HEPATOPROTECTIVE ACTIVITY OF A POLYHERBAL FORMULATION "BONJIGAR®" AGAINST DICLOFENAC SODIUM-INDUCED HEPATOTOXICITY IN ALBINO RABBITS

Tanweer Khaliq*, Humaira Naseer, Faiza Mumtaz

ABSTRACT Polyherbal formulations are available with a large number of indications although they are widely used scientific work related to their safety and efficacy is scarce. The objective of the current investigation was to study the hepatoprotective effect of Bonjigar®, a polyherbal mixture; on Diclofenac sodium-induced liver toxicity in experimental rabbits. A total of thirty rabbits were divided into five groups, each containing six animals. Group, I was kept in control. Group II was given Diclofenac Sodium (20 mg/ml/kg body weight orally). Group III was treated with Bonjigar® (1.5 ml/kg body weight orally) while group IV and V were administered with Silymarin (100 mg/5ml/kg body weight orally) + Diclofenac Sodium (20 mg/ml/kg) and Bonjigar® (1.5 ml/kg) + Diclofenac Sodium (20 mg/kg) respectively for 15 days. Blood samples were taken at 0, 1st, 7th, and 15th days intervals. The effect of Bonjigar® was monitored for different serum biochemical parameters like alanine transferase (ALT), aspartate transferase (AST), bilirubin, blood urea nitrogen (BUN), creatinine, total oxidant status (TOS) and catalase (CAT) by following the standard methods. Histopathological analysis was also performed. Results demonstrated that Bonjigar® significantly reduced the elevated levels of ALT, AST, bilirubin, creatinine, and total oxidant status (TOS), and enhanced the reduced hepatic catalase (CAT) activity. Histopathological studies of liver sections also indicated the protective effect of Bonjigar® in reducing the hepatic unwanted effects of Diclofenac. It has been concluded that Bonjigar® syrup is effective to improve liver and kidney functions against Diclofenac sodium-induced toxicity in rabbits.

Department of Physiology and Pharmacology, Faculty of veterinary sciences, University of Agriculture, Faisalabad, Pakistan
Corresponding Author: Tanweer Khaliq,
Email: t_khaliqch@yahoo.com

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INTRODUCTION

The liver is one of the largest organs in the human body for detoxification and disposition of endogenous substances. If the natural protective mechanisms of the liver are disturbed during all such exposures, resulting will be hepatic injury (1). Diclofenac sodium is a no-steroidal anti-inflammatory (NSAID) agent and has been widely used as an anti-inflammatory and analgesic. It is a potent inhibitor of cyclooxygenase as well as lipo-oxygenase enzyme (2).

Adverse effects of Diclofenac Sodium include hepatotoxicity, increased levels of transaminases, renal failure, weight loss, and a gastrointestinal ulcer (3). On the other hand, Silymarin (seed extract of *Silybum marianum*; family *Asteraceae*) is one of the most commonly used synthetic hepatoprotective drugs which correct liver toxicity. Active flavonolignans (silymarin, silybin, dehyrosilybin, silydianin, isosilybin and silychristin) are, present in Silymarin extract, responsible for hepatoprotective activity (4).

Several polyherbal formulations are available in the market worldwide for the treatment of various liver disorders but the majority of them have not yet scientifically been validated. The quality profile of herbal products involves the complete information of phytoconstituents present in the final product and the Food and drug administration (FDA) has passed the GMP and quality control regulations exclusively for ayurvedic products 2003 (5). At present, large numbers of herbal products are available commercially possessing hepatoprotective activity mainly Iksir-e-Jigar, Jigarine, Liv 52, Livergen and Octogin.

Bonjigar® is one of them, a polyherbal mixture of *Silybum marianum* (Ount Katara), *Eclipta alba* (Bhangara), *Cichorium intybus* (Kasni), *Berberis aristata* (Chitra), *Picrorrhiza kurrooa* (Kutki), *Tamarix gallica* (Jhaau), *Boerhavia diffusa* (Gadapurna), *Solanum nigrum* (Makoi) and *Raphanus sativus* (Mooli). These plants have already been reported to have hepatoprotective activity. But no scientific work related to the hepatoprotective activity of Bonjigar® has been reported. Thus the present study was conducted to explore the use of Bonjigar® as a hepatoprotective formulation against Diclofenac sodium induced liver toxicity.

MATERIALS AND METHODS

Drugs and Chemicals

Diclofenac Sodium (Dicloran® Disperlet Tablets. Sammi Ltd., Karachi), Silymarin (Siliver® Suspension Abbott Lab. Ltd., Karachi), and Bonjigar® syrup (Herbion Pvt. Ltd., Karachi) were purchased from local market of Faisalabad, Pakistan.

Preparation of solution

Each tablet of Diclofenac sodium contains 50 mg of the active ingredient. It was dissolved in distilled water and administered to animals at the dose of 20 mg/ml/kg orally with the help of a stomach tube by following the method defined by Das and Roy, (6).

Animals

Thirty, healthy adult rabbits of the same sex weighing 1430g (1.43±0.07) were procured from the local market of Faisalabad and kept in an animal room of the Department of Physiology and Pharmacology, University of Agriculture, Faisalabad, Pakistan. The rabbits were housed in individual iron cages with a period of light and dark alternatively, at ambient temperature ((22±2ºC) with proper ventilation facility. Rabbits were fed with seasonal fodder (lucern) and water ad-libitum till completion of the experiment.

Allocation of Groups and Experimental Procedure

A total of thirty rabbits were divided into five groups, each containing six animals. Group, I was kept in control. Group II was given Diclofenac Sodium (20 mg/ml/kg body weight orally). Group III was treated with Bonjigar® (1.5 ml/kg body weight orally) while group IV and V were administered with Silymarin (100 mg/5ml/kg body weight orally) + Diclofenac Sodium (20 mg/ml/kg) and Bonjigar® (1.5 ml/kg) + Diclofenac Sodium (20 mg/kg) respectively for 15 days.

Blood Sampling

Blood samples were collected at 0, 1st, 7th, and 15th days intervals. Up to 5-7ml of blood was collected with the help of a syringe from the jugular vein of each rabbit in Eppendorf tubes. These blood samples were centrifuged at 3000 r.p.m for 15 minutes to separate the serum which was stored at -4°C for biochemical analysis.

Biochemical Estimations

The biochemical analysis in serum was done by using commercially available kits from Randox Laboratories Ltd, United Kingdom. The serum biochemical parameters include ALT, AST, Bilirubin, Blood Urea Nitrogen (BUN), Creatinine, Total Oxidant Status (TOS), and Catalase.

Histopathological studies

Tissue samples (liver and kidney) fixed in 10% Formalin were processed by the graded concentration of Xylin and embedded in Paraffin. Tissue sections with a thickness 5µm were stained with Hematoxylin and Eosin. Mounted glass slides were examined and a photographic assessment was carried out under a light microscope (Olympus Optical Co., Tokyo, Japan).

STATISTICAL ANALYSIS

Analysis of variance (ANOVA) under a completely randomized design (CRD) was applied on the data to observe the difference in different groups and at different sampling days (7). Duncan Multiple Range Test was applied for

significant difference among the means of different groups at different sampling days. The level of significance was p≤0.05.

RESULTS AND DISCUSSION

The liver regulates various metabolic processes in the body. Many traditional curement use herbal preparations for the prevention of hepatic ailments but management therapy for liver disorder through an herbal drug system is still considered to be an intriguing problem (8). Liver toxicity caused by Diclofenac is related to the oxidative metabolism of the drug which results in the production of minor oxidative metabolites (9) There was a significant (P<0.01) increase in ALT, AST, and bilirubin levels in Diclofenac treated group as compared to the control group. The ALT and AST activities increased significantly (P<0.01) in Bonjigar® treated group as compared to the control group. Bonjigar® + Diclofenac treated group significantly (P<0.01) alleviated the levels of ALT, AST, and bilirubin as compared to Diclofenac treated group. The ALT, AST, and bilirubin values decreased significantly (P<0.01) in Silymarin+Diclofenac treated group when compared with Diclofenac treated group (table-1). There was a significant (P<0.01) increase in BUN, creatinine, and total oxidant status (TOS) levels treated with Diclofenac as compared to the control group. Bonjigar® + Diclofenac treated group significantly (P<0.01) alleviated the levels of BUN and creatinine as compared to Diclofenac treated experimental group. The BUN and creatinine values decreased significantly (P<0.01) in Silymarin+Diclofenac treated group (table-2). There was a significant (P<0.01) increase in total oxidant status (TOS) level treated with Diclofenac as compared to the control group. Bonjigar® + Diclofenac treated group significantly (P<0.01) alleviated the level of TOS in comparison to Diclofenac-treated experimental group. The TOS level decreased significantly (P<0.01) in Silymarin+Diclofenac treated group (table-2). There was a significant (P<0.01) decrease in catalase (CAT) activity treated with Diclofenac as compared to the control group. Bonjigar® + Diclofenac treated group significantly (P<0.01) elevated the level of CAT in comparison to Diclofenac-treated experimental group. The CAT level increased significantly (P<0.01) in Silymarin+Diclofenac treated group (table-2). Results provided evidence that Diclofenac has adverse effects on liver functions. Enzymes, AST (SGOT), and ALT (SGPT) elevation were observed in the serum of rabbits. In the body fluids estimation of enzyme level is a beneficial indicator of overt disease of genetic predisposition of a disease state. Thus there was no ambiguity that Diclofenac causes liver toxicity in experimental rabbits (10). In the present study, ALT and AST values increased in Diclofenac treated group as compared to the control and all experimental groups as reported by Das and Roy (6). Significant (P<0.01) increase in bilirubin level was observed in Diclofenac treated group as compared to control group. Same observations were found by (11) in the elevation of bilirubin level. A significant (P<0.01) reduction in aminotransferases (ALT, AST) and bilirubin levels was observed when animals were treated with Bonjigar® as compared to toxic group (Diclofenac) which might be due to antioxidant properties of preparation. Hepatoprotective effect of Silymarin was probably because of antioxidant potential of their constituents namely silybin, isosilybin, silychristin and silydianin (12). Elevated levels of urea and serum creatinine are indicative markers of nephrotoxicity. Blood urea nitrogen and Serum creatinine values increased significantly (P<0.01) in Diclofenac treated group. Baravalia *et al*., (11) and Nabarawy, (11) investigations supported the results.

An elevation in BUN and creatinine levels might be due to the inhibition of prostaglandin synthesis or the production of reactive oxygen species (14). Reactive oxygen species (ROS) have the potential to cause deleterious effects in vivo

environment. Different endogenous antioxidant enzymes like glutathione peroxidase, catalase, and superoxide dismutase regulate these reactive species' damage (15). Oxidant status was found to be elevated in Diclofenac treated group as compared to the control group while Bonjigar® effectively decreased the oxidants level which might be due to the antioxidant properties of the constituents of Bonjigar®. In the present study, the level of catalase was depleted in the group which was fed with Diclofenac as reported by Das and Roy, (6). Liver catalase activity was restored significantly by Bonjigar® administration which manifested that Bonjigar® has a free radical scavenging ability. istopathological view of liver tissues showed a normal arrangement of hepatocytes and mononuclear cell infiltration in the portal area in control animals (Fig-A1). Liver hepatocyte vacuolar degeneration was observed in the group treated with Diclofenac as compared to the control group (Fig-B1). While hepatic changes caused by Diclofenac were improved with Bonjigar® administration to rabbits which indicated the protective action of Bonjigar® against liver degeneration and necrosis (Fig-E1). Silymarin treated group also exhibited the protective effect (Fig-D1).

In control group renal tissues were found with intact nuclei. Some have condensed hyper chromatic nuclei with foamy tuft of capillaries (Fig-A2). Diclofenac treated group revealed kidney tissues with massive vacuolar degeneration in proximal convoluted tubule (Fig-B2). Bonjigar® corrected the variations induced by Diclofenac in animals (Fig-E2). Protective measures were also seen in Silymarin-treated rabbits (Fig-D2). Histopathological view of liver sections showed the same results as reported by (11) and (15) among the animals of their study treated with Diclofenac. Bonjigar® corrected the histopathological variations induced by Diclofenac in animals which might be due to its antioxidant potential.

CONCLUSION

The results obtained in the current study have indicated that Bonjigar® syrup possesses significant hepatoprotective properties against Diclofenac sodium-induced hepatotoxicity as compared to the standard drug, Silymarin. It is also effective in reducing the toxic effects of Diclofenac on the kidney. All the findings are in coordination with histopathological studies which showed the protective activity of Bonjigar® against Diclofenac sodium induced toxicity on liver.

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TABLE AND FIGURES

Table 1: Effect of Bonjigar® on ALT, AST and Bilirubin levels in Diclofenac sodium induced Hepatotoxic rabbits model.

Group (n)	ALT (U/L)	AST (U/L)	Bilirubin (mg/dL)
Control	41.42±0.4**E**	52.17±0.31**C**	0.58±0.02**B**
Diclofenac treated	65.33±4.57**A**	63.37±2.24**A**	0.76±0.04**A**
Bonjigar®	45.46±0.42**D**	54.08±0.38**C**	0.58±0.02**B**
Silymarin+Diclofenac	55.96±3.28**C**	57.04±0.76**B**	0.62±0.03**B**
Bonjigar® + Diclofenac	58.54±3.42**B**	58.46±0.89**B**	0.65±0.03**B**

Mean+SEM values, n=6. Data were analyzed statistically using Analysis of variance (ANOVA) under two factor factorial completely randomized designs (CRD).

Table 2: Effect of Bonjigar® on BUN, creatinine, TOS and CAT levels in Diclofenac sodium induced Hepatotoxic rabbits model.

Group (n)	BUN (mg/dL)	Creatinine (mg/dL)	TOS (µmol/L)	CAT (kU/L)
Control	17.36±0.26**C**	0.73±0.02**B**	2.35±0.12**B**	38.85±0.86**A**
Diclofenac treated	26.08±1.54**A**	0.87±0.02**A**	3.18±0.2**A**	35.39±0.88**B**
Bonjigar®	17.93±0.30**C**	0.75±0.02**B**	2.34±0.07**B**	39.68±0.80**A**
Silymarin+Diclofenac	18.96±0.44**BC**	0.78±0.02**B**	2.44±0.08**B**	39.07±0.72**A**
Bonjigar® + Diclofenac	20.37±0.57**B**	0.80±0.02**AB**	2.59±0.07**B**	37.58±0.79**AB**

Mean+SEM values, n=6. Data were analyzed statistically using Analysis of variance (ANOVA) under two factor factorial completely randomized designs (CRD). Capital letters are used for overall mean.

Fig-1. Effect of Bonjigar® on Diclofenac - induced Hepatotoxicity in Rabbits (Histopathology).

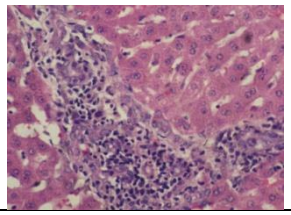
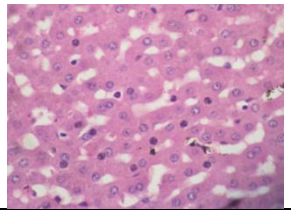
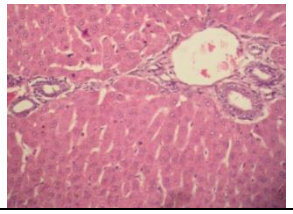
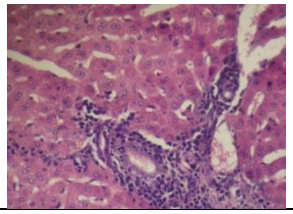
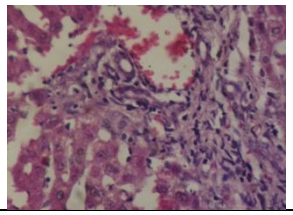
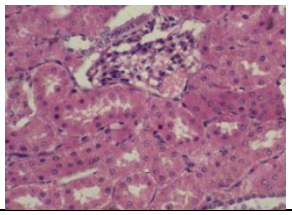
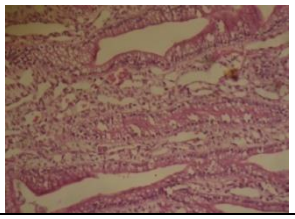
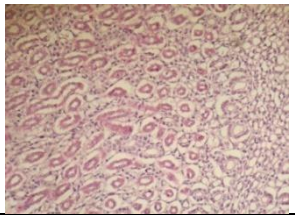
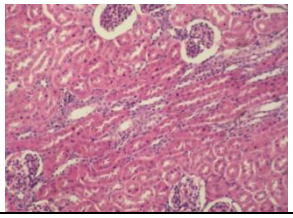
A1	Control	
B1	Diclofenac treated	
C1	Bonjigar®	
D1	Silymarin+Diclofenac	
E1	Bonjigar® + Diclofenac	

Fig-2. Effect of Bonjigar® on Diclofenac - induced Nephrotoxicity in Rabbits (Histopathology).

A2	Control	
B2	Diclofenac treated	
C2	Bonjigar®	
D2	Silymarin+Diclofenac	
E2	Bonjigar® + Diclofenac