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Evaluation of comparative antioxidant and hepatoprotective activities of three commonly used polyherbal formulations

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A B S T R A C T

Out of the major health issues worldwide, liver disorders are significant. Liver diseases have become one of the major causes of morbidity and mortality. Besides viruses, liver disorders can arise due to xenobiotics, excessive drug therapy, environmental pollution and alcohol intoxication but drug-induced hepatotoxicity appears to be the most common contributing factor. Modern medicine introduced a bulk of hepatoprotective therapies yet they have various side effects. Despite the widespread use of the traditional medicine, there is a lack of scientific evidence on their efficacy and safety. Therefore, this study was undertaken to evaluate the hepatoprotective activity of three commercially available formulations namely Jigarine, iksir-e-jigar and sharbat-e-deenar. In the present study these three randomly selected polyherbal formulations were investigated for their potential hepatoprotective (*in-vivo*) and antioxidant activities (*in vitro*). Hepatoprotective activity was evaluated in paracetamol-induced liver damage in Swiss albino male mice model. Morphological parameter (liver weight), biochemical parameters (ALT, AST, ALP, TB, and TP) and histological parameters (macroscopic and microscopic changes of liver tissues) were evaluated. Test polyherbal formulations showed significant (<0.01) antioxidant activity as measured by DPPH free radical scavenging method and significant hepatoprotection as well. The present findings verified the efficacy of polyherbal formulations in paracetamol-induced hepatotoxicity in mice. Therefore, it may be suggested that a dose adjustment may be necessary to optimize the effects in clinical settings.

Keywords: Antioxidant activities, hepatoprotective activity, polyherbal formulations

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INTRODUCTION

Liver plays a surprising range of fundamental physiological functions in the regulation, maintenance, and performance of homeostasis in the human body. It has been found to be linked with about all the biochemical pathways required for growth, supply of nutrients, fight against diseases, and energy requirements of the human body (Ahsan et al., 2009; Eipel et al., 2010). Liver has an exclusive tendency to detoxify and excrete most of the xenobiotics and their metabolites, along with therapeutic drugs; neutral, anionic

or cationic, hydrophilic or hydrophobic in nature, other contaminants and many other chemicals that have been proved to be potentially toxic to the human body. According to WHO about 18,000 people die every year due to liver diseases. It is projected that two billion people around the world are infected with hepatitis B and about 350 million of them may develop chronic liver disease. About 90 percent of patients suffering from cirrhosis develop liver tumors. One of the tenth most common tumor in the world with over 250,000 new cases each year is hepatocellular carcinoma(Gupta and Neelam Misra, 2006). Despite the fact that liver has regeneration ability it suffers a lot of ailments leads to end stage liver diseases and even hepatic failure. Hepatic damage may progress to cardiac decompensation, disseminated cancer and extra hepatic infections.

The massive functional reserve of the liver masks the clinical impact of mild liver damage. Corticosteroids and immunosuppressant are commonly used to treat liver disease in modern medicine. Moreover, the treatment success for liver diseases is disappointing (Jain et al., 2008). Unfortunately, all drugs used in the treatment of liver diseases are inadequate and have serious side effects such as immunosuppression and bone marrow depression (Rao et al., 2006). It has been well documented that due to increasing side effects of modern medicine, trend has been shifted towards use of alternative treatment options. Herbal medicines have a number of active constituents such as phenolic and polyphenolic compounds as well as flavonoids, phyenylpropanoids, phenolic acids, tannins, etc that are responsible for their hepatoprotectiveactions. Hence herbal coded formulations of hepatoprotective plants i.e. Jigarine, Iksir-e-jigar and Sharbat-e-deenar were selected for the study of hepatoprotective activity in paracetamol induced hepatotoxic rats.

MATERIAL AND METHODS

Tested Herbal formulations

The three herbal formulations were randomly selected for the present study, Jigarine (Hamdard Laboratories Pvt Limited, Karachi),Iksir-e-jigar (Qarshi Laboratories Pvt Limited, Lahore), Sharbat-e-deenar (Ashraf Laboratories Pvt Limited, Faisalabad) (table 1).

Chemicals and Drugs

The chemicals used were methanol (Sigma Aldrich), Petroleum ether (Merck, Germany), paracetamol (Sigma Aldrich), silymarin (Sigma Aldrich), 1% Methyl Cellulose (Merck Chemicals, Germany). All the other chemicals used were of analytical grade. Diagnostic kits for biochemical analysis were purchased from Merck Chemical Co., Germany.

Experimental animals used

Healthy adult albino male mice, weighing 20-30 g procured from University of Veterinary and Animal Sciences, Lahore were housed in the metal cages in the animal house of Department of Pharmacy, University of Sargodha, Sargodha. After procurement, animals were divided into different groups and left for seven days so that the animals can acclimatize to experimentation room where they were provided standard husbandry conditions; temperature was maintained at 25±2 ˚C, 12-h light: 12-h dark cycle and free access to laboratory feed and tap water was provided *ad libitum* throughout the experiment (Ibrahim et al., 2008). The experimental protocols employed were got approved from the institutional ethical committee of University of Sargodha, Sargodha.

Experimental procedures followed

Preparation of test and reference drug solutions

Polyherbal formulation Jigarine and standard drug silymarin were suspended in an aqueous solution of 1% carboxymethylcellulose (CMC) daily prior to administration, Iksir-e-jigar and Sharabat-e-deenar were already in liquid form.

Grouping of paracetamol induced hepatotoxicity study

Animals were divided into 9 groups (n=6/group) (Girish et al., 2009). as normal control receiving distilled water, negative control receiving PCM 500 mg/kg, positive control receiving silymarin 50 mg/kg/day, group 4,5 receiving jigarine in 50 and 100 mg/kg/day doses respectively. Group 6 and 8 receiving iksir e jigar & Sharbat e deenar 2.60 ml/kg /day while group 7 and 9 receiving iksir e jigar & Sharbat e deenar 5.20 ml/kg /day respectively for 7 days and on 8th day PCM was administered to all groups except normal control. The study parameters were determined on 9th day. The biochemical parameters were measured by using the standard diagnostic kit. The histopathological studies were also conducted. The antioxidant activity was measured by using the DPPH radical scavenging method (Choudhary et al., 2013).

Biochemical and histological parameters

After 24 h of PCM administration, animals were anaesthetized using chloroform and about 1 ml of blood was collected by cardiac puncture. The blood was allowed to clot and centrifuged at 350 g for 10 min. The serum was separated and used for biochemical assay (Girish et al., 2009). AST, ALT, ALP, billirubin levels and total protien were determined by using kits.

Histopathology

The animals were sacrificed and livers were excised, washed with phosphate buffer and dried with tissue paper. The liver was weighed by using electronic balance and transferred to a 10 % formalin solution. The liver tissues were processed for paraffin embedding and sections of 5-micron thickness were taken in a microtome. After staining with haematoxylin and eosin (H&E), slides were examined under microscope for histopathological changes (Girish et al., 2009).

Evaluation of Anti-Oxidant Activity

DPPH free radical scavenging activity

The free radical scavenging activity of Polyherbal formulations were measured in vitro by 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) assay (Choudhary et al., 2013).

Table 1. Composition of formulations

Formulation	Plants
Jigarine	*Achilleamille folium* and *Artemisia absinthium*
Iksir-e-jigar	*Violaodorata, Foeniculumvulgare, Chicoriumintybus, VitisvineferaLinn, Boragoofficinalis, Solonumnigrum* and Ammonium Chloride
Sharbat-e-deenar	*Cichoriumintybus*(root bark),*Cichoriumintybus* (seeds), *Rosa damascene* (flowers), *Nymphaea alba* (flowers), *Onosmabracteatum*, *Cuscutareflexa* (seeds) and *Rheum emodi*.

RESULTS

In-vitro antioxidant activity of test Polyherbal formulations using DPPH free radical scavenging method

Table 1 described *in-vitro* antioxidant activity of test polyherbal formulations that has been performed using DPPH free radical scavenging method.

Effect of test Polyherbal formulations and Silymarin in Paracetamol-induced hepatotoxic mice model

Paracetamol treatment in mice markedly raised the biochemical parameters including ALT, AST, ALP, TB and TP. Liver weight was also increased by this treatment. Silymarin used as a reference control drug produced the decrease in the said parameters. Furthermore, the animal groups treated with test Polyherbal formulations decreased the parameters in a dose dependant manner as shown in Table 2.

DISCUSSION

In all over the world hepatic diseases have grown to be one of the most important causes of morbidity and mortality in human. The most common causative factors are the hepatotoxic adverse effects of the drugs (Cheemerla and Balakrishnan, 2021). Paracetamol is one of the most commonly used hepatotoxin in the experimental study of hepatic diseases. It significantly raised the ALT, AST and ALP levels in the serum, showing hepatotoxicity. The ALT, AST and ALP activity and serum bilirubin level are generally used as biochemical markers to assess hepatic damage (Girish et al., 2009). A leading cause of cirrhosis and liver cancer is hepatitis (Van Lerberghe, 2008). In the 21st century, therapeutic modalities are usually being shifted towards the development of natural products in hepatic diseases by assimilating the supremacy of traditional medicines with strength of scientific notions of rational, valid, evidence-based medicinal assessment, standardization and controlled clinical trials to file the effectiveness and safety profile (Thyagarajan et al., 2002). The current study is about hepatoprotective and antioxidant effect of marketed Polyherbal formulations for the treatment of liver diseases, without any major side effects. In current study Paracetamol administration raised serum enzyme levels significantly, resulting in damage to the structural integrity and hepatotoxicity. Results of ALT, AST ALP, and bilirubin levels are shown in table 2. Treatment with the three different Polyherbal formulations (Jigarine, Iksir-e-jigar and sharbat-e-deenar) decreased the paracetamol-induced elevated liver enzymes like ALT, ALP, AST and bilirubin levels significantly ($P < 0.01$) at all doses except Jigarine which reduced the ALP and bilirubin levels significantly ($P < 0.05$) only at a dose of 100mg/kg that is in line with study (Valko et al., 2007).

Thus the decrease in enzyme levels clearly points out the effectiveness of these herbal formulations to normalize the functional state of the diseased liver. This is supported by the view that serum levels of transaminases return to normal with the healing of hepatic parenchyma and regeneration of hepatocytes. Furthermore, a significant reduction in liver total protein levels (5.1±0.04) was observed in paracetamol-challenged mice when compared to the normal control (5.5±0.04) as shown in table 2. This validates the reported translation inhibition effect of paracetamol overdose. However, pretreatment of all three Polyherbal formulations

resulted in a dose-dependent suppression of paracetamol-induced adverse effects on liver. Antioxidants play an important role in liver diseases, aging, cancer and inflammatory disorders due to their free radical scavenging activity (Hardik Soni et al., 2014). These natural antioxidants can be formulated as nutraceuticals, to bear oxidative stress in the human body. Use of the free radical DPPH is a one way to determine antioxidant activity (Choudhary et al., 2013).

Table 1: the DPPH scavenging activity of test samples: Percent inhibition ± S.E.M

Sr. No.	**Concentration (µg/ml)**	**Control (Ascorbic acid)**	**Jigarine**	**Iksir-e-jigar**	**Sharbat-e-deenar**
1	10	17.0±0.58	25.33±2.37*	37.25±5.2****	29.57±0.44***
2	20	29.0±1.16	37.40±5.37*	47.87±1.21****	43.48±4.11****
3	40	48.0±1.16	56.47±0.78*	59.6±0.99***	63.13±1.26****
4	60	60.7±1.45	71.10±1.00**	74.63±1.18***	74.90±1.46****
5	80	78.0±2.31	72.03±1.22^{ns}	80.57±0.91^{ns}	83.07±0.94^{ns}
6	100	85.0±1.16	85.07±1.90^{ns}	90.33±0.54^{ns}	90.87±0.75^{ns}

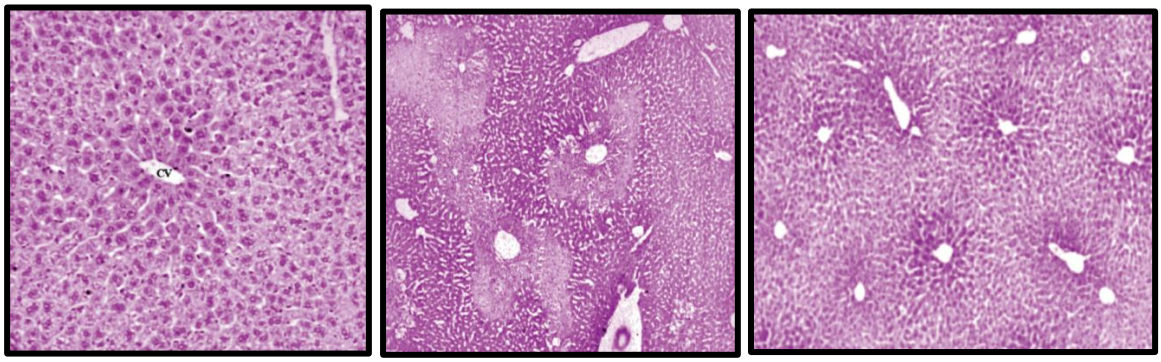
Values are represented as means % inhibitions ± S.E.M (n=3), *P<0.05, **P<0.001, ***P<0.0005, ****P<0.0001, ^{ns}P>0.05 compared to the control group by two-way ANOVA followed by Dunnett's multiple comparison test

Table 3: Effects of test Polyherbal formulations and Silymarin on paracetamol-induced increase of serum increase of ALT, AST, ALP, total bilirubin level, total protein level(decrease), increase in liver weight

GROUP	ALT U/L	AST U/L	ALP U/L	Total Bilirubin mg/dl	Total Protein g/dl	liver weight (g % of b.w)
G1	59.0±2.19	94.0±2.56	199.67±7.49	0.7±0.00	5.5±0.04	4.04±0.07
G2	364.16±40.43^{a}	262.50±15.84^{a}	288.67±6.49^{a}	1.3±0.18^{a}	5.1±0.04^{a}	5.25±0.25^{a}
G3	48.0±3.59**	95.50±5.76**	174.00±1.83**	0.6±0.00**	5.75±0.02**	4.62±0.13^{NS}
G4	127.0±51.4**	155.0±43.64**	247.67±2.1*	1.13±0.91^{NS}	5.20±0.04^{NS}	5.28±0.26 ^{NS}
G5	65.0±4.12**	153.33±2.11**	225.33±8.29**	0.6±0.00**	5.80±0.04**	4.76±0.12 ^{NS}
G6	44.0±1.46**	90.66±3.84**	241.33±1.38**	0.6±0.00**	5.63±0.20**	4.71±0.14 ^{NS}
G7	51.0±7.49**	85.67±2.17**	198.0±3.71*	0.63±0.05*	6.2±0.0**	4.46±0.12*
G8	52.66±5.22**	75.67±5.71**	246.67±23.19*	0.6±0.00**	5.60±0.15**	4.71±0.18 ^{NS}
G9	73.33±15.90**	92.33±5.73**	215.33±12.23**	0.6±0.00**	5.70±0.07**	4.58±0.14*

Values are presented as mean ± SEM (n= 6 mice/ group). ^{a} P<0.05 compared to the normal control group, * P<0.05, **P<0.001, ^{NS} P>0.05 compared to paracetamol group, by One-way ANOVA followed by Dunnett's multiple comparison test.

Figure 1: Histopathological Examination.



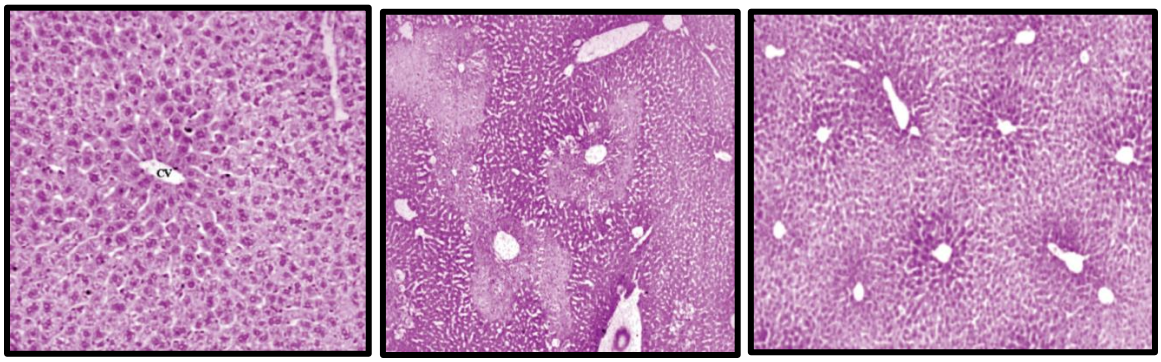
A: normal control; ***B***: Paracetamol treated mice; **C.** Silymarin treated mice (50 mg/ kg)

Alcohol extract of *Capparis sepiaria* stem already reported in a study hepato-protective effect against CCl4-induced toxicity in Albino rats (Satyanarayana et al., 2009). The test drug was given at a dose of 100 mg/kg daily and Silymarin was used as standard drug at a dose of (25 mg/kg) orally for 7 days. As a result, elevated levels of (AST), (ALT), total blirubin were significantly reduced and sharp decreased in total protein level when compared with the toxic control (Satyanarayana et al., 2009). In a study conducted increased levels of marker enzymes like ALT, AST and ALP due to thioacetamide, ranitidine and paracetamol administration were significantly reduced by C*uscuta. reflexa* stem extract administration (Katiyar et al., 2015).

The results of current study are highly correspondant with the conclusions of several studies that have reported the hepatoprotective effects of different plants such as; *Carica papaya* (Sadeque et al., 2010), *Carissa spinarum* (Hegde et al., 2010), *Suaeda fruticose* (Rehman et al., 2014), *Cocculus hirsutus* (Thakare et al., 2009), *Dodonaea viscose* (Khan et al., 2013), *Ipomoea staphylina* (Bag et al., 2013), *Alcea rosea* and *Malva sylvestris* (Hussain et al., 2014a) (Hussain et al., 2014b), *Trianthema decandra* (Geethalakshmi et al., 2010) All of the above mentioned plants have shown the more or less likely same effects of hepato protection against paracetamol-induced hepatotoxicity under different experimental conditions. The hepatoprotective activity of these medicinal plants have confirmed by various studies, but none of the studies reported presence of such phytochemical constituent that exactly exhibit the hepatoprotective activity.

The results given in table 1 have shown that the activities of test formulations are more comparable to L-ascorbic acid. This is also understandable, since L-ascorbic acid is already in a pure form, while these Polyherbal formulations still need to be processed in order to isolate the active compounds responsible for their antioxidant activity. The biochemical observations were further supported by histopathological studies of liver sections of the mice. Finally, the histological study showed that treatment with Polyherbal formulations (Jigarine, Iksir-e-jigar and sharbat-e-deenar) caused enhanced cells regeneration in the liver and restoration of normal cellular size of the cells in extract treated group with no hemorrhage. It further confirms the hepatoprotective activity of these three formulations. Increased serum enzyme levels were more effectively reduced by Iksir-e-jigar than sharbat-e-deenar and lastly by Jigarine in paracetamol-induced toxicity in mice. More effective results are shown in order Iksir-e-jigar>sharbat-e-deenar>Jigarine. Iksir-e-jigar and sharbat-e-deenar showed almost similar antioxidant activity as compared to Jigarine, when measured by DPPH free radical scavenging method by using L-ascorbic acid as a reference standard. More effective antioxidant activity was observed in order orderIksir-e-jigar = sharbat-e-deenar>Jigarine. Pretreatment with Jigarine, Iksir-e-jigar and sharbat-e-deenar for 7 days reduced the histopathological damage associated with hepatotoxicity from paracetamol intoxicated treatment. However, more effective results are shown in order Iksir-e-jigar>sharbat-e-deenar>Jigarine.

Histopathological Examination

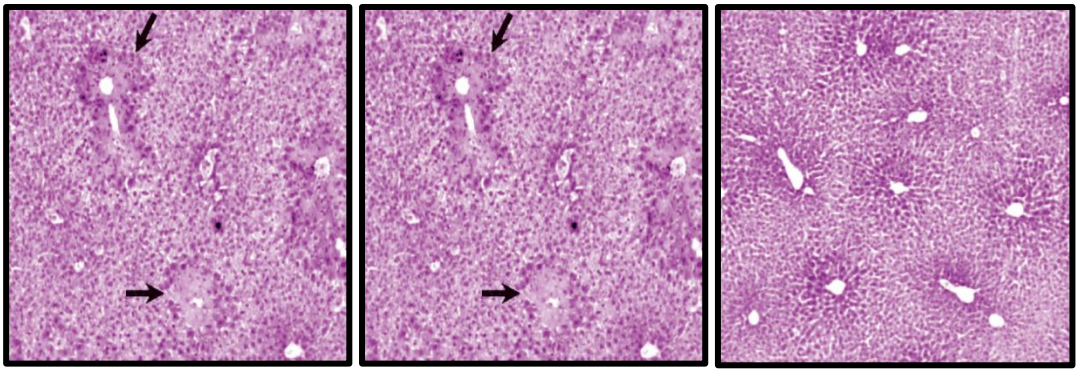


A: normal control Hepatic cords and cells are normal in shape.

B: Paracetamol treated mice. Shows extensive necrosis & fatty changes.

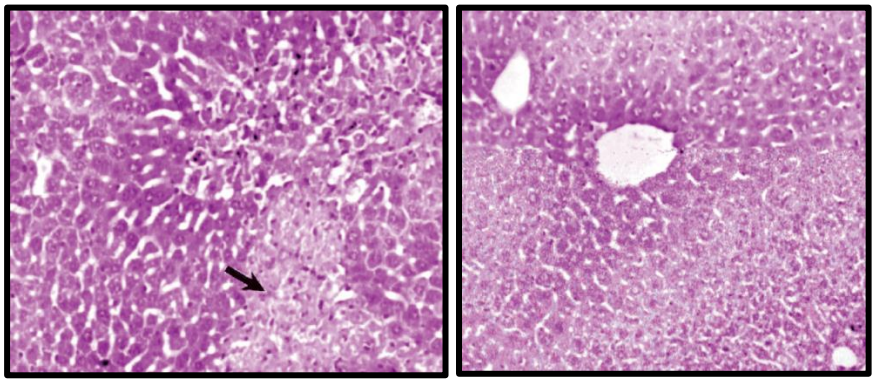
C.Silymarin treated mice (50 mg/ kg) Showing protection of hepatocytes

Figure: 1 Histopathological examination of Silymarin treated liver of mice.



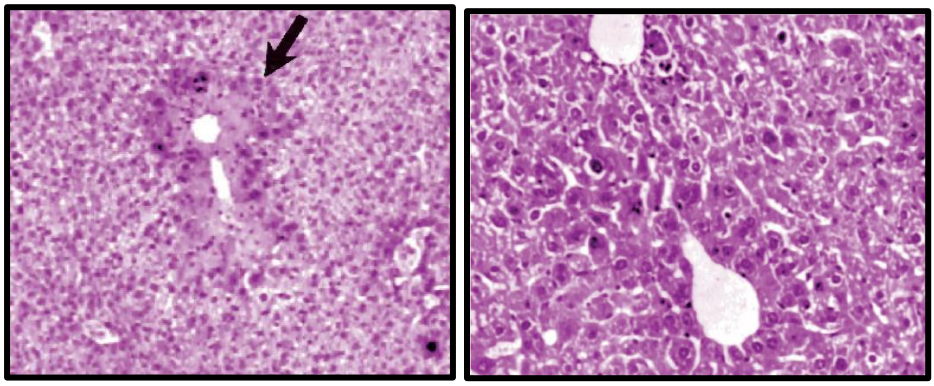
A. Jigarine treated mice (50mg/kg) ***B.*** Jigarine treated mice (100mg/kg)
Focal necrotic areas indicated with arrow complete normalization of liver architecture

Figure 2: Histopathological examination of jigarine treated liver of mice.



A. Iksir-e-jigar treated mice (2.6ml/kg) ***B.*** Iksir-e-jigar treated (5.20ml/kg)
Focal necrotic area is indicated with arrow Complete normalization of liver architecture

Figure 3*:* Histopathological examination of iksir-e-jigar treated liver of mice.



A. Sharbat-e-deenar treated mice (2.6ml/kg) ***B.*** Sharbat-e-deenar treated mice (5.20ml/kg)
Focal necrotic area is indicated with arrow Complete normalization of liver architecture
Figure 4: Histopathological examination of sharbat-e-deenar treated liver of mice.

CONCLUSION

On the basis of results obtained, it can be concluded that all three Polyherbal formulations namely Jigarine, Iksir-e-jigar and sharbat-e-deenar have shown significant hepatoprotective and antioxidant activities. When their hepatoprotective and antioxidant activities were comparatively evaluated, Iksir-e-jigar was found to be most effective among all three Polyherbal formulations
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