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Role of Ashwagandha (*Withaniasomnifera*) root extract in attenuating hepaticdeficits in Diclofenac sodium-induced Swiss albino mice(*Mus musculus*)

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ABSTRACT
The established hepatotoxicity resulting from diclofenac sodium administration in humans and animals necessitates the exploration of potential therapeutic interventions. *Withania somnifera*, known for its hepatoprotective properties, was investigated for its restorative effects on diclofenac sodium-induced hepatotoxicity in Swiss albino mice. Following diclofenac sodium induction, significant elevation was observed in Aspartate transaminase (AST), 2.3 times; Alanine aminotransferase (ALT), 2.6 times; Bilirubin 18.9 times and alkaline phosphatase (ALP) 2.27 times. Conversely, serum globulin and albumin levels decreased significantly 7.47 times and 13.18 times, respectively. Upon treatment with *W. somnifera*, a substantial hepatoprotective activity was also evident, with notable reductions in AST, 2.06times; ALT, 1.86times; bilirubin, 3.61times and ALP, 1.78 times. Additionally, a significant increase in serum globulin, 10.36 times and albumin, 11.37 times was observed post-treatment. *W. somnifera* demonstrated a significant restoration of hepatic function in diclofenac-induced hepatotoxicity. The reparative effect of *W. somnifera* is attributed to its intrinsic antioxidant and free radical scavenging properties. Looking ahead, biologically active compounds, specifically withanolides present in W. somnifera, hold promise as novel therapeutic molecules for addressing hepatic disorders. Further exploration of these compounds may contribute to the development of innovative treatments for diclofenac-induced hepatotoxicity and related hepatic complications. Similar hepatoprotective role of Silymarin was also observed.

KEYWORDS: Diclofenac sodium, Hepato protective, Hepato-toxicity, Silymarin, Withania somnifera, Withanolides

I. INTRODUCTION

Diclofenac Sodium is a non-steroidal anti-inflammatory drug that was developed in the late 1970s and has been clinically approved to treat a range of rheumatic conditions;it also serves as an analgesic and an anti-inflammatory agent [5]. Like other non-steroidal anti-inflammatory medicines, diclofenac has also been associated with a modest but considerable incidence of hepatotoxicity. Hepatotoxicity can vary from jaundice and many fatal cases of hepatitis [19, 24].The liver controls a number of crucial metabolic processes. These metabolic function distortions are related to hepatic damage [21]. Herbs are crucial in the treatment of a variety of liver disorders [12] because allopathic medicine lacks effective liver protecting mechanism.*Withania somnifera*(Family Solanaceae) commonly known as Ashwagandha is an herb that has Hepatoprotectiveproperties [12]. Ayurveda traditional medicine claims that the root of *W. somnifera* has strong aphrodisiac[26], sedative [7] and energy-boosting tonic effects [27]. Frequent studies have revealed that *W. somnifera*has anabolic, anti-serotonergicand anticancer properties [10]. Additionally, it is helpful in the management of stress, geriatric issues, arthritis, and male sexual dysfunctions [6]. Additionally, it contains anticoagulant [18], adaptogenic, cardiotropicand cardio protectiveeffects [9].It has been demonstrated that *W. somnifera* inhibits Lipid peroxidase (LP) in mice exposed to stress [22].It is well known asadaptogenic [9], anti-inflammatory [17],antioxidant [4], anti-platelet, anti-hypertensive, hypoglycemic and hypolipidemic. [22].*W. somnifera* may also play a role in its cardio protectiveand Hepato protective properties [9].

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II. MATERIALS AND METHODS

Preparation of Extract

W.somnifera roots were collected and washed in running tap water and were allowed to dry in the shade. The roots were finely grinded in mortar and pestle and sieved twice. After that, 250ml of distilled water and 50 gm of dry powder were boiled for 30 minutes. For 24 hours, this solution was allowed to stand. The solution was filtered and evaporated the following day to obtain dry extract. This dried extract was further pulverized and then kept at 25°C. To attain a concentration of 1% as needed, the extract was suspended in water [25].

Test drug preparation

Diclofenac Sodium tablets was crushed to fine powder and mixed in distilled water at a concentration of 1 mg/ml. The LD_{50} of Diclofenac Sodium in Mice was reported as 95 mg/kg B.W [3].

Positive Control treatment

Silymarin (Milk thistle) was used as positive control, which was dissolved in distilled water before administration [16].

Experimental Protocol

Animals were divided into five groups having 5 mice in each group.

Group I: Untreated; Group II -Induced diclofenac sodium at a dose of 9.5 mg/kg B.Wfor 15 days;Group III -Administered 300mg/kg of *W. somnifera*to diclofenac sodium treated mice for 10 days;Group IV -Administered 600 mg/kg of W. somnifera to diclofenac sodium treated mice for 10 days; Group V -This group was administeredSilymarin (1mg/kg/day) orally for 10 days, to diclofenac sodium treated mice.

In order to assure appropriate absorption Diclofenac sodium and *W. somnifera* was given via gavage to animals that had been fasting for two to three hours before and one hour after receiving test medications. At the end of experiments animals from all groups were fasted for 12 hours before sacrificing (only water was provided). After that, animals were sacrificed while being given intraperitoneally injection with ketamine (75 mg/kg) for anesthesia. The blood was drawn from retro-orbital plexus to perform liver function tests viz. total Bilirubin, Alanine transaminase (ALT), Aspartate transaminase (AST) alkaline phosphatase (ALP), Globulin and Albumin.

III. STATISTICAL ANALYSIS

The data obtained from the control and treated groups of mice were expressed in Mean± SE and further analyzed by ANOVA and t-test by using SPSS software.

IV. RESULTS AND DISCUSSION

Clinical Manifestation

In the course of the research study, mice within the control group consistently exhibited heightened levels of energy and a robust appetite across the experimental duration. While the administration of diclofenac sodium did not result in fatality, salivation, or seizures among the experimental mice. Howeverother notable symptoms were observed viz. reduced feed intake, hind limb jerking, labored breathing, and a decline in body weight (Table-1).

Table 1: Effect of drug Diclofenac sodium on the clinical manifestation of *Mus musculus*

Clinical signs	Group I	Group II	Group III	Group IV	Group V
Death	×	×	×	×	×
Decrease feed intake	×	✓	✓	×	×
Hind limb jerk	×	×	×	×	×
Laboured breathing	×	✓	×	×	×
Salivation	×	×	×	×	×
Seizures	×	×	×	×	×

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Table 2: Effect of *Withania somnifera* on the liver function parameters of *Mus musculus* with Diclofenac Sodium induced hepatic dysfunction

Parameters	Group I	Group II	Group III	Group IV	Group V
AST (IU/L)	30.03 ± 0.008	69.17 ± 1.81**	54.82 ± 0.49**	33.53 ± 0.38**	34.31 ± 0.44
ALT (IU/L)	25.00 ± 2.09	65.26 ± 0.99**	54.81 ± 0.51**	35.05 ± 0.53**	34.37 ± 0.48
Bilirubin (mg/dL)	0.13 ± 0.008	2.46 ± 0.07**	0.93 ± 0.20**	0.68 ± 0.04**	0.54 ± 0.02*
Globulin (g/dL)	2.84 ± 0.19	0.38 ± 0.05**	1.77 ± 0.01**	3.94 ± 0.08**	4.00 ± 0.04
Albumin (g/dL)	4.22 ± 0.02	0.32 ± 0.05**	2.05 ± 0.01**	3.64 ± 0.14**	3.78 ± 0.08
ALP (IU/L)	46.4 ± 0.67	105.4 ± 3.32**	84.2 ± 0.37**	59 ± 1.51**	49.4 ± 1.56*

****- highly significant by t-test ($p < 0.001$) *- not significant by t-test between Group IV and Group V ($p < 0.01$)**

Figure 1: Effect of Withania somnifera on Diclofenac sodium induced changes in the liver parameters of Swiss albino mice (*Mus musculus*)

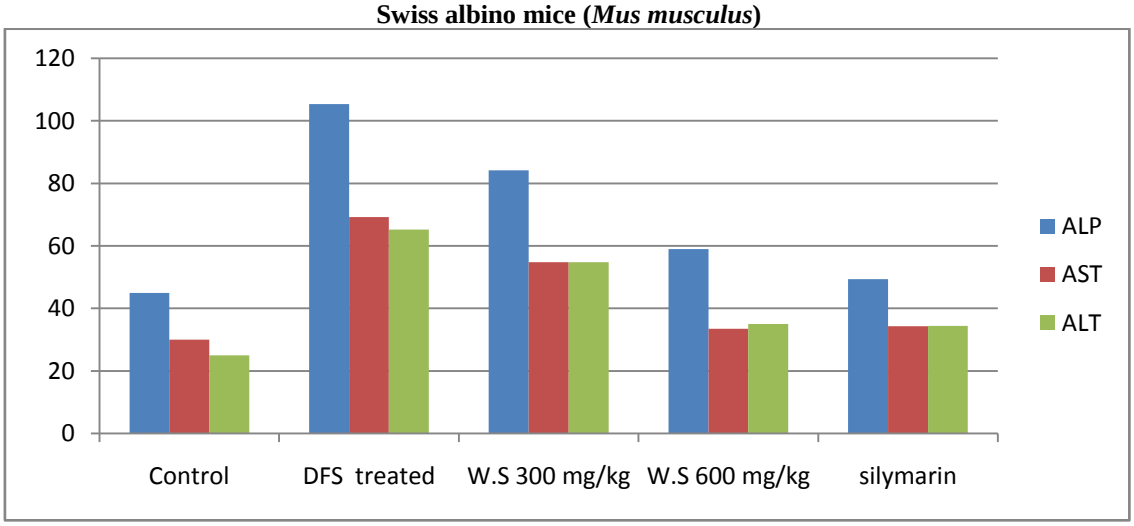

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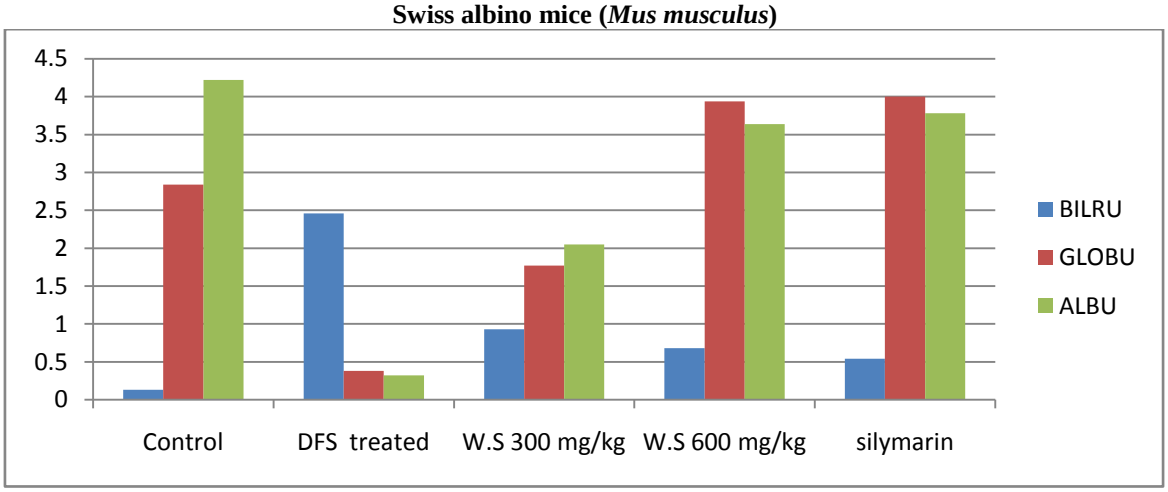
Figure 2: Effect of *Withania somnifera* on Diclofenac sodium induced changes in the liver parameters of Swiss albino mice (*Mus musculus*)



In our investigation, Group II, treated exclusively with Diclofenac sodium, exhibited a substantial increase in biochemical parameters (AST, 2.3times ALT, 2.6timesALP, 2.27timesand Bilirubin, 18.9 times) as compared to the control group. However, few parameters depreciated significantly (Serum Globulin, 7.47times; Serum Albumin, 13.18 times) relative to the control (Table-2). These alterations were statistically significant ($p < 0.001$) and indicative of pronounced hepatic damage, consistent with previous findings [8, 14]. The observed rise in serum transaminase (AST and ALT) levels signifies hepatocellular damage, corroborated by structural and morphometric changes within hepatocytes, as reported [1]. Elevation in AST and ALT levels is a characteristic manifestation of liver disorders, with a marked increase observed in conditions such as jaundice and hepatitis [29].The elevation in Serum Bilirubin and ALP levels observed in our study is indicative of liver impairment, suggesting cholestatic alterations in the liver biliary ducts, possibly attributable to the toxic nature of diclofenac sodium. The increase in ALP levels is consistent with cholestasis or biliary obstruction, commonly employed as a marker of liver health [29]. Similar findings were also reported by several workers in Swiss albino mice [1, 11]. The substantial rise in serum Bilirubin in the treated groups may be attributed to liver impairments resulting from diclofenac sodium (DFS) toxicity. Serum Bilirubin serves as a crucial marker for hepatic excretory function, with its elevation indicative of liver damage [13].The elevation in bilirubin levels observed in this study may be associated with an excessive breakdown of erythrocytes, indicative of hemolytic anemia, and potentially induced by the toxic effects of diclofenac sodiumas reported [20]. The marked decrease in both Serum Albumin and Globulin levels suggests potential liver impairment. Serum Albumin, being the predominant protein synthesized by hepatocytes, serves as a quantitative marker reflecting the functioning liver cell mass.[23] Hepatocellular injury caused by diclofenac sodium may disrupt protein synthesis pathways, leading to diminished production and secretion of albumin into the bloodstream[28].Further investigation was done with the diclofenac sodium treated mice by administrating W. somnifera at a dose of 600 mg/kg body weight. The treated group exhibited a protective effect on hepatocellular integrity, as evidenced by a substantial reduction inparameters (AST, 2.06timesALT, 1.86 times). Additionally, there was a marked decline in ALP (1.78 times), indicating potential alleviation of cholestatic stress. The considerable decrease in bilirubin (3.61times) further supported the hepatoprotective role of W. somnifera.Furthermore, *W. somnifera* administration demonstrated a notable improvement in serum albumin (11.37 times) and globulin (10.36 times). The increase in albumin levels suggested enhanced hepatic protein synthesis, while the rise in globulin levels indicated a positive modulation of immune and inflammatory responses. The decrease in AST, ALT, Bilirubin, ALP and increase in Albumin and Globulin are highly significant statistically ($p < 0.001$, Table-2). The Hepato-protection of W. somnifera was very likely to Silymarin (Standard) which depicts Hepato protective activity by ameliorating the altered biochemical parameters (AST, 2.01times;ALT, 1.89times;ALP,

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2.13times;bilirubin, 4.55times;globulin, 10.52times and albumin, 11.81times)..In conclusion, W. somnifera, particularly at a dose of 600 mg/kg, exhibited a significant remedial effect against diclofenac sodium-induced hepatotoxicity in Mus musculus. The marked decline in AST, ALT, Bilirubin, ALP and increase in Albumin and Globulin by treating W. somnifera and Silymarin are not much significant statistically (Table-2).

Effect of *Withania somnifera* on Histo-architecture of Liver in diclofenac sodium challenged *Mus musculus*

Histological experiments conducted to correlate the damaging effect of diclofenac sodium as well as reparative effect of *W. somnifera* at two consecutive doses for the same day length in relative to a control group and a standard group used as reference. The control group showed well organized hepatocytes with well scattered bile ducts, central vein without any congestion and presence of few mononuclear inflammatory cells (Plate-1). Diclofenac treated group showed swelling of hepatocytes, constricted central vein and slightly accumulation of bile duct (Plate-2). Inter-hepatic space was also found to increase as reported [2]. Large number of mononuclear inflammatory cells was also found. Massive disorganization of hepatic cells showing inter hepatic cellular space, aggregation of bile ducts, conjunction in central vein and large number of mononuclear inflammatory cells were noticed (Plate -2). In *W. somnifera*(at conc. of 300 mg/kg B.W) treated test group mice, architecture of hepatic cell was recovered as randomly dispersed bile ducts, negligible appear of conjunction of central vein, less intercellular space and a smaller number of inflammatory mononuclear cells were observed[15] (Plate-3). The test group pretreated with drug and later exposed to herbal extract showed a remarkable recovery especially at higher dose concentration of *W. somnifera* (600 mg/kg B.W) was approximately very similar to the positive control Silymarin acting as an Hepato-protectant (Plate-4)[29].

Figure 1: Histological changes exhibited by the administration of drug Diclofenac sodium and it's remedial by *W. somnifera* in relative to control (Plate 1-4).

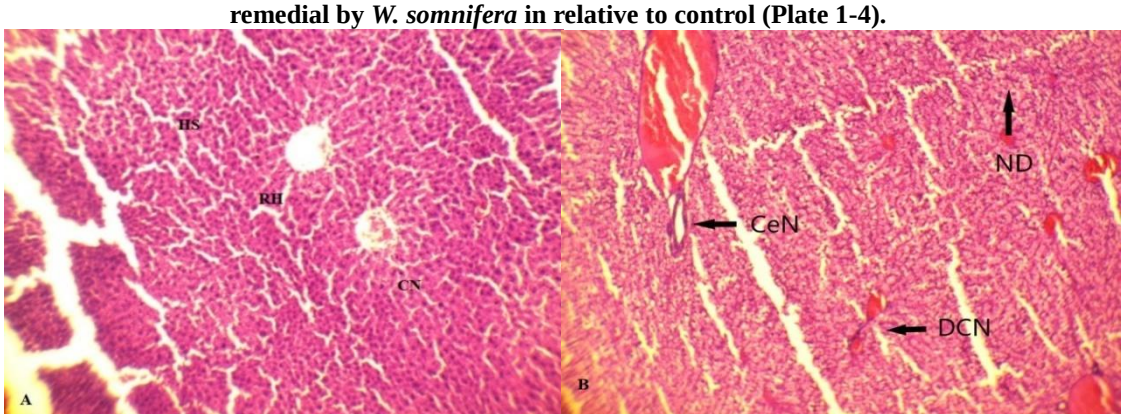

Plate 1 **Plate 2**

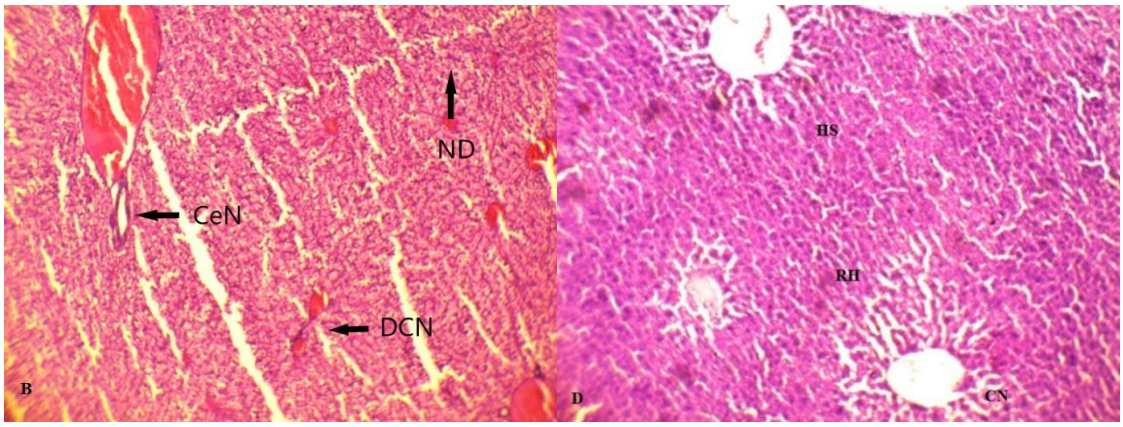

Plate 3 **Plate 4**

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V. CONCLUSION

In conclusion, our study investigated the impact of diclofenac sodium-induced toxicity in Swiss albino mice and explored the reparative potential of *W.somnifera*. The administration of diclofenac sodium at 9.5 mg/kg body weight resulted in significant hepatotoxic effects, as evidenced by elevated levels of hepatic markers such as AST, ALT, ALP, bilirubin, and disrupted levels of serum albumin and globulin. These alterations indicated substantial hepatic impairment and underscored the toxic nature of diclofenac sodium. *W.somnifera*, administered at two consecutive doses of 300 mg/kg and 600 mg/kg for 10 days, exhibited a considerable reparative effect against diclofenac sodium-induced toxicity. The higher dose, 600 mg/kg, emerged as particularly effective, comparable to the standard Silymarin at 1 mg/kg. This finding highlights the potential of W. somnifera as a healing adjunct in mitigating the adverse effects of diclofenac sodium on liver function. The remedial action of W.somnifera was evident in the normalization of biochemical parameters, including AST, ALT, ALP, and bilirubin levels, reflecting the restoration of hepatic integrity. Furthermore, W. somnifera administration led to a notable improvement in serum albumin and globulin levels, suggesting enhanced hepatic protein synthesis and positive modulation of immune and inflammatory responses. Overall, the findings from this study support the hepatoprotective properties of *W. somnifera*against diclofenac sodium-induced toxicity in Swiss albino mice. Further molecular and histopathological investigations are recommended to elucidate the underlying mechanisms of *W.somnifera's* reparative effects, paving the way for potential therapeutic interventions in diclofenac sodium-induced liver injury.

Conflict of interest

We declare that there is no conflict of interest with any other authors regarding the content of this paper.

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