver enzyme, but also found in brain muscle, heart, or kidney. In hepatocellular injury, this enzyme elevation is less specific because other organ damage also results in an increased level of SGOT. To differentiate other organ injury from liver injury ratio of serum AST TO ALT can be calculated. Excessive productions of ALP lead to Hepatobiliary injury and cholestasis [28]. Elevated levels of both ALP and GGT a specific indications of hepatobiliary injury, especially biliary effects and cholestasis [29]. Bilirubin level increases with little change in ALT and an unchanged increase in ALT indicates cholestasis. total bilirubin is a better indicator as compared to ALT in the case of Acute liver disease [30].

The current study shows SGPT level was calculated as statistically significant in the treated group and the standard group. SGOT level also decreases as compared to control but it is calculated insignificant statistically. ALP level was calculated as moderately significant in the standard and significant results in the Treated group. GT and TB level showed moderately significant results while for standard it shows highly significant results. After induction of liver toxicity by administration of acetaminophen for 6 days, there was a significant elevation of enzyme level in the treated and standard groups. The treated group has statistically significant results when compared to the control, standard group was significant statistically in comparison to treated group.

On day 21st again extract and standard drug was administered to the respective group and blood was analyzed on the 28th day of the study. The result shows a significant decrease in SGOT, SGPT, and ALP. The enzyme levels show significant reduction ($P \leqslant 0.05$). The experimental model shows the Hepatoprotective activity of formulation same as the standard drug Silymarin.

The activity was supported by histopathological results; the microscopic studies show multiple sections of the liver tissue reveal fairly preserved architecture. Multiple portal tracts included in the biopsy reveal no significant pathology. The photomicrography of the hepatoprotective treated group reveals mild scattered lymphocytic infiltrate along with bile ductules and radicals of the hepatic artery and veins. The hepatocytes are arranged in single-cell hepatic cords. The sinusoids are congested and reveal scattered Kuffer cells. Focal areas reveal large bile ducts infiltrated by lymphocytes, and no fatty changes are seen, after detailed examination, it is concluded that no significant pathology is seen. The histopathology of the standard group as a hepatoprotective potential: shows foci of mild to moderate lymphocytic

infiltrate, the sinusoid is congested and reveals scattered Kuffer cells; studies concluded that liver tissue shows Foci of mild portal tract Inflammation.

Conclusion

The present investigation indicates aqueous extract of Polyherbal formulation containing *Cymbopogon citratus, Commiphora mukul, Tribulus terrestris,* and *Berberis aristata* exert significant hepatoprotective activity by reducing elevated enzyme levels against Acetaminophen-induced toxicity and mild Hepatocurative effect. This formulation is effective in treating drug-induced hepatocellular necrosis. This activity is due to the presence of flavonoids and saponins. It was concluded that polyherbal formulation is more effective than standard Silymarin. This study also shows that polyherbal formulation can be used for treating different hepatic disorders.

Further studies are required to isolate the active principle responsible for this hepatoprotective activity.

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