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Protective Effect of Polyherbal Formulation on Simvastatin Hepatotoxicity in Rats

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ABSTRACT

Objective: This study was designed to assess the effect of polyherbal formulation (PHF) on simvastatin hepatotoxicity in rats and to assess the possibility of co-administration of PHF along with hepatotoxic drugs. **Methods:** Hepatotoxicity in rats was induced by simvastatin (20 mg/kg p.o. for 30 days) and the protective effect of PHF (0.25 ml/kg/p.o. and 0.5 ml/kg/p.o. either along with drug or followed by inducing hepatotoxicity) was identified by estimating marker enzymes for liver function such as aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, and γ glutamic transpeptidase; oxidative stress markers such as lipid peroxidation, reduced glutathione, super oxide dismutase, and catalase; and protein profile such as total bilirubin, direct bilirubin, total albumin, and total protein. A histopathological study was also carried out to confirm hepatotoxicity. **Results and Discussions:** Simvastatin hepatotoxicity was characterized by a significant increase in oxidative stress along with marker enzymes of liver function and depletion of proteins. Administration of PHF either along with simvastatin or followed by inducing hepatotoxicity significantly improved the level of marker enzymes for liver function, oxidative stress, and protein profile. **Conclusion:** The study suggests a protective role of PHF on simvastatin hepatotoxicity and it can be utilized to treat hepatotoxicity with long-term clinically useful drugs.

Key words: Hepatotoxicity, liver function, oxidative stress, protein profile, polyherbal formulation, simvastatin

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INTRODUCTION

Drug-induced hepatotoxicity (DIH) accounts for 9.5% of all suspected adverse drug reactions and are the most common reason for withdrawal of a drug from the market. The liver is a central metabolizing organ so it more susceptible to metabolism-dependant injury. Thus, injury may be a direct toxic effect or immunological reaction to either the drug or an active metabolite formed by bioactivation.[1] It is reported that 62% of withdrawn drugs have toxic metabolites.[2] Although, with the exception of rare cases, DIH subsides after cessation of treatment with the drug, which represents an important diagnostic and therapeutic challenge for physicians.

Simvastatin (SMT) competitively inhibits HMG-CoA to mevalonate. Mevalonate is also a precursor of Coenzyme Q10 (CoQ10). Thus, treatment with statins could also lower its levels. CoQ10 acts as an antioxidant, has membrane stabilising effects, and is important for cellular mitochondrial respiration, which is essential for energy production in organs.[3,4] Thus, simvastatin causes oxidative

stress mediated hepatotoxicity by depleting antioxidant enzymes.[5]

Polyherbal formulation (PHF) contains the plant extracts of Himsra, Kasani, Kakamachi, Arjuna, Kashmarda, Birranjshipa, Jharuka and is processsed in 50 mg herbs each in the powder form of Bhringraja, Punarnava, Guduch, Daruharidra, Mulaka, Amalaki, Chitraka, Vidanga, Haritaki, and Parpata. Each individual herb has reported hepatoprotective, liver tonic, antioxidant, and anti-inflammatory activity.

In our previous study, *in-vivo* evaluation of simvastatin hepatotoxicity was carried out and we have reported that SMT causes oxidative stress mediated hepatotoxicity in rats.[5] The main objective of this study was to assess the hepatoprotective effect of PHF in simvastatin hepatotoxicity and to assess the possibility of co-administration along with such hepatotoxic drugs.

MATERIALS AND METHODS

Animals

The protocol described in this study was approved by the Institutional Animal Ethics Committee (IAEC) of M. S. University, Baroda and received permission from the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India. Healthy adult male Wistar rats weighing 150-200 g were used. Rats were housed in polypropylene cages, maintained under standardized condition (12 hr light/dark cycles, 24°C, 35 to 60% humidity) and provided free access to palleted CHAKKAN diet (Nav Maharashtra Oil Mills Pvt. Ltd., Pune) and purified drinking water ad libitum.

Experimental protocol

Animals were divided into seven different groups, each having 6 rats and treated accordingly. Group 1: rats received a normal standard diet for 24 days; Group 2: rats received PHF (0.25 ml/kg/p.o. alone for 30 days); Group 3: rats received SMT (20 mg/kg p.o. for 30 days) (5); Group 4: rats received SMT along with PHF (0.25 ml/kg/p.o. for 30 days); Group 5: rats received SMT along with PHF (0.5 ml/kg/p.o. for 30 days); Group 6: rats received SMT followed by PHF (0.25 ml/kg/p.o. for 1 week); Group 7: rats received SMT along with silymarin (20 mg/kg/p.o.).

Collection of serum

Blood samples were withdrawn from the retro-orbital plexus under light ether anesthesia without any anticoagulant and allowed to clot for 10 minutes at room temperature. The blood was centrifuged at 2500 rpm for 20 minutes. The serum obtained was kept at 4ºC until used. All the animals were euthanized after blood collection using the spinal dislocation method and the liver was removed for study of histopathology.

Estimation of liver function

Estimation of marker enzymes for liver functions such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) were done by using a kit provided by Span Diagnostic Ltd., India and γ glutamic transpeptidase (γGTP) was done using a kit from Dade Behring Ltd., UK. Estimation of lactate dehydrogenase (LDH) was done using a kit fom Enzopak-Reckon diagnostics. Protein profile such as total bilirubin, direct bilirubin, total albumin, and total protein were done using a kit from Span Diagnostic Ltd, India.

Estimation of oxidative stress markers

All the animals were euthanized after blood collection with the spinal dislocation method under light ether anesthesia and the liver was removed for study of oxidative stress markers and histopathological evaluation. The liver was kept in cold conditions (precooled in inverted petridish on ice). It was cross-chopped with a surgical scalpel into fine slices in chilled 0.25 M sucrose and quickly blotted on a filter paper. The tissue was minced and homogenized in 10 mM Tris-HCl buffer, pH 7.4 (10%w/v) with 25 strokes of tight teflon pestle of glass homogenizer at a speed of 2500 rpm. The clear supernatant was used for oxidative stress marker assays like lipid peroxidation,[6] reduced glutathione,[7] super oxide dismutase,[8] and catalase.[9]

Histopathological study

The liver was collected after the rats were sacrificed. After blotting free of blood and tissue fluids, the liver was kept in 5% formalin. A 5-15 μm thick section was serially cut on a leitz microtome in a horizontal plane and mounted on a glass slide with the help of egg albumin in glycerin solution (50% v/v). They were then stained with 10% hematoxyline for 3-5 minutes and placed in running water intensified the staining. The hematoxyline stained sections were stained with 10% eosin for 2 minutes. The sections were observed and desired areas were photographed using an Olympus photomicroscope. The sections were viewed under 40X magnification.

Statistical analysis

All the data are expressed as mean ± SEM. Data were analyzed by analysis of variance (ANOVA) followed by Bonferroni's multiple comparison tests using the computer based fitting program (Prism, Graphpad). Differences were considered to be statistically significant when $P < 0.05$.

RESULTS

The effect of PHF on marker enzymes of liver function [Table 1]

Serum levels of ALT and AST were significantly increased ($P<0.001$) after treatment with simvastatin compared with control. Correspondingly, there was a significant increase ($P<0.001$) in the ratio of ALT/AST after administration of simvastatin [Figure 1A]. Treatment with simvastatin showed a significant increase ($P<0.001$) in serum γGTP, ALP, and LDH compared with control. PHF (0.25 ml/kg) alone did not have any effect on the levels of ALT, AST, γGTP, ALP, and LDH but PHF (0.25 ml/kg and 0.5 ml/kg) when administrated along with or after treatment with simvastatin produced a significant decrease ($P<0.001$) in the levels of ALT, AST, γGTP, ALP, and LDH. Silymarin treatment also produced a significant decrease ($P<0.001$) in the levels of these enzymes but was less than PHF. Correspondingly, there was a significant decrease ($P<0.001$) in the ratio of ALT/AST after treatment with PHF and Silymarin [Figure 1A].

The effect of PHF on the protein profile in SMT treated rats [Table 2]

Simvastatin administration caused a significant increase ($P<0.001$) in serum total bilirubin and indirect bilirubin and a significant decrease ($P<0.01$) was found in direct bilirubin compared with control. Simvastatin produced a significant decrease ($P<0.001$) in serum total protein compared with control. There was no significant change observed in serum globulin and albumin after treatment with simvastatin. PHF (0.25 ml/kg and 0.5 ml/kg) along with simvastatin, PHF (0.25 ml/kg) followed by simvastatin, and silymarin showed a significant decrease ($P<0.001$) in total bilirubin and indirect bilirubin and a significant increase ($P<0.01$) was observed in direct bilirubin compared with simvastatin control. PHF (0.25 ml/kg and 0.5 ml/kg) along with simvastatin was found to cause a significant increase ($P<0.001$) in total protein compared with simvastatin control. PHF (0.25ml/kg) followed by simvastatin and silymarin treatment caused a significant increase ($P<0.01$) in total protein. PHF (0.25 ml/kg and 0.5 ml/kg) along with simvastatin, followed by simvastatin did not have any significant effect on the levels of globulin and albumin. Correspondingly, the changes were observed in the ratio of albumin/ globulin but that was not statistically significant [Figure 1B].

Table 1: The effect of PHF on marker enzymes of liver function in SMT hepatotoxicity

Groups	ALT (IU/L)	AST (IU/L)	γGTP (IU/L)	ALP (IU/L)	LDH (IU/L)
Control	35.17± 2.613	40.00± 1.414	40.83± 1.014	152.5± 1.478	345.3± 4.702
PHF (0.25ml)	32.50± 2.262	37.33± 1.476	39.00± 0.9661	151.0± 2.221	340.0± 3.347
SMT Alone	91.17± 2.786+++	72.50± 0.991+++	72.67±1.229+++	248.0± 2.098+++	508.3± 3.095+++
SMT + PHF (0.25ml)	39.50± 1.285***	48.00± 1.932**	47.50± 0.763***	158.3±1.606***	354.0±1.844***
SMT + PHF (0.5ml)	38.17± 2.786***	43.83± 1.869***	44.17± 3.016***	154.2± 3.016***	339.8± 4.658***
SMT followed by PHF (0.25ml)	45.17± 2.227***	44.17± 1.641***	45.83± 2.822***	159.8± 2.892***	369.3± 5.439***
SMT + SLM	44.17± 1.078***	53.33± 0.881*	51.00± 0.966***	165.2± 1.195***	366.3± 2.418***

Values are expressed as mean ± SEM of 6 animals in each group, analyzed by one way ANOVA followed by Bonfferoni's multiple comparison tests. ***, +++: $P<0.001$, ++,**: $P<0.01$, +,*: $P<0.05$, + compared with control, *compared with SMT control.

Table 2: The effect of PHF on the protein profile in SMT hepatotoxicity

Groups	Total Bilirubin (mg/dl)	Direct Bilirubin (mg/dl)	Indirect Bilirubin (mg/dl)	Total Protein (mg/dl)	Albumin (mg/dl)
Control	0.7033± 0.008	0.3017± 0.006	0.4050± 0.014	7.497± 0.116	4.995± 0.058
PHF (0.25ml)	0.6900± 0.023	0.3100± 0.009	0.3817± 0.024	7.330± 0.075	4.900± 0.096
SMT Alone	1.407± 0.046+++	0.5000± 0.083++	0.9067± 0.084+++	6.483± 0.135+++	4.660± 0.083
SMT+ PHF (0.25ml)	0.7233± 0.006***	0.3433± 0.010*	0.3800± 0.012***	7.087± 0.038**	4.900± 0.022
SMT+ PHF (0.5ml)	0.7183± 0.027***	0.3117± 0.020**	0.4067± 0.0084***	7.183± 0.13***	5.017± 0.14
SMT followed by PHF (0.25ml)	0.7417± 0.018***	0.3217± 0.013*	0.4200± 0.007***	7.000± 0.136*	4.817± 0.26
SMT +SLM	0.8217± 0.009***	0.3083± 0.009**	0.5133± 0.008***	6.835± 0.036*	4.578± 0.044

Values are expressed as mean ± SEM of 6 animals in each group, analyzed by one way ANOVA followed by Bonfferoni's multiple comparison tests. ***,+++: $P<0.001$, ++,**: $P<0.01$, +,*: $P<0.05$, + compared with control, * compared with SMT control.

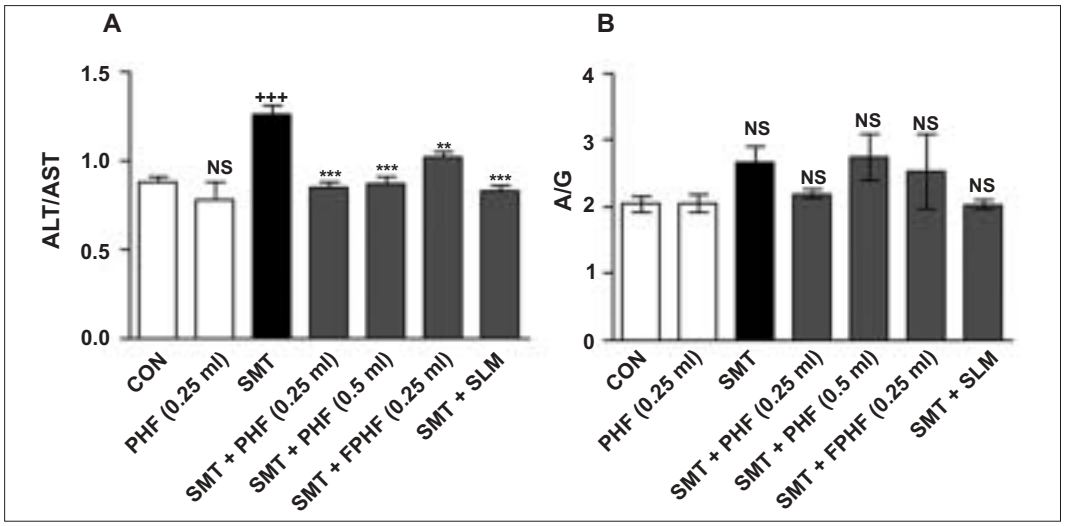

Figure 1: The effect of PHF on ALT/AST (A) and A/G (B) in SMT hepatotoxicity. Values are expressed as mean ± SEM of 6 animals in each group end analyzed by one way ANOVA followed by Bonfferoni's multiple comparison tests. ***,+++: *P*<0.001, **: *P*<0.01, NS: Not Significant, +: compared with control, *: compared with SMT control

The effect of PHF on oxidative stress markers in SMT treated rats [Table 3]

Simvastatin significantly increased (p<0.001) lipid peroxidation compared with control [Figure 1]. Tissue levels of GSH, SOD, and CAT were significantly decreased (p<0.001) after treatment with simvastatin compared with control. PHF (0.25 ml/kg and 0.5 ml/kg) along with simvastatin and PHF (0.25 ml/kg) followed by simvastatin was found to cause a significant decrease (p<0.001) in lipid peroxidation and a significant increase (p<0.001) in the levels of GSH, SOD, and CAT as compared with simvastatin control. Silymarin along with simvastatin produced an effect similar to PHF.

The effect of PHF on the histopathological changes in SMT hepatotoxicity

The liver section of the control rats revealed normal hepatic hexagonal lobules and normal morphology [Figure 2A]. Liver tissue of simvastatin-treated rats showed degeneration of hepatocytes [Figure 2C]. PHF and silymarin treatment improved the structural integrity of liver cells [Figures 2D-2G].

DISCUSSION

As the cells are damaged, ALT and AST are enzymes produced within the cells of the liver that leaks into the bloodstream leading to a rise in the serum levels. ALP is an enzyme that is associated with the biliary tract and is elevated; biliary tract damage and inflammation should be considered. It is often used to confirm that alkaline phosphatase is of hepatic etiology by γGTP. Mild to moderate elevation of ALT, AST, and ALP (1-3 times) are usually seen in drug toxicity.[10-12] This decrease in elevated serum ALT, AST, ALT/AST, γGTP, ALP, and LDH levels in PHF along with or followed by simvastatin-treated animals in part may be due to the protective effect of PHF on liver cells following the restoration of liver cell membrane permeability.[13]

This protective effect indicates a reduction in enzymes present in the extra cellular milieu of the liver cell. The protective effect of the component of PHF has also been observed in several experimental studies.[14,15] It can be stated that PHF contains *Tamarix gallica* and crude herbal extracts of *Capparis spinosa, Cichorium intybus, Solanum nigrum,*

Table 3: The effect of PHF on oxidative stress markers in SMT hepatotoxicity

Groups	MDA (Nmole/gm of tissue)	GSH (µmole/gm of tissue)	SOD (Unit/gm of tissue)	CAT (µmole H2O2 consumed/ min/gm of tissue)
Control	5.102± 0.094	106.0± 1.633	43.67± 1.282	295.0±1.592
PHF(0.25ml)	5.100± 0.093	104.0± 2.266	44.00± 1.673	308.7±16.87
SMT Alone	13.33±1.667+++	78.17±2.151+++	22.67±1.145+++	193.2±8.765+++
SMT + PHF (0.25ml)	5.700±0.23***	95.00±1.461***	42.000.816***	287.5±1.648***
SMT + PHF (0.5ml)	5.367±0.308***	95.17±2.892***	40.50±2.604***	279.8±2.892***
SMT followed by PHF (0.25ml)	5.750±0.270***	97.67±3.232***	31.17±1.662**	269.8±2.892***
SMT +SLM	6.350±0.178***	94.00±1.238***	38.83±0.60***	273.0±2.436***

Values are expressed as mean ± SEM of 6 animals in each group, analyzed by one way ANOVA followed by Bonfferoni's multiple comparison tests. ***,+++: p<0.001, **: p<0.01, + compared with control, *compared with SMT control.

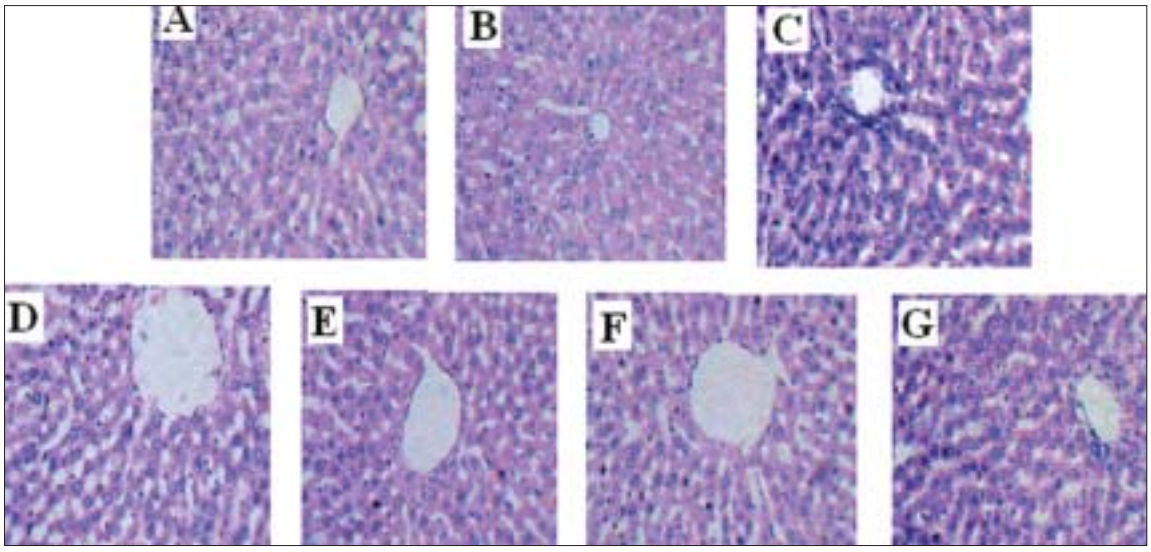

Figure 2: The effect PHF on the histopathological change in SMT hepatotoxicity. Hematoxylin and eosin-stained sections of rat liver (magnification X 100): (A) Control ; (B) PHF (0.25 ml/kg) treated; (C) SMT (20 mg/kg) treated; (D) SMT with PHF (0.25 ml/kg) treated; (E) SMT with PHF (0.5ml/kg) treated; (F) SMT followed by PHF (0.25 ml/kg) treated; (G) SMT with silymarin (20 mg/kg) treated

T. arjuna, and *A. millefolium*. These medicinal herbs alone or in combination can influence a restoration of the cellular functions and structural integrity of the liver.

PHF along with and followed by simvastatin significantly normalized total bilirubin, total protein, direct bilirubin, and albumin indicating liver curative effect. The curative and hepatoprotective effect of *Cassia occidentalis, Cichorium intybus,* and *Solanum nigrum* of PHF were observed against chemically induced liver damage in experimental animals.[16] The diuretic effect of *T. arjuna* and the anti-inflammatory and anti-immunotoxicity effects of *Cichorium intybus* have been shown in clinical and experimental studies.[17,18]

In our previous study, we reported that simvastatin caused oxidative stress mediated hepatotoxicity.[5] PHF along with and followed by simvastatin significantly reduced lipid peroxidation and increased antioxidant enzymes such as GSH, SOD, and CAT as compared with simvastatin-treated animals indicating modification of oxidative stress by PHF. The protection of liver cells against toxic materials including drugs, lipid peroxidation, and free radical injury may decrease inflammation.[19] Immune dysfunction is a component of liver disease and thus immunomodulation by herbal therapy prevents oxidative stress and inflammation and strengthens the detoxifying power of the liver cell.[20] The anti-oxidative property of esculetin and p-methoxybenzoic acid, the main constituents of *Cichorium intybus* and *Capparis spinosa*, respectively, have been reported in chemically-induced hepatotoxicity in experimental animals.[21,22] *Achillea millefolium*, another component of PHF, contains flavonoids and terpenoids with anti-oxidative and anti-inflammatory properties.[23,24] Furthermore, the anti-oxidative property of the flavonoid content of *Tamarix gallica* and the inhibitory effect *Solanum nigrum* crude extracts on free radical-mediated DNA damage increases the hepatoprotective effect of PHF.[25] In addition, the antioxidative, anti-lipoproxidative and the increase in glutathione content of the liver cells was observed with arjunolic acid and flavonoids present in *T. Arjuna.*[26]

Although there is insufficient information to establish the mechanism of action of PHF protection, this could be due to its anti-inflammatory, anti-oxidative immunomodulating as well as restorative effects.

CONCLUSION

The study suggests a protective role of PHF (Livpep) in drug-induced hepatotoxicity and this effect may be due to its anti-inflammatory, anti-oxidative as well as restorative effects. PHF can be utilized to treat hepatotoxicity with long-term clinically useful drugs, which are at risk of developing hepatotoxicity.

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Natural products have proven to be a great source of new biologically active compounds. Thus, in an effort to discover new lead anti-malarial compounds, several research group screen plant extracts to detect secondary metabolites with relevant biological activities that could served as templates for the development of new drugs. Flavonoids have been isolated and characterized from many medicinal plants used in malaria endemic areas.[10] However, controversial data have been obtained regarding their antiplasmodial activity, probably because of their structural diversity.[11-13] More recently, several flavonoids have been isolated from Artemisia afra[14,16] and Artemisia indica,[17-20] two plants related to Artemisia annua, the famous traditional Chinese medicinal plant from which artemisinin is isolated.

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