LIVINA, A POLYHERBAL PREPARATION PROTECTS LIVER AGAINST ACECLOFENAC-INDUCED HEPATIC INSULT IN SPRAGUE-DAWLEY RATS: A COMPARISON WITH SILYMARIN

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Summary

Aceclofenac, a widely used nonsteroidal anti-inflammatory drug (NSAID), has been associated with a number of severe cases of clinical hepatotoxicity. Protective activity of polyherbal formulation Livina was compared beside a well-established standard hepatoprotective drug Silymarin against Aceclofenac induced hepatic injury in Sprague-Dawley rats. To clarify its effect on liver functions, adult male rats were injected with Aceclofenac at the single dose of 60 mg kg^{-1} body weight orally once daily for 60 days. The hepatoprotective effect of polyherbal formulation (Livina) was comparatively evaluated with Silymarin (25 mg kg^{-1}) by measuring levels of serum markers such as alanine aminotransferase (ALT), aspertate aminotransferase (AST), γ-glutamyle transpeptidase (GGT), alkaline phosphatase (ALP), lipid peroxidase (MDA), δ-Aminolevulinate dehydratase activity (δ-ALA-D), serum total protein and bilirubin (total and direct) levels in rats serum along with the histopathological studies to support the above mentioned parameters. The study found that the supplementation of Livina significantly ($P<0.05$, $P<0.001$) reduced the damaging effects on liver by Aceclofenac. Histopathological changes (congestion of central vein, centrilobular necrosis and sinusoidal congestion) induced by Aceclofenac were also reduced to a moderate extent in Livina treatment. In both the cases the effectiveness of Livina were found to be almost parallel with Silymarin, indicating the herbal formulation to be almost as effective as the standard drug.

Keywords: Aceclofenac, hepatotoxicity, liver enzymes, Livina, Silymarin.

Running Title: Hepatoprotective effect of Livina

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Introduction

Liver diseases are considered to be serious health problem, as the liver has one of the highest value of importance for the systemic detoxification and deposition of endogenous and exogenous substances. Steroids, vaccines and antiviral drugs, which are commonly employed as therapies for hepatic diseases, have potential adverse effects, especially when administered for long terms [1]. Therefore, it became necessary to screen a battery of new drugs, which can primarily act as an anti-hepatotoxic agent, and side-by-side ameliorates drug induced critical hepatic damage as a secondary rescuer. Thus to introduce a group of alternative drugs, for the treatment of liver diseases and to replace the currently used drugs of doubtful efficacy and safety. In recent years, there has been a substantial increase in the use of so-called complementary and alternative therapies that utilize herbal medicines by patients with liver disease [2-4].

Several non-steroidal anti-inflammatory drugs (NSAIDs) have been associated with liver damage [5]. Aceclofenac is a common and well established NSAID which is chemically designated as [{2-(2', 6'- dichlorophenyl) amino } phenylacetoxyacertic acid], a phenyl acetic acid derivative. It is effective in the treatment of painful inflammatory diseases and has been used to treat more than 75 million people worldwide. Chronic intake of Aceclofenac, damages GI mucosa by irritant action, causing alteration in mucosal permeability and/or suppression of prostaglandin (PGE_2) synthesis [6]. The mode of action of Aceclofenac has been recently clarified in that the compound was shown to elicit preferential inhibition of COX-2 as a result of limited but sustained biotransformation to diclofenac [7-10]. In our previous studies we have established the fact that Aceclofenac induced hepatotoxicity and GI toxicity has been partially ameliorated by using combinatorial vitamin and herbal supplementation [11,12].

Modern medical science does not have, at present, a therapeutic agent that could cure the different liver disorders without showing any accountable side effect. In fact, the available remedies are from the traditional system of medicine. Herbs have recently attracted attention as health beneficial food and as essential materials for some critical disease remedy. Herbal medicines derived from plant extracts are being increasingly utilized to treat a wide variety of clinical disorders including liver disease [13,14], ischemia, reperfusion injury, atherosclerosis, acute hypertension, haemorrhagic shock, diabetes mellitus and cancer [15,16] with relatively little knowledge regarding their molecular mode of action [17].

Livina, a polyherbal formulation is very useful as a natural hepatoprotective medicine [18]. Our recent work also established that Livina protects against gastric mucosal damage and maintain mucosal lipid profile [19]. It also revels that Livina has an antioxidant property that protect liver and stomach against oxidative damage [20]. Recent work established that Livina prevent high fat diet induced obesity in experimental rats [12]. Moreover Livina composed of several Indian medicinal plant extract those have hepatoprotective activity.

Silymarin is a composite combination of four flavonolignan isomers, which are silybin, isosilybin, silydianin and silychristin with an empirical formula $C_{25}H_{22}O_{10}$. The structural resemblance of silymarin to steroid hormones is assumed to be accountable for its protein synthesis facilitator measures. Among the isomers silybin is the main and most active component and represents about 60-70 per cent, followed by silychristin (20%), silydianin (10%), and isosilybin (5%) (Saller et al). Silipide (IdB 1016) is the silybin - phosphatidylcholine complex that ensures a huge increase in the bioavailability of silybin [21]. Various researchers against partial hepatectomy models and toxic models in experimental animals have established hepatoprotective activity of silymarin by using acetaminophen, carbon tetrachloride, ethanol, D-galactosamine, and amanita phalloides toxin [22,23].

Keeping the above information in view, the present study was designed to demonstrate Aceclofenac induced hepatotoxicity and the possible protective role of Livina, a polyherbal formulation in compared with Silymarin a known hepatoprotective drug in experimental rats.

Materials and Methods

Chemicals and Drugs

Aceclofenac was procured from Nulife Pharmaceuticals, Pune. Silymarin was purchased as Silybion-140 tablets from Micro labs (Hosur, Tamilnadu, India). Bovine serum albumin (Sigma chemical St. Louis, MO, USA), thiobarbituric acid and TCA (Loba Chemie, Mumbai, India) were procured from standard company as mentioned. Livina syrup was obtained from Dey’s Medical Stores (Mfg.) Ltd., 62, Bondel Road, Kolkta-700019, India. AST, ALT, GGT, ALP, total protein and bilirubin kits were obtained from Merck, Germany. All other chemicals and solvents were of analytical grade commercially available.

Animals

Male Sprague-Dawley rats weighing 152 ± 5g were used in the experiment. They were inbreed and obtained from our CPCSEA approved animal house (Registration No. 50/CPCSEA/1999). The animals were grouped and housed in cages and maintained under standard laboratory conditions (temperature 25 ± 2°) with dark and light cycle (12h/12h). They were allowed free access to standard dry pellet diet (Hindustan Lever, Kolkata) and water *ad libitum*. The experiment was carried out according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India and approved by the Institutional Animal Ethics Committee (IAEC) (Approval No. Dey's/IAEC/001/07, dated 14.08.2007).

Experimental Design

The rats were divided into the following groups each containing 6 rats (n=6).

Group-I: Control Rats, which fed normal diet and water.

Group-II: Rats treated with Aceclofenac (60 mg kg^{-1}, orally) [24] for 60 days.

Group-II: Rats treated with Aceclofenac (60 mg kg^{-1}, orally)) along with 0.25 ml/day Livina orally once daily for 60 days.

Group-IV: Rats treated with Aceclofenac (60 mg kg^{-1}, orally)) along with 0.5 ml/day Livina orally once daily for 60 days.

Group-V: Rats treated with Aceclofenac (60 mg kg^{-1}, orally)) along with 1.0 ml/day Livina orally once daily for 60 days.

Group-VI: Rats treated with Aceclofenac (60 mg kg^{-1}, orally)) along with Silymarin (25 mg kg-^{1}, orally.) for 60 days.

Each 2ml of Livina syrup contains extracts of:

1.	*Solanum nigrum*	20 mg
2.	*Holarrhena antidysenterica*	10 mg
3.	*Tephrosia purpurea*	40 mg
4.	*Andrographis paniculata*	10 mg
5.	*Phyllanthus niruri*	20 mg
6.	*Tinospora cordifolia*	10 mg
7.	*Terminalia chebula*	10 mg
8.	*Asteracantha longifolia*	20 mg
9.	*Alstonia scholaris*	20 mg
10.	*Berberis aristata*	40 mg
11.	*Cichorium intybus*	10 mg
12.	*Picrorhiza kurroa*	20 mg

Measurement of body weight

The body weight intake of each mouse was recorded every day using a sensitive balance.

Blood collection

The total duration of experiment was 60 days, at the end of which the animals were fasted overnight. The animals were anaesthetized by ether, and blood was withdrawn through retro-orbital plexus. After collection of blood the serum was separated by centrifuging at 3,000 rpm for 10 min for biochemical estimation. For histological study, liver tissue was quickly removed after autopsy and fixed in 10% formal saline.

Separation of serum

Immediately after death, blood samples were collected in heparinized test tubes and plain tubes and centrifuged. Blood cells and other solid matters settled down as pellet and the clear supernatant containing only serum were carefully removed without disturbing the pellet. This clear serum was used for biochemical testing of hepatic markers.

Preparation of tissue homogenate

Liver tissue was washed with ice-cold saline. The tissues were then cut into fragments and homogenized with 3 volumes (w/v) of the appropriate buffer using a Potter-Elvehjam homogenizer with a Teflon pestle and centrifuged at 12000g for 20 min at 4°C [25], the supernatant was used for the estimation of lipid peroxidation.

Biochemical estimation

Serum ALT [26], AST [26], GGT [26], ALP [27], and bilirubin [28] (total and direct) were determined by kinetic method using the kit obtained from Merck, Germany in a double beam spectrophotometer (Elico SL-164, Elico Limited, Hyderabad, India). The transaminases activities were determined as change in absorbance/min at 340 nm. Serum ALP activity was determined from the rate of release of paranitrophenol at 405 nm. Total protein and bilirubin (total and direct) were measured at 540 nm. Serum total protein was measured according to the method of Lowry et al 1951 [29].

Estimation of Lipid peroxidation

Lipid peroxidation was estimated by the method of Ohkawa et al. (1979) in tissue homogenates. Briefly, the reaction mixture contained Tris-HCl buffer (50 mM, pH 7.4), ter-butyl hydroperoxide (BHP) (500 μM in ethanol) and 1 mM $FeSO_4$. After incubating the samples at 37°C for 90 min, the reaction was stopped by adding 0.2 ml of 8% sodium dodecyl sulfate (SDS) followed by 1.5 ml of 20% acetic acid (pH 3.5). The amount of malondialdehyde (MDA) formed during incubation was estimated by adding 1.5 ml of 0.8% TBA and further heating the mixture at 95°C for 45 min. After cooling, samples were centrifuged, and the TBA reactive substances (TBARS) were measured in supernatants at 532 nm by using 1.53 X 10^5 M^{-1} as extinction coefficient. The levels of lipid peroxidation were expressed in terms of nmoles of TBARS per 90 min/mg protein [30].

Estimation of δ-Aminolevulinate dehydratase activity (δ-ALA-D)

The livers of treated Rats were quickly removed, placed on ice and homogenized in 15 mM NaCl (1:10 w/v). The homogenate was centrifuged at 4000 g for 10 min at 4ºC to yield a low-speed supernatant fraction that was used for enzyme assay. Enzyme assay was carried out as described by Sassa (1982) with slight modification [31]. Reaction was started 10 min after the addition of the liver homogenate by adding the substrate and carried out over 60 min at 39ºC. The reaction product was determined using modified Ehrlich's reagent at 555 nm, with molar absorption coefficient of 6.1 x 10^4 M^{-1} for the Ehrlich-porphobilinogen salt.

Histopathological examination

Small portions of the liver tissue from all the groups were immediately collected after sacrificed. Tissues were fixed in 10% formalin in phosphate buffer (pH 7.0) for 24 hr. at room temperature for histopathology [32]. Tissues were embedded in paraffin wax and sections were cut at 3-5 μm

slices and were stained with haematoxylin and eosin (H&E) and observed under light microscope.

Statistical analysis

Data were analysed by one way analysis of variance followed by Duncan's multiple range test using a commercially available statistics software package (SPSS for Windows, ver. 11.0;SPSS Inc., Chicago, IL, USA). Results were presented as mean ± S.D. Values of P<0.05 were regarded as statistically significant.

RESULTS

Effects of Livina on body weight and liver weight

The mean body weight was significantly reduced in Aceclofenac-intoxicated rats compared with control animals (Table 1). Supplementation of Livina 0.5ml/day and 1ml/day significantly increased mean body weight compared with Aceclofenac treated rats. Mean liver weight significantly elevated in Aceclofenac treated rats as compared with the control and that was restored as normal in 0.5ml/day and 1ml/day Livina pretreatment (Fig.1).

Table 1. Effects of Livina and Aceclofenac on body weight of Sprague-Dawley rats after 60 days treatment

Groups	Initial BW (g)	Final BW (g)	% Weight gain	% Weight loss
I	154.28 ± 1.58	213.48 ± 2.15	38.37	
II	155.51 ± 1.67	134.50 ± 1.93$^{\#\#}$	-	13.51
III	154.73 ± 1.38	199.64 ± 2.55*	29.02	
IV	155.95 ± 1.78	192.31 ± 2.17*	23.31	
V	155.68 ± 1.51	206.73 ± 1.61**	32.79	
VI	154.87 ± 1.73	209.24 ± 1.89**	35.11	

All values are mean ± S.D., n=6 rats in each group. $^{\#\#}$P<0.05 as compared with the control animal *P<0.01, **P<0.05, as compared with the Aceclofenac treated animal.

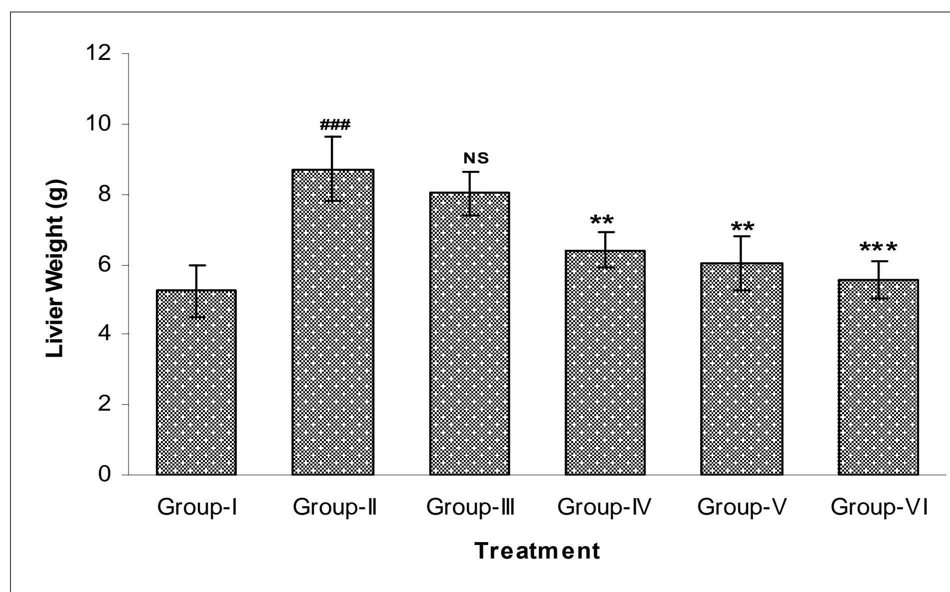

Fig.1: Effect of Livina on liver weight in Aceclofenac induced hepatotoxicity in rats. $^{###}$P<0.001 significantly different from control group. **P<0.01, ***P<0.05 significantly different from Aceclofenac treated animals. NS = Not significant.

Effects of Livina on biochemical investigation

Table 2 and 3 shows the biochemical parameters of control and experimental animals. The results of AST, ALT, GGT, ALP, and bilirubin (total and direct) in control rats were 66.42 ± 4.89, 42.73 ± 2.57, 3.73 ± 0.24, 72.51 ± 11.51, 0.86 ± 0.05 and 0.06 ± 0.02 respectively, whereas in Aceclofenac treated rats these levels were elevated to 132.73 ± 8.13, 90.48 ± 4.52, 8.04 ± 0.91, 228.62 ± 18.55, 5.07 ± 0.07 and 0.65 ± 0.04 respectively. Livina pretreatment at the dose of 0.5 ml/day significantly (P<0.001) prevented the Aceclofenac induced rise in the AST, ALT, GGT, ALP, and bilirubin (total and direct) to 85.44 ± 4.75, 68.73 ± 3.23, 5.71 ± 0.51, 112.55 ± 12.72, 2.15 ± 0.06 and 0.13 ± 0.03 respectively being compared to Aceclofenac treated group. With higher dose of Livina (1.0 ml/day) further reduction of AST, ALT, GGT, ALP, and bilirubin (total and direct) to 73.59 ± 4.09, 52.61 ± 3.61, 4.54 ± 0.43, 95.33 ± 11.44, 1.36 ± 0.05 and 0.09 ± 0.01 respectively were noted. Silymarin (25 mg kg^{-1}, orally.) pretreatment also prevented the Aceclofenac induced rise in AST, ALT, GGT, ALP, and bilirubin (total and direct) to 71.78 ± 5.64, 46.44 ± 2.52, 4.26 ± 0.44, 88.74 ± 9.83, 1.14 ± 0.03 and 0.10 ± 0.02 respectively.

Table 2. Effects of Livina and Aceclofenac on serum AST, ALT and GGT of Sprague-Dawley rats after 60 days treatment

Groups	Treatment	AST (IU/L)	ALT (IU/L)	GGT (IU/L)
I	Control	66.42 ± 4.89	42.73 ± 2.57	3.73 ± 0.24
II	Aceclofenac (60 mg kg^{-1})	132.73 ± 8.13$^{\#}$	90.48 ± 4.52$^{\#}$	8.04 ± 0.91$^{\#}$
III	Livina (0.25 ml)	102.61 ± 6.29*	76.33 ± 4.26*	6.25 ± 0.57*
IV	Livina (0.5 ml)	85.44 ± 4.75**	68.73 ± 3.23**	5.71 ± 0.51**
V	Livina (1.0 ml)	73.59 ± 4.09***	52.61 ± 3.61***	4.54 ± 0.43***
VI	Silymarin (25 mg kg-^{1})	71.78 ± 5.64***	46.44 ± 2.52***	4.26 ± 0.44***

All values are mean ± S.D., n=6 rats in each group. $^{\#}$P<0.001 as compared with the control animal *P<0.01, **P<0.05, ***P<0.001 as compared with the Aceclofenac treated animal.

Table 3. Effects of Livina and Aceclofenac on serum ALP, total bilirubin and direct bilirubin of Sprague-Dawley rats after 60 days treatment

Groups	Treatment	ALP (IU/L)	Total bilirubin (mg/dl)	Direct bilirubin (mg/dl)
I	Control	72.51 ± 11.51	0.86 ± 0.05	0.06 ± 0.02
II	Aceclofenac (60 mg kg^{-1})	228.62 ± 18.55$^{\#}$	5.07 ± 0.07$^{\#}$	0.65 ± 0.04$^{\#}$
III	Livina (0.25 ml)	163. 12 ± 14.78*	3.56 ± 0.05*	0.27 ± 0.04*
IV	Livina (0.5 ml)	112.55 ± 12.72**	2.15 ± 0.06**	0.13 ± 0.03**
V	Livina (1.0 ml)	95.33 ± 11.44***	1.36 ± 0.05***	0.09 ± 0.01***
VI	Silymarin (25 mg kg-^{1})	88.74 ± 9.83***	1.14 ± 0.03***	0.10 ± 0.02***

All values are mean ± S.D., n=6 rats in each group. $^{\#}$P<0.001 as compared with the control animal *P<0.01, **P<0.05, ***P<0.001 as compared with the Aceclofenac treated animal.

Effects of Livina on serum total protein

Figure 2 shows serum total protein level of control and experimental animals. The results of total protein (mg dl^{-1}) in control rats was 7.66 ± 0.58, whereas in Aceclofenac treated rats this value was significantly (P<0.001) reduced 4.52 ± 0.65. Livina pretreatment at the dose of 0.5 ml/day significantly (P<0.001) prevented the Aceclofenac induced reduced total protein 6.67 ± 0.64 being compared to Aceclofenac treated group. With higher dose of Livina (1.0 ml/day) further elevation of total protein 6.99 ± 0.37 was observed. Silymarin (25 mg kg^{-1}, i.p.) pretreatment also prevented the Aceclofenac induced total protein reduction 6.81 ± 0.75 as compared with Aceclofenac alone treated group.

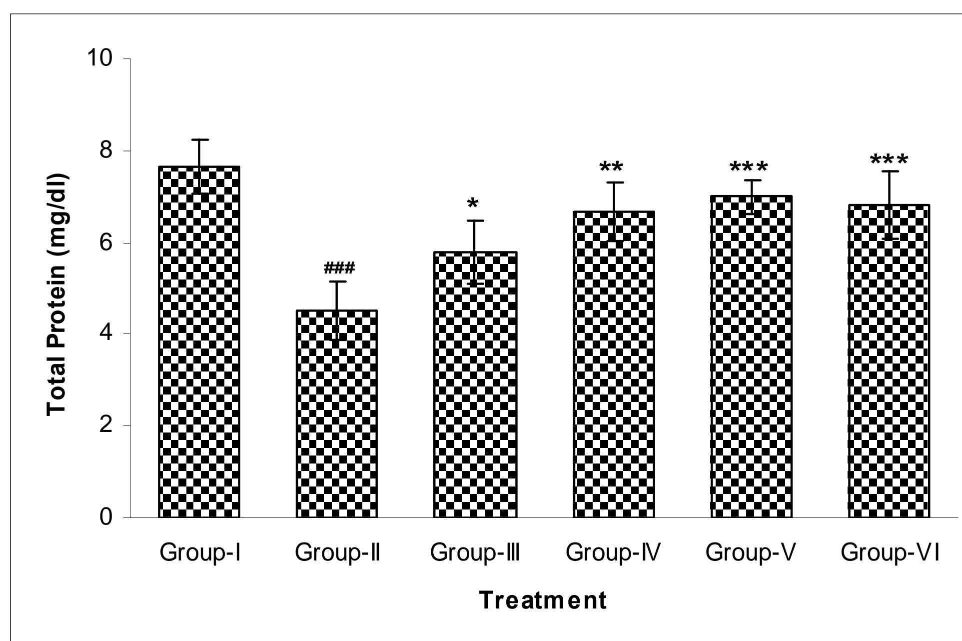

Fig.2: Effect of Livina on total protein in Aceclofenac induced hepatotoxicity in rats. ###P<0.001 significantly different from control group. *P<0.01, **P<0.05, ***P<0.001 significantly different from Aceclofenac treated animals.

Effects of Livina on liver lipid peroxidation

Figure 3 shows liver lipid peroxidation (MDA content) level of control and experimental animals. The result of lipid peroxidation (MDA content) in liver in control rats was 1.72 ± 0.21, whereas in Aceclofenac treated Rats these levels was elevated to 7.86 ± 0.32. Livina

pretreatment at the dose of 0.5 ml/day significantly (P<0.001) prevented the Aceclofenac induced rise in the lipid peroxidation 5.44 ± 0.28 being compared to Aceclofenac treated group. With higher dose of Livina (1.0 ml/day) further reduction of lipid peroxidation 3.21 ± 0.18 was noted. Silymarin (25 mg kg^{-1}, i.p.) pretreatment also prevented the Aceclofenac induced rise in 3.08 ± 0.16 as compared with Aceclofenac alone treated group.

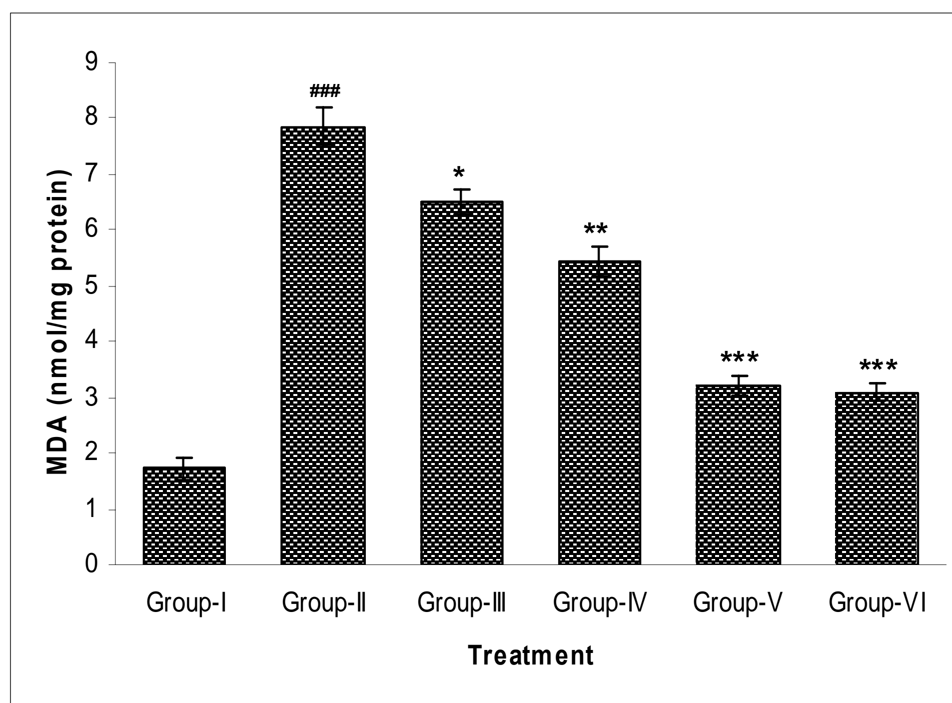

Fig.3: Effect of Livina on lipid peroxidation (MDA Content) in Aceclofenac induced hepatotoxicity in rats. $^{###}$P<0.001 significantly different from control group. *P<0.01, **P<0.05, ***P<0.001 significantly different from Aceclofenac treated animals.

Effects of Livina on δ-Aminolevulinate dehydratase activity (δ-ALA-D)

Figure 4 shows liver δ-Aminolevulinate dehydratase activity (δ-ALA-D) activity of control and experimental animals. The result of δ-Aminolevulinate dehydratase activity (δ-ALA-D) in liver in control rats was 25.41 ± 1.56, whereas in Aceclofenac treated rats these levels was significantly reduced to 12.64 ± 1.42. Livina pretreatment at the dose of 0.5 ml/day significantly (P<0.001) prevented the Aceclofenac induced decreased in the δ-ALA-D 17.65 ± 1.43 being compared to Aceclofenac treated group. With higher dose of Livina (1.0 ml/day) further

elevation of δ-ALA-D 22.86 ± 1.61 was noted. Silymarin (25 mg kg^{-1}, i.p.) pretreatment also prevented the Aceclofenac induced reduced in 21.52 ± 1.77 as compared with Aceclofenac alone treated group.

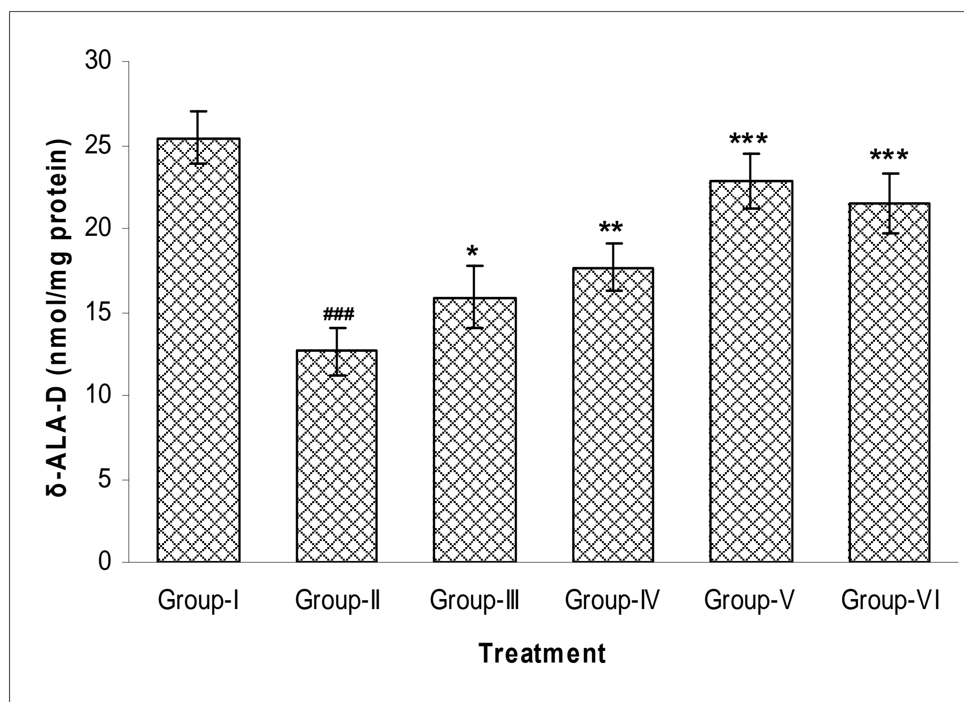

Fig.4: Effect of Livina on δ-ALA-D in Aceclofenac induced hepatotoxicity in rats. $^{###}P<0.001$ significantly different from control group. $^{*}P<0.01$, $^{**}P<0.05$, $^{***}P<0.001$ significantly different from Aceclofenac treated animals.

Effects of Livina on histopathological changes

Figure 5 shows the photomicrographs of control, Aceclofenac and Livina treated liver section. Histological observation of liver tissue of the normal animal showed hepatic cells with well-preserved cytoplasm, nucleus, nucleolus and central vein. In Aceclofenac treated group, histological observation showed fatty degeneration, damage of parenchymal cells, steatosis and hydropic degeneration of liver tissue (Table 4). Prominent damage of central lobular region appeared in the liver. Livina restored the histopathological abnormality induced by Aceclofenac.

Table 4. Histopathological changes in Aceclofenac induced liver injury in rats

Microscopic observation	Control	Aceclofenac treated	Livina (0.25 ml)	Livina (0.5 ml)	Livina (1.0 ml)	Silymarin
Nuclear disintegration	-	+++	-	-	-	-
Chromatolysis	-	++	-	-	-	-
Cytoplasmic vacuolation	-	++	+	+	-	-
Necrobiosis	-	+	-	-	-	-
Necrosis	-	+++	-	-	-	-
Kuppfer cell hyparplasia	-	+++	++	+	+	-
Portal inflammation	-	-	-	-	-	-
Sinusoidal dialation	-	++	-	-	-	-
Central venous dialation	-	+	+	-	-	-
Increased cytoplasmic eosinophilia	-	+++	+	+	-	+

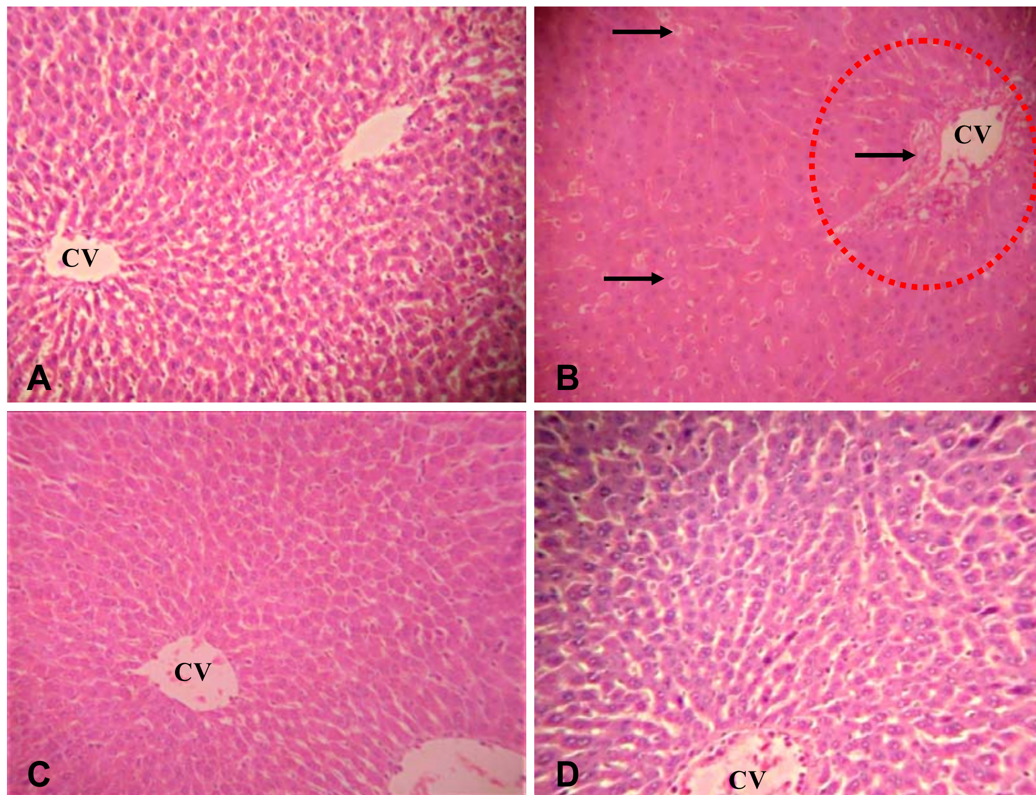

Fig.5: Photomicrographs of rats liver obtained from different treatment groups. A: Control; B: Aceclofenac (60 mg kg^{-1}) treated; C: Livina treatment (0.5 ml/day); C: Livina treatment (1 ml/day). CV= Central vein;

Discussion

Hepatotoxicity from NSAIDs can occur at any time after drug administration, but like most adverse drug reactions, it commonly occurs within 6-12 weeks of initiation of therapy. There are two main clinical patterns of hepatotoxicity due to NSAIDs. The first is an acute hepatitis with jaundice, fever, nausea, greatly elevated transaminases and sometimes eosinophilia. The alternative pattern is with serological (ANF-positive) and histological (periportal inflammation with plasma and lymphocyte infiltration and fibrosis extending into the lobule) features of chronic active hepatitis [33-36].

Two main mechanisms are responsible for liver injury: hypersensitivity and metabolic aberration. Recently, a number of in vitro animal models have been used to investigate the possible mechanisms of NSAID-related hepatotoxicity. Studies using rat liver mitochondria and

freshly isolated rat hepatocytes showed that diphenylamine, which is common in the structure of NSAIDs, uncouples oxidative phosphorylation, decreases hepatic ATP content and induced hepatocyte injury [37,38]. Aceclofenac was reported to be most commonly associated with hepatotoxicity. In recent study shows that administration of Aceclofenac at a dose of 60 mg kg^{-1} produce severe liver damage in experimental animals [39].

The elevated levels of serum enzymes are indicative of cellular leakages and loss of functional integrity of cell membrane in liver. It is established that serum enzymes such as ALT and AST levels were elevated in NSAID-induced hepatotoxicity [1]. Serum ALP and bilirubin levels on the other hand are related to the function of hepatic cells [41]. Increase in serum level of these hepatic markers signifies structural and functional catastrophe of the hepatic systems, in our current study we are focusing on the magnitude of recovery of the Aceclofenac injured hepatic unit by Livina.

Serum aminotransferase activities have long been considered as sensitive indicators of hepatic injury [42]. Injury to the hepatocytes alters their transport function and membrane permeability, leading to leakage of enzymes from the cells [43]. Hepatocytic necrosis or abnormal membrane permeability, these enzymes are released from the cells and their levels in the blood increases. ALT is a sensitive indicator of acute liver damage and elevation of this enzyme in non-hepatic diseases is unusual. ALT is more selectively a liver paranchymal enzyme than AST [44]. Therefore, the marked release of AST, ALT and GGT into the circulation indicates severe damage to hepatic tissue membranes during Aceclofenac intoxication. In the present study administration of Aceclofenac caused a dramatic elevation in serum AST, ALT and GGT activities, indicating subchronic hepatotoxicity induced by administration of Aceclofenac. Pretreatment with Livina, a polyherbal formulation efficiently prevented the Aceclofenac - induced elevation of serum AST, ALT and GGT activities in a dose-dependent manner, indicating the hepatoprotective activity of Livina against the intoxication of Aceclofenac. The results were compared with the standard hepatoprotective drug Silymarin where Livina showed almost similar results.

Bilirubins, which are enzymes originally present at higher concentration in cytoplasm. When there is heaptopathy, bilirubin comes into the blood stream in conformity with the extent of liver damage [45]. Moreover, it is well known that narcotizing agents produce sufficient injury to

hepatic parenchyma to cause elevation in bilirubin content in plasma [46]. However, bilirubin is one of the most useful clinical clues to the severity of necrosis and its accumulation is a measure of binding, conjugation and excretory capacity of hypatocyte. These effects induced by Aceclofenac, were confirmed by our results. Livina at the dose of 0.5ml/day and 1ml/day orally for twenty-eight days significantly restored the altered ALP and total bilirubin levels, which is quantitively comparable with the efficacy shown by Silymarin and also directly indicated the gross effectiveness of the herbal formulation on functional status of the liver.

Lipid peroxidation has been postulated to be the destructive process of liver damage due to Aceclofenac intoxication [47]. Lipid peroxide levels were significantly increased in Aceclofenac -intoxicated rats. The increase in MDA (in terms of TBARS) suggests enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defense mechanisms to prevent the formation of excessive free radicals [48,49]. Aceclofenac intoxicated animals treated with 0.5ml/day and 1ml/day Livina orally for 28 days, had significantly reduced lipid peroxide levels in compared with rats treated with Aceclofenac only. Aceclofenac intoxification significantly lowered total protein levels and 0.5ml/day and 1ml/day Livina supplementation significantly increased protein levels more or less up to the normal benchmark. It is known that δ-ALA-D activity is susceptible to a variety of treatment that are associated with oxidative stress [50,51]. Recent research established that paracetamol treatment inhibits δ-ALA-D activity [52]. In this study we observed a decrease in hepatic δ-ALA-D activity in Aceclofenac-treated rats but pretreatment with Livina restored it.

Histopathology of the liver samples revealed that the necrosis was reduced to few inflammatory cells in the rats treated with Livina. Cytoplasmic vacuolations and hydropic changes were less prominent. Inflammation of portal veins was also reduced. Thus the histopathological study shows reduction of degree of necrosis in the Rats treated with Livina as compared with Aceclofenac treatment.

Conclusion

In conclusion, the results of this study demonstrate that Livina, a polyherbal formulation has a potent hepatoprotective action on Aceclofenac induced hepatic damage in Rats. In order to confirm their antioxidant potential and to identify various enzymes involved in generating oxygen free radicals further studies are essential.

Acknowledgement

The authors are thankful to Prof. (Dr.) T.K.Pal, Department of Pharmaceutical Technology, Jadavpur University, Kolkata-700032 for his valuable suggestions and Mr. Gautam Dey, M.D., Mr. Ranajit Dey, Jt. M.D., Mr. Subharthee Dey, Whole time Director, and Mr. S.K. Dasgupta, Advisor, H.R., Mr. Ambarish Mukherjee, General Manager Works for facilities and encouragement during this investigation. They are also thankful to Mr. Swapan Kumar Mitra, Manager, MIS, Mr. S.P. Basuchoudhury, Manager Purchase and Mr. Utpal Dutta, Assistant manager for their co-operation and ungrudging help in carrying out this study.

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