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Evaluation of hepatoprotective activity of polyherbal formulation PN01 against paracetamol and isoniazid induced liver damage

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ABSTRACT

The main objective was to scientifically evaluate the possible hepatoprotective activity of polyherbal formulation PN01. In paracetamol induced model wistar albino rats were divided into three groups. Group I animals were fed orally with 1.0 ml/kg/day of normal saline for seven days. Group II and III animals were treated with herbal formulation (200 mg/kg p. o) and Silymarin (50 mg/kg/p. o) respectively for 7 days. On 7th day Paracetamol suspension was given orally at a dose of 750 mg/kg p. o). In Isoniazid induced hepatotoxicity model, animals were divided into four groups (n=6). Group I animals were fed with standard diet. In Group II, animals were treated with INH (54mg/kg p. o). Group III animals were treated with herbal formulation at the dose of (200mg/kg p. o.) and simultaneously received INH (54mg/kg p. o.) once daily for a period of 30 days. Group IV animals were treated with standard drug Silymarin (50mg/kg p. o.) and received INH (54mg/kg p. o.) 1h after administration of standard drug once daily for a period of 30 days. In paracetamol induced hepatotoxicity, silymarin and test treated group showed a significant ($p<0.01$) decrease in cholesterol level as compared to Isoniazid treated group. Triglyceride level was significantly ($p<0.001$) increased in Isoniazid treated group as compared to control group. In Isoniazid induced hepatotoxicity triglyceride level was significantly ($p<0.001$) decreased in silymarin and test treated groups as compared to Isoniazid treated group.
The results suggest that polyherbal formulation may have the potential therapeutic value in the treatment of isoniazid and paracetamol induced hepatic damage and some liver diseases.

INTRODUCTION

The most crucial role of liver is the detoxification of poisonous substances. Common cause of liver disease is inflammation, which often results from abuse of alcohol and malnutrition [1] and over dose of acetaminophen and Isoniazid [2]. Several other factors like microorganisms and chemicals may trigger hepatotoxicity.

The INH induced hepatotoxicity is initiated by CYP-450-mediated metabolism to toxic metabolites such as acetyl hydrazine and hydrazine. Hydrazine reacts with sulfhydryl group, which result in glutathione depletion within the hepatocyte leading to cell death [3]. Acetaminophen is metabolized together with cytochrome P450 As a result, N-actyl-p-benzoquinone imine (NAPQI) or N-acetyl-p-benzosemiquinone imine (NAPSQI) appears in the body`s system [4].Currently plant based medicines are gaining importance in the treatment of liver diseases like hepatitis, cirrhosis, and loss of appetite.

MATERIALS AND METHODS

Animals

Wistar albino rats were procured from the animal house facility of Smt. Kashibai Navale College of Pharmacy, Kondhwa, Pune. Animals were kept at 22±2 ^{0} C with a 12 h day light/ dark cycle under humid tropical conditions. All procedures involving use of laboratory animals were as per the CPCSEA guidelines. Study was approved by IAEC.

Poly herbal formulation

Polyherbal formulation PN01 containig Punarnava ghana, Katuki ghana, Guduchi ghana, Daruharidra ghana, Triphala ghana, Haridra ghana, Tamalaki ghana, Kalmegh ghana, Sharpunkha ghana, Kakamachi ghana, Bhringraj ghana, Yavakshsr choorna, Navayas loha, Tamra bhasma and Saptarangi ghana was selected for the study.

Acute oral toxicity

Acute toxicity study of PNO1 was carried out according to OECD 423 guidelines [5]

Experimental models

Paracetamol induced hepatotoxicity:

A total of 18 animals were selected. Group I animals were fed orally with 1.0 ml/kg/day of normal saline for seven days. Group II and III animals were treated with herbal formulation (200 mg/kg p. o) *and* Silymarin (50 mg/kg/p. o) respectively for 7 days. On 7th day Paracetamol suspension was given orally at a dose of 750 mg/kg p. o). At the end of the treatment period, blood samples were collected by direct cardiac puncture and the serum was used for the assay of marker enzymes *viz.*, aspartate aminotranferase (AST), alanine aminotranferase (ALT), alkaline phosphatase (ALP) and total proteins [6] etc.

INH induced Hepatotoxicity :

Animals were divided into four groups (n=6). Group I animals were fed with standard diet and were administered saline solution once daily for period of 30 days. In Group II, animals were treated with INH (54mg/kg p. o) once daily for period of 30 days. Group III animals were treated with herbal formulation at the dose of (200mg/kg p. o.) and simultaneously received INH (54mg/kg p. o.) once daily for a period of 30 days. Group IV animals were treated with standard drug Silymarin (50mg/kg p. o.) and received INH (54mg/kg p. o.) 1h after administration of standard drug once daily for a period of 30 days. At the end of the experiment, rats were sacrificed and blood was collected for biochemical estimations.

RESULTS

In paracetamol treated group, significant ($p<0.05$) increase in cholesterol level was observed as compared to control group. Silymarin and test treated groups showed significant ($p<0.01$) decrease in cholesterol level as compared to paracetamol. Silymarin and test treated groups showed significant ($p<0.01$) decrease in triglyceride level. HDL level was found to be significantly ($p<0.01$) decreased in paracetamol treated group as compared to control group. No significant increase in HDL level was observed in silymarin and test treated groups. No significant difference in the LDL level was observed in paracetamol, silymarin and test treated groups. VLDL level was found to be significantly ($p<0.05$) increased in paracetamol treated group as compared to control group as shown in Table 1. Bilirubin and protein level was found to be significantly ($p<0.05$) increased in paracetamol treated group as compared to control group as shown in Table 2.

Significant increase in alkaline phosphatase level was

Table 1 : Effect of polyherbal formulations on lipid profile in paracetamol treated animals

Treatment	Cholesterol (mg/dl)	Triglyceride (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
Control	88.75 ± 8.86	43.50 ± 1.75	40.75 ± 2.72	39.30± 7.47	8.10± 0.53
Paracetamol	124.3 ± 6.22*	86.00 ± 8.19***	25.00 ± 2.91**	45.27± 1.24	19.70 ± 4.11*
Paracetamol + Silymarin	76.75 ± 1.70$^{\#\#\#}$	52.75 ± 5.48$^{\#\#\#}$	34.50 ± 1.04	61.75± 9.16	11.00 ± 1.34
Paracetamol +Test	85.50 ± 8.06$^{\#\#}$	56.75 ± 2.83$^{\#\#}$	27.25 ± 3.63	78.35± 17.16	12.15 ± 1.14

Values are expressed as mean+ SEM* ($p<0.05$), ** ($p<0.01$) and ***($p<0.001$) indicates comparison with control group and # ($p<0.05$), ## ($p<0.01$) and # # # ($p<0.001$) indicates comparison with paracetamol group.

Table 2 : Effect of polyherbal formulation on bilirubin and total protein in paracetamol treated animals.

Treatment	Bilirubin (mg/dl)	Total protein (mg/dl)
Control	0.19 ± 0.01	5.12 ± 0.44
Paracetamol	0.52 ± 0.05*	6.60 ± 0.23*
Paracetamol + Silymarin	0.48 ± 0.02	5.55 ± 0.13
Paracetamol +Test	0.70 ± 0.13	5.52 ± 0.04

Values are expressed as mean+ SEM. * ($p<0.05$) indicates comparison with control group and #indicates comparison with paracetamol group.

Table 3 : Effect of polyherbal formulations on liver enzymes in paracetamol treated animals

Treatment	ALP (IU/L)	SGOT (IU/L)	SGPT (IU/L)
Control	126.3 ± 35.85	78.75 ± 6.79	77.75 ± 6.95
Paracetamol	195 ±14.48	330.5 ± 50.32***	140.8 ± 13.99**
Paracetamol + Silymarin	319.3 ±17.05	188.8 ± 27.41#	110.0 ± 5.81
Paracetamol +Test	458.3 ± 84.06##	221.0 ± 14.60	85.00 ± 7.99##

Values are expressed as mean+ SEM. * ($p<0.05$) indicates comparison with control group and #indicates comparison with paracetamol group.

Table 4 : Effect of polyherbal formulations in lipid profile in isoniazid treated animals

Treatment	Cholesterol (mg/dl)	Triglyceride (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
Control	88.75 ± 8.86	43.50 ± .75	34.50 ± 1.04	45.27 ± 1.24	8.10 ± 0.53
Isoniazid	122.0 + 10.42*	116.9 + 3.61***	27.75 + 3.11	46.15 + 9.62	28.70 + 4.11***
Isoniazid + Silymarin	73.75 ± 2.01##	49.75 ± 6.88###	24.00 ± 2.91	37.55 ± 5.01	9.95 ± 1.37###
Isoniazid + Test	78.50 ± 6.64##	48.25 ± 1.25###	33.75 ± 2.95	35.10 ± 3.57	15.25 ± 1.99##

Values are expressed as mean+ SEM. * ($p<0.05$), ** ($p<0.01$) and ***($p<0.001$) indicates comparison with control group and # ($p<0.05$), ## ($p<0.01$) and # # # ($p<0.001$) indicates comparison with isoniazid group.

Table 5 : Effect of polyherbal formulation on bilirubin and total protein in isoniazid treated animals

Treatment	Bilirubin (mg/dl)	Protein (mg/dl)
Control	0.19 ± 0.01	5.12 ± 0.44
Isoniazid	0.49 + 0.04	7.70 +1.03*
Isoniazid + Silymarin	0.44 ± 0.02	7.00 ± 0.17
Isoniazid + Test	0.55 ± 0.04	7.00 ±0.22

Values are expressed as mean+ SEM. * indicates ($p<0.05$) comparison with control group.

observed as compared to paracetamol treated group. Significant ($p<0.001$) increase in SGOT level was observed in paracetamol treated group as compared to control group. SGOT level was significantly ($p<0.05$) decreased in silymarin treated group as compared to paracetamol treated group. Significant ($p<0.01$) increase in SGPT level in paracetamol treated group as compared to control group. SGPT level was significantly ($p<0.01$) decreased in test treated group as compared to paracetamol treated group as shown in Table 3.

Cholesterol level was significantly ($p<0.05$) increased in Isoniazid treated group as compared to control treated group. Silymarin and test treated group showed significant ($p<0.01$) decrease in cholesterol level as compared to Isoniazid treated group. Triglyceride level was significantly ($p<0.001$) increased in Isoniazid treated group as compared to control group. Triglyceride level was significantly ($p<0.001$) decreased in silymarin and test treated group as compared to Isoniazid treated group. No significant increase in level of HDL and LDL was observed in silymarin and test treated group. VLDL level was significantly ($p<0.001$) increased in Isoniazid treated group as compared to control group. VLDL level was significantly ($p<0.01$) ($p<0.01$) increased in silymarin and test treated groups respectively as compared to Isoniazid treated group presented in Table 4. Protein level was significantly ($p<0.05$) increased in Isoniazid treated groups as compared to control group presented in Table 5 . No significant increase in the level of alkaline phosphatase in Isoniazid, silymarin and test treated groups was observed. SGOT level was significantly ($p<0.01$) increased in Isoniazid treated group as compared to control group. Silymarin and test treated group showed significant ($p<0.05$) decrease in SGOT level as compared to Isoniazid treated group. No significant increase in SGPT level was observed in Isoniazid, silymarin and test treated groups as shown in Table 6.

DISCUSSION

Many of the polyherbal formulations are available in the

Table 6 : Effect of polyherbal formulations on liver enzymes in isoniazid treated animals

Treatment	ALP (IU/L)	SGOT (IU/L)	SGPT (IU/L)
Control	195.0 ± 14.48	78.75 ± 6.79	77.75 ± 6.95
Isoniazid	184.0 ± 25.69	220.3 ± 44.86**	133.5 ± 10.41
Isoniazid+ Silymarin	168.8 ± 25.74	101.8 ± 3.35#	65.50 ± 7.93
Isoniazid + Test	133.3 ± 19.37	105.0 ±17.33#	82.25 ± 11.12

Values are expressed as mean+ SEM. ** ($p<0.01$) indicates comparison with control group and # ($p<0.05$) indicates comparison with isoniazid group.

market and are widely prescribed for the liver disorders but no scientific quantitative and qualitative data is available recommending the use of these herbal formulations. Polyherbal formulations with active chemical constituents for intended effect may undergo some undesired changes due the chemical or physical chemical interactions over the period of time and can lose the therapeutic activity. Taking into the considerations the above possibility an attempt was made to scientifically evaluate the hepatoprotective activity of marketed polyherbal formulation PN01.

The study reveals that PN01 decreases the elevated total cholesterol and triglycerides level. No significant decrease was observed on levels of VLDL and LDL. HDL level was also not found to be increased. Determination of liver enzymes showed decrease in ALP level in test group. SGOT level were found to be decreased in silymarin treated group. SGPT levels were decreased in test group. Histopathological findings showed only mild venous congestion in silymarin and test groups Isoniazid treatment elevated total cholesterol, triglycerides and VLDL level which were reversed by silymarin and test group. SGOT level was decreased in test group. These results indicate that the polyherbal formulation PN01prevents the liver damage. Presence of the polyherbs in PN01 with variety of chemical constituents have reduced the liver damage probably through anti-inflammatory, antioxidant, membrane stabilizing, immunomodulatory , antiallergic, antistress activity which are the essential qualities of any drug which prevents liver damage[7]. Decrease in the leaking of SGOT and SGPT is an indication of reduction in the generation of free radicals and reduction in cell necrosis as well as immune suppression and glutathione reduction activity. PN01 in both the models showed the hepatoprotective activity by controlling the level of cholesterol and liver enzymes.

CONCLUSION

The polyherbal formulation PN01 at the dose of 200mg/kg showed hepatoprotective activity against paracetamol and isoniazid induced liver damage. PN01 can be used to prevent liver damage induced by chemical or drug.

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