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EVALUATION OF EFFICACY OF PUNARNAVA (BOERHAVIA DIFFUSA) IN DICLOFENAC INDUCED HEPATOTOXICITY IN WISTAR RATS

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Abstract

This research paper explores the potential hepatoprotective properties of Punarnava (Boerhavia Diffusa), a plant scientifically classified in the Ekasara Gana of Vishaghna Dravya by Acharya Sushruta, known for exhibiting rasayana gunakarma [2] in accordance with Ayurvedic principles. Traditionally, the roots of Boerhaavia diffusa L., commonly referred to as 'Punarnava,' have been employed by various tribes in India for treating diverse hepatic disorders. The study focuses on evaluating Punarnava's medicinal efficacy, particularly its in vivo antioxidant activity and hepatoprotective effects [8]. Experimental hepatic damage was induced in Wistar rats using diclofenac (DF) [1,4,5]. Significantly, pretreatment with Punarnava root powder demonstrated a noteworthy reduction in the elevated body weight, serum bilirubin levels, and hematological factors induced by diclofenac (DF) [1,4,5]. These findings suggest that Punarnava acts as a safeguard for liver cells against DF-induced damage, primarily through its antioxidant effects on hepatocytes.

Keywords- Anti-stress activity, Diclofenac NSAIDs Drugs, Dushivisha, Garvisha, Liver Toxicity, Punarnava Hepatoprotective, Ykrut Raktavaha Strotas

1. Introduction

NSAID drugs are commonly used to treat a variety of medical conditions. However, they can also cause adverse side effects, including nephrotoxicity (kidney damage) and hepatotoxicity

(liver damage). Diclofenac [1,4,5], a type of NSAID, is known to have a higher risk of hepatotoxicity compared to other NSAIDs. This is because diclofenac is an oxidizing substance that can damage various organs, including the liver and kidneys. The liver and kidneys are essential organs that play a crucial role in metabolism, detoxification, and the elimination of metabolic waste products. As a result, they are particularly vulnerable to drug-induced harm.

Drug-induced liver injury is a prevalent complication, particularly among NSAIDs, due to their widespread use in various treatments. Diclofenac, an NSAID, stands out for its high incidence and severity of liver toxicity. This drug is extensively used worldwide, both with and without prescriptions. Diclofenac has been shown to cause clinically significant liver damage based on established criteria for hepatotoxicity assessment. Its liver toxicity rate surpasses that of other NSAIDs. Diclofenac's therapeutic applications span various conditions, including antipyretic use and treatment of musculoskeletal disorders. However, diclofenac administration disrupts the body's antioxidant system, leading to oxidative stress generation. This oxidative stress can trigger liver dysfunction, acute liver impairment, and even death.

The liver plays a vital role in the body's functioning, encompassing storage, metabolism, and detoxification processes. It is crucial for regulating red blood cell production, glucose synthesis, and bilirubin levels. Bilirubin, prothrombin time (PT), and albumin tests are commonly used to assess liver function and identify potential damage. In both Ayurveda and modern medicine, the liver is considered an essential organ. Ayurveda refers to the liver as "yakrut," a critical component of the "raktvaha strotas," the blood circulatory system. Damage to the raktvaha strotas can manifest as cyanosis, fever, anemia, hemorrhage, and red discoloration of the eyes. Diclofenac, with its oxidative stress-inducing properties, can negatively impact the raktavaha strotas. The liver's role in regulating blood elements (raktadhatu) means that liver disorders can disrupt blood composition, leading to symptoms such as abdominal pain, weakness, dark urine, nausea, and loss of appetite. In healthy individuals, the liver's crucial role as the root of the raktvaha strotas (blood circulatory system) may not be apparent. However, in cases of infective jaundice and elevated bilirubin levels, the liver's function becomes evident. Ayurveda classifies drug-induced hepatotoxicity as Garavisha and Dushivisha. Garavisha refers to a slow-acting, artificial poison, while Dushivisha involves a combination of two poisons. The signs and symptoms of Garavisha [13,14] mirror those of drug- induced hepatotoxicity, making it a fitting analogy for

diclofenac. The toxins present in dialog accumulate in the body, gradually affecting the liver and leading to hepatotoxicity. Excessive or prolonged use of diclofenac can also trigger liver toxicity. Dushivisha [11-12], on the other hand, represents the cumulative effects of partially detoxified diclofenac. This incomplete detoxification process disrupts the raktadhatu (blood elements), contributing to hepatotoxicity. Punarnava (Boerhavia diffusa) [8,9,10] emerges as a promising hepatoprotective agent due to its antioxidant properties. Its ability to combat oxidative stress makes it valuable in treating hepatic, renal, and blood-related disorders. Punarnava offers a multitude of benefits and pharmacological actions. Punarnava possess Madhur, Tikta, Kashay, Ushna virya, Laghu ruksha guna.

2. Aim

Punarnava (Boerhavia Diffusa) hepatoprotective activity against diclofenac induced hepatotoxicity in WISTAR RATS.

3. Methodology

Name	Punarnava (Boerhavia diffusa)
Physical Appearance	Light Brown Powder
Storage conditions	Room Temperature
Handling Precautions	Standard Laboratory Precautions

3.1. Material:

Diclofenac Injection 10 mg/ Kg bw

Species	Wistar Rats
Sex	Male and Female
Age	6 weeks
Initial Body Weight	200 ± 20 gm
Source	Authentic Research Center

Environmental Conditions	Room temperature maintained between 22+3ºC; Relative humidity 50±5 and illumination cycle set to 12 hours light and 12 hours dark.
Accommodation	Three Rats per cage housed in polypropylene cages with stainless steel grill top, facilities for food and water bottle, and bedding of Corn Cob.
Diet	Standard Pelleted feed (Maintenance Diet)
Water	Normal Water

1.1. Vivo Study:

The study was performed as per previously reported protocol (Alabi & Akomolafe, 2020). Wistar rats were divided randomly into different groups as shown in following table [3].

Sr. No.	Group Name (n=6)	Treatment specifications
1.	Vehicle Control	-
2.	Disease Control	Diclofenac sodium 10 mg/kg bw
3.	Test	Diclofenac sodium 10 mg/kg bw + Punarnava (514 mg/kg bw)

Hepatotoxicity was induced in animals by intramuscular administration of Diclofenac 10 mg/kg bw per day for 7 days. On 7th day blood parameters such as serum bilirubin levels. Depending upon the levels of serum bilirubin level were randomized into above groups and treatment initiated. Treatment with Test Item continued for next 14 days. Blood withdrawn on day 7 & 14 and at the end of experiment to evaluate serum bilirubin.

1.1. Body Weight

Body weights of all animals were recorded weekly throughout the experiment.

1.2. Food Consumption

Food Consumption was recorded weekly throughout the experiment as food consumed in 24 hours.

1.3. Hematology

Whole blood was collected at the end of study for hematology. Hematology was performed by semi-automatic hematology analyzer (Triscan Vet Hematology Analyzer).

1.4. Serum Biochemical Parameters

Serum Bilirubin levels were analyzed using commercially available kits (Pathozyme) and as per kit manufacturers protocol using Biochemistry Analyzer ChemXpress.

1.5. Data Analysis and Report Preparation

Statistical tool was used for the data analysis. GraphPad Prism Ver 8.42 was used for the statistical analysis. Results were expressed as Mean±SD. The data were analyzed by one- way or two-way analysis of variance (ANOVA) followed by Tukey's multiple comparisons test. Statistical significance was considered at #P<0.05, ##P<0.01, ###P<0.001 as compared to vehicle control group & *P<0.05, **P<0.01, ***P<0.001 as compared to disease control group in all cases.

Above data suggests that there is no significant decrease in level of body weight in diclofenac control group as compare to vehicle control group. Showing that they was no remarkable effect over body weight even after disease induction. And even level of body weight in Punarnava were not showing any significant improvement as compared to diclofenac control group which indicates they was no positive improvement in body weight after administration of treatment. Since observation p value is greater than 0.05 hence we conclude that there is no significant difference in 3 groups.

Table 3: Food Consumption: Male (gm)

Group	Day 0	Day 7	Day 14	Day 21	Day 28
Vehicle Control	11.67	10.00	12.67	11.67	10.33
Disease Control	10.00	9.33	11.33	10.33	11.67
Punarnava	10.33	11.00	12.00	11.67	10.67

Table 4: Food Consumption: Female (gm)

Group	Day 0	Day 7	Day 14	Day 21	Day 28
Vehicle Control	10.33	11.67	12.00	11.60	9.67
Disease Control	10.67	10.00	9.33	12.00	12.67
Punarnava	9.67	10.33	10.00	10.33	10.67

Above data suggests that there is no significant decrease in level of food consumption in diclofenac control group as compare to vehicle control group. Showing that they was no remarkable effect over food consumption even after disease induction. And even level of food consumption in Punarnava were not showing any significant improvement as compared to diclofenac control group which indicates they was no positive improvement in food consumption after administration of treatment. Since observation p value is greater than 0.05 hence we conclude that there is no significant difference in 3 groups.

Table 5: Serum Biochemistry: Bilirubin (mg/dl) (Male)

Group	Animal ID	Day 0	Day 7	Day 14	Day 21
Vehicle Control	1	0.36	0.37	0.35	0.39
	2	0.31	0.41	0.39	0.38
	3	0.39	0.44	0.42	0.44
	Mean	0.35	0.41	0.39	0.40
	SD	0.04	0.04	0.04	0.03
Disease Control	1	0.36	0.85	0.81	0.71
	2	0.37	0.86	0.82	0.81
	3	0.36	0.83	0.79	0.77
	Mean	0.36	0.85	0.81	0.76
	SD	0.01	0.02	0.02	0.05
Punarnava	1	0.37	0.83	0.60	0.64
	2	0.36	0.75	0.54	0.47
	3	0.38	0.82	0.59	0.51
	Mean	0.37	0.80	0.58	0.54
	SD	0.01	0.04	0.03	0.09

Figure 3: Bilirubin: Male

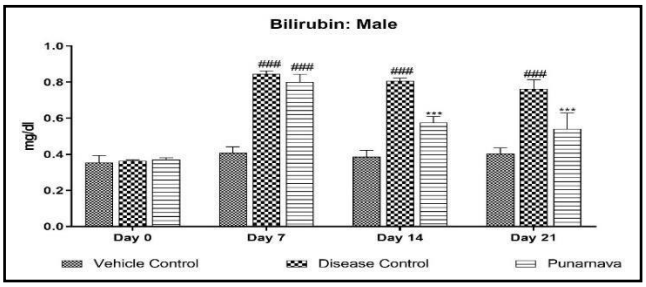
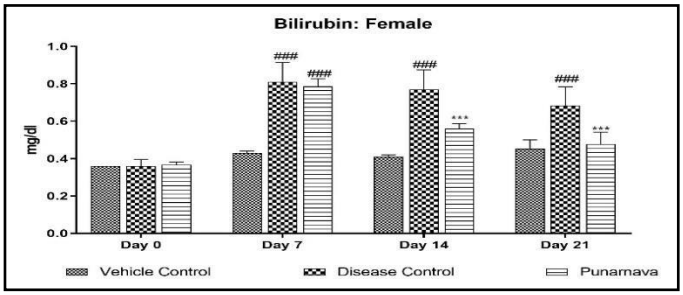


Table 6: Serum Biochemistry: Bilirubin (mg/dl) (Female)

Group	Animal ID	Day 0	Day 7	Day 14	Day 21
Vehicle Control	4	0.36	0.42	0.40	0.40
	5	0.36	0.44	0.42	0.48
	6	0.36	0.43	0.41	0.48
	Mean	0.36	0.43	0.41	0.45
	SD	0.00	0.01	0.01	0.05
Disease Control	4	0.34	0.76	0.72	0.63
	5	0.34	0.74	0.70	0.62
	6	0.40	0.93	0.89	0.80
	Mean	0.36	0.81	0.77	0.68
	SD	0.03	0.10	0.10	0.10
Punarnava	4	0.37	0.75	0.54	0.45
	5	0.35	0.78	0.55	0.55
	6	0.38	0.83	0.59	0.43
	Mean	0.37	0.79	0.56	0.48
	SD	0.02	0.04	0.03	0.06

Figure 4 Bilirubin: Female



Compare to normal control group, there were significant $p < 0.001$ increase in bilirubin level activities in diclofenac control group from day 7 of study. Relative to diclofenac control group, significant decrease in bilirubin level activities $p<0.001$ were documented in Punarnava group from 14th day.

Table 7 Haematology: Male

Group	Animal ID	WBC (10^3/µl)	LYM (10^3/µl)	NEUT (10^3/µl)	RBC (10^6/µl)	HGB (g/dl)	HCT (%)	PLT (10^3/µl)	PCT (%)
Vehicle Control	1	3.70	2.70	0.80	6.17	13.50	31.90	366.00	0.31
	2	6.80	4.50	2.00	7.02	15.20	37.00	284.00	0.23
	3	3.00	1.90	1.00	6.07	13.80	32.40	216.00	0.18
	Mean	4.50	3.03	1.27	6.42	14.17	33.77	288.67	0.24
	SD	2.02	1.33	0.64	0.52	0.91	2.81	75.11	0.07
Disease Control	1	3.50	1.90	1.40	5.63	13.10	30.40	214.00	0.16
	2	6.10	3.30	2.30	5.84	12.60	30.00	297.00	0.24
	3	3.10	2.00	1.00	5.89	13.20	31.30	249.00	0.20
	Mean	4.23	2.40	1.57	5.79	12.97	30.57	253.33	0.20
	SD	1.63	0.78	0.67	0.14	0.32	0.67	41.67	0.04
Punarnava	1	3.70	2.50	1.00	5.63	12.80	30.60	258.00	0.19
	2	4.10	2.30	1.50	5.63	12.30	29.20	214.00	0.16
	3	6.20	3.40	2.40	5.99	12.90	30.90	306.00	0.24
	Mean	4.67	2.73	1.63	5.75	12.67	30.23	259.33	0.20
	SD	1.34	0.59	0.71	0.21	0.32	0.91	46.01	0.04

Table 8 Haematology: Female

Group	Animal ID	WBC (10^3/µl)	LYM (10^3/µl)	NEUT (10^3/µl)	RBC (10^6/µl)	HGB (g/dl)	HCT (%)	PLT (10^3/µl)	PCT (%)
Vehicle Control	4	5.40	1.50	2.00	5.82	12.70	30.50	280.00	0.21
	5	3.60	1.30	1.30	5.94	12.70	30.00	243.00	0.19

	6	5.50	1.90	1.30	6.29	14.00	32.90	276.00	0.23
	Mean	4.83	1.57	1.53	6.02	13.13	31.13	266.33	0.21
	SD	1.07	0.31	0.40	0.24	0.75	1.55	20.31	0.02
Disease Control	4	4.00	1.80	1.90	4.72	9.90	24.40	338.00	0.27
	5	4.90	2.00	2.60	4.85	11.90	27.30	232.00	0.19
	6	2.90	1.60	1.20	6.38	13.90	33.50	201.00	0.19
	Mean	3.93	1.80	1.90	5.32	11.90	28.40	257.00	0.22
	SD	1.00	0.20	0.70	0.92	2.00	4.65	71.84	0.05
Punarnava	4	3.10	1.60	1.30	5.94	13.30	32.00	325.00	0.26
	5	2.10	1.30	0.70	5.45	12.80	29.50	98.00	0.08
	6	2.90	1.50	1.20	6.81	14.00	35.00	267.00	0.23
	Mean	2.70	1.47	1.07	6.07	13.37	32.17	230.00	0.19
	SD	0.53	0.15	0.32	0.69	0.60	2.75	117.94	0.10

Above data suggests that there is no significant decrease in level of lymphocytes, neutrophil, HCT, PCT, platelets count, RBC and WBC of diclofenac control group as compare to vehicle control group. Showing that they was no remarkable effect over lymphocytes, neutrophil, HCT, PCT, platelets count, RBC and WBC even after disease induction. And even level of lymphocytes, neutrophil, HCT, PCT, platelets count, RBC and WBC in Punarnava were not showing any significant improvement as compared to diclofenac control group which indicates they was no positive improvement in lymphocytes, neutrophil, HCT, PCT, platelets count, RBC and WBC after administration of treatment. Since observation p value is greater than 0.05 hence we conclude that there is no significant difference in 3 groups. There is significant decrease in level of hemoglobin in diclofenac control group as compare to vehicle control group and relative to diclofenac control group significant increase in hemoglobin level in Punarnava group in female wistar rats.

3. Discussion

This study aimed to assess the preventive effects of Punarnava (Boerhavia Diffusa) against diclofenac-induced liver toxicity in Wistar Rats, focusing on elucidating its mechanisms of

action through the evaluation of oxidative stress, the antioxidant defense system, and inflammation.

The administration of diclofenac (DF) in this study resulted in an imbalance in the antioxidant system, a pro-inflammatory response, and a substantial increase in serum bilirubin levels. diclofenac (DF) administration was associated with heightened oxidative stress due to the down- regulation of the antioxidant system. Inflammation and oxidative stress were closely linked events. Oral administration of Punarnava to Wistar rats injected with diclofenac led to a marked decrease in the elevated serum total bilirubin levels, indicating an improvement in liver function and integrity. These improvements in biochemical parameters related to liver function.

Punarnava is anti-stress and antioxidant and contains flavonoids chemical constituents, anti-filamentary (shothaghni) opposite to diclofenac drug. Hence however further clinical investigation are required to access the efficacy and safety of Punarnava in Human beings.

4. Conclusion

In summary, the study concludes that prolonged administration of diclofenac resulted in liver toxicity. The use of Punarnava (Boerhavia Diffusa) exhibited significant potential in mitigating diclofenac-induced liver injury and toxicity. This protective effect was attributed to the enhancement of the antioxidant defense system, anti-inflammatory properties, and the suppression of oxidative stress. Nevertheless, additional clinical investigations are imperative to assess the efficacy and safety of Punarnava (Boerhavia Diffusa) in human subjects.

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