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Preventive effect of polyherbal mixture against rifampicin and pylorus ligation-Induced gastric ulcers and liver necrosis in rats.

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Abstract

Antitubercular drug is produced hepatotoxicity is major drawback of an effective treatment of treatment of tuberculosis. In this paper we review the incidence, pathology and clinical features of antituberculosis drug-induced hepatotoxicity, discuss the metabolism and mechanisms of toxicity of Rifampicin, and describe risk factors and management of antituberculosis drug induced hepatotoxicity, the antiulcer activity was assessed by determining and comparing the ulcer index in the test drug groups with that of the vehicle control and standard Omeprazole. In case of pylorus ligation induced ulcers, the polyherbal mixture showed significant reduction of ulcers in a dose dependent manner. The parameters taken to assess antiulcer activity were % inhibition ulcer index. The results indicated that polyherbal mixture significantly ($p<0.001$) decreased the % inhibition ulcer index as well as hepatoregenrative action.

Keywords:polyherbal mixture, liver toxicity, ulcer index, Antitubercular, hepatoregenrative

Introduction

Tuberculosis (TB) is one of the major causes of death from a curable infectious disease. About 9 million new TB cases occurred in 2004 and 1.7 million people died from TB that year. Sub-Saharan Africa has the highest incidence and mortality rates, mainly due to HIV/AIDS, whereas the South-East Asian region has the largest number of both new cases and deaths from TB. Recommended standard treatment for adult respiratory TB is a regimen of isoniazid, rifampicin, and pyrazinamide for 2 months, followed by 4 months of isoniazid and rifampicin. Ethambutol is usually added to this regimen and streptomycin is recommended by the World Health Organization (WHO) in retreatment cases in most developing countries. The most frequent adverse effects of antituberculosis treatment are hepatotoxicity, skin reactions, gastrointestinal and neurological disorders. Hepatotoxicity is the most serious one and is the focus of the present review. Antituberculosis drug-induced hepatotoxicity (ATDH) causes substantial morbidity and mortality and diminishes treatment effectiveness. Asymptomatic transaminase elevations are common during antituberculosis treatment, but hepatotoxicity can be fatal when not recognized early and when therapy is not interrupted in time [1].

There is a growing interest in the pharmacological evaluation of various plants used in Indian traditional systems of medicine. Polyherbal Mixture contains five drugs like Trichosanthes dioica, Pistacia khinjuk, Cheilanthes albomarginata, Cheilanthes *farinose* and *Clematis gouriana.* All these drugs are have pharmacological properties like lipid lowering, in hyperglycemia, asthma, gastro-intestinal disorders [2]. skin diseasesn [3]. anti-inflammatory and anti-nociceptive [4]. Anthelminitic [5]. antioxidant, antimicrobial [6]. The powdered fronds of *Cheilanthes farinosa*, mixed with butter are used for the treatment of eczema, scabies and various other skin disorders which might result in lesions and wounds [7]. The aerial parts from this genus are particularly used in Asia and Europe as a remedy to reduce pain and fever, as diuretic, in the treatment of rheumatic pain, eye infections, gonorrhoeal symptoms, bone illnesses, chronic skin disorders, gout and varicosity [7,8]. One of the species *C. gouriana* from the genus is found in India. It is not yet much evaluated for its phytochemical constitution and pharmacological activities. This species is found in the Western Ghats of the India. *Clematis gouriana* Roxb. is a climbing shrub, from the family Ranunculaceae which is commonly known as the "Indian traveller's joy". The fruits are tonic and stomachic. The roots of *C. gouriana* are the traditionally used in cardiovascular disease and the leaves of *C. gouriana* are recommended in folk medicine as the antipyretic with wound healing properties and in skin diseases. [9,10]. Anti-inflammatory, antipyretic, antibacterial, antiviral, in treatment diarrhoea and throat infection [11-14]. the plant has a root like stem which is a creeping rhizome and grows in a variety of situations particularly along water streams and near waterfalls. *Trichosanthes dioica* Roxb being very rich in protein and vitamin A, it has certain medicinal properties, and many reports are available regarding its role in lowering of blood sugar and serum triglycerides [15]. The fruits are easily digestible and diuretic in nature. They are also known to have antiulcer effects it grows as a vegetable all over India. [16]. It is prescribed to improve appetite and digestion [17]. The decoction of *Trichosanthes dioica* Roxb. is useful as a valuable alterative tonic, and as a febrifuge, which is given for boils and other skin diseases [18,19]. The juice of the leaf

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is applied to patches of alopecia areata [20]. The root is used as a hydragogue cathartic, tonic and febrifuge. [20]

Methods

Preparation of polyherbal mixture

The leaves, fruits and stems of *Trichosanthes dioica, Pistacia khinjuk, Cheilanthes albomarginata, Cheilanthes farinose* and *Clematis gouriana* were shade dried at room temperature. The dried aerial parts were then crushed in a mill and stored in a dry place until use. The polyherbal mixture was soxhleted by using methanol for 48hrs, at 55^{0}C temperature. The residue was removed by filtration. The solvent was then evaporated, under reduced pressure, on a rotary evaporator at 42-45^{0}C. The concentrated extract was dried on water bath and transferred to Petri dishes. The solid extract was scrapped before complete drying; and then dried to a constant weight.

Acute toxicity study and dose selection of drug was done by OECD Guidelines-423, 2004

1/10th of the maximum tolerable dose (2000 mg/kg) i.e. 100, 200 and 400mg/kg were taken for the studies.

Animal

All experimental procedures were carried out in strict accordance with the guidelines prescribed by the Committee for the polyherbal drug mixture of Control and Supervision on Experimentation on Animals (1012/c//06/CPCSEA) and were approved by the Institutional Animal Ethics Committee. Wistar rats weighing between 180 to 200 gm of either sex were used. Animals had free access to standard pellet diet and water *ad libitum.*

Rifampicin Induced Hepatotoxicity

Treatment schedule

Group I: Normal control Group-The animals in this group received distilled water (1ml/100gm, p.o.) as vehicle from day 1st to 2nd Day. The biochemical estimations were done on after 48 hr. after overnight fasting of animals[21].

Group II: Negative Control Group- The animals in this group received rifampicin (1gm/kg, p.o. in gum 5% acacia mucilage) at day 1st. The biochemical estimations were done on after 48 hr. after overnight fasting of animals.

Group III: Positive control Group- The animals in this group received rifampicin (1gm/kg, p.o. in gum 5% Acacia mucilage) at day 1st after the 30 min, Silymarin (100 mg/kg, p.o.) at day 1st. The biochemical estimations were done on after 48 hr. after overnight fasting of animals.

Group IV: Polyherbal mixture - The animals in this group received rifampicin (1gm/kg, p.o. in gum 5% acacia mucilage) at day 1st after the 30 min. in 4 divided doses of polyherbal mixture (100mg/kg, p.o.) 48 hr. The biochemical estimations were done on after 48 hr. after overnight fasting of animals.

Group V: Polyherbal mixture - The animals in this group received rifampicin (1gm/kg, p.o. in gum 5% acacia mucilage) at day 1st after the 30 min. in 4 divided doses of polyherbal mixture (200mg/kg, p.o.) 48 hr. The biochemical estimations were done on after 48 hr. after overnight fasting of animals.

Group VI: Polyherbal mixture - The animals in this group received rifampicin (1gm/kg, p.o. in gum 5% acacia mucilage) at day 1st. after the 30 min. in 4 divided doses of polyherbal mixture (400mg/kg, p.o.)

48 hr. The biochemical estimations were done on after 48 hr. after overnight fasting of animals.

Biochemical estimations

On the 10th day after 2hr. of $FeSO_4$ administration all the animals were anesthetized with anesthetic ether and blood was withdrawn by puncturing retro-orbital plexus by using fine glass capillary and collected in plain sterile centrifuge tubes and allowed to clot. Serum was separated by centrifugation at 10000 rpm for 15 min. at 5^{0}C. The separated serum was used for estimation of AST, ALT, ALP, and TB.

Pyloric ligation induced gastric ulcer [22]

Treatment schedule

Group I: Treated with of 4% v/v aqueous tween 80 (10 ml/kg p.o),

Group II: Treated with Polyherbal mixture 100mg/kg p.o) respectively for 14 days

Group III: Treated with Polyherbal mixture 200mg/kg p.o) respectively for 14 days.

Group IV: Treated with Polyherbal mixture 400mg/kg p.o) respectively for 14 days

Group V: Treated with Omeprazole (20 mg/kg p.o) were administered 30min prior to induction of gastric ulcer.

On the 14th day, all groups' rats were fasted 24 h prior to induction of gastric ulcer. Pyloric ligation was done by ligating the pyloric end of the stomach of rats 1 h after drug administration. (Karimulla et *al*) Animals were allowed to recover and stabilized in individual cage and were deprived of water during post-operative period. After 4 h of surgery, rats were sacrificed by cervical dislocation and ulcer index were examined on the dissected stomachs as described below.

Measurement of Ulcer Index

The stomachs were excised and were examined for hemorrhagic lesions in glandular mucosa. Immediately after the animals were sacrificed, their stomachs were dissected out, cut along the greater curvature and the mucosa was rinsed with cold normal saline to remove blood contaminant, if any. The sum of the length (mm) of all lesions for each stomach was used as the ulcer index (UI), and the percentage of inhibition (%*I*) was calculated as described by using the following formula.

$$\%I = \frac{(USc - USt)}{USc} \times 100$$

Where USc = ulcer surface area in control
USt = ulcer surface area in treated animals

Statistical analysis

The results are expressed as mean ± S.E.M. Data was analyzed by one-way ANOVA followed by Dunnett test. Value of *p* less than 5% (i.e. $p<0.05$) was considered statistically significant.

Result and Discussion

Table no. 1: Effect of Polyherbal Mixture Treatment on Different Biochemical Parameters in Rifampicin Induced Hepatotoxicity

Sr. No.	Serum Biochemical parameters	Groups					
		Normal Control	Negative control	Positive control	Polyherbal mixture - 100	Polyherbal mixture - 200	Polyherbal mixture - 400
1	AST (U/ml)	41.84 ± 1.12	121.06 ## ± 1.14	52.44 ** ± 1.85	119.28 ± 1.06	111.6** ± 1.19	62.38** ± 1.45
2	ALT (U/ml)	35.32 ± 1.92	98.86 ## ± 1.23	56.22 ** ± 1.80	88.8* ± 1.52	78.6 ** ± 1.30	65.66** ± 1.97
3	ALP (KA units/dl)	11.12 ± 1.18	48.94 ## ± 1.24	12.38 ** ± 1.46	46.3 ± 1.49	37.48 ** ± 1.75	27.92** ± 1.20
4	TP (gm/dl)	9 ± 0.83	7.94 ## ± 1.14	9.7 ** ± 0.57	6.76 ± 1.12	7.24 ** ± 2.03	8.04 ** ± 0.82
5	TB (gm/dl)	0.76 ± 0.11	3.46 ## ± 1.60	0.56 ** ± 0.11	2.48 ± 0.61	1.18 ** ± 0.44	1 ** ± 0.15

n=6. Values are expressed as Mean ± S.E.M.
* = $p < 0.05$, **=$p < 0.01$ when compared with Negative control
= $p < 0.01$, when compared with Normal control
Statistically analyzed by One Way ANOVA followed by Dunnett test

Table no.2: Effect of Polyherbal Mixture Treatment on Pylorus Ligation Induced Gastric Ulcer

Sr. no.	Groups	Treatment	Ulcer Index	Percentage Inhibition
1	I	Control	23.8 ±2.09	----
2	II	PM-100	14.92 ± 1.04*	37.31
3	III	PM-200	9.68 ± 1.37**	59.32
4	IV	PM-400	5.94 ± 1.13**	75.04
5	V	Omeprazole	3.68 ± 0.97**	84.53

n=6. Values are expressed as Mean ± S.E.M.
* = $p < 0.05$, **=$p < 0.01$ when compared with Negative control
= $p < 0.01$, when compared with Normal control
Statistically analyzed by One Way ANOVA followed by Dunnett test

The levels of number of hepatic enzymes are used as diagnostic indicators of hepatic injury. SGOT, SGPT and serum bilirubin are the most sensitive tests employed in the diagnosis of hepatic diseases. Elevated levels of serum enzymes are indicative of cellular leakage and loss of functional integrity of the cell membrane in liver. The hepatoprotective activity of drug against rifampicin may be due to inhibitory effects on formation of the active metabolite, 25-desacetyl rifampicin which in turn reduces drug-metabolizing enzymes and actively and specifically binds to RNA polymerases and thereby inhibits the nucleic acid and protein synthesis. [22].

In the present study, the AST, ALT levels were increased by Rifampicin. Post treatment with Polyherbal mixture of PM 400 mg/kg after 48 hr. significantly ($p<0.01$) reduced the elevated AST,

ALT levels. ALP activity is related to functioning of the hepatocytes. This ALP level was significantly ($p<0.01$) reduced by Polyherbal mixture of PM 400 mg/kg after 48 hr. The total bilirubin and total protein level was also restored to the normal level by the Polyherbal mixture of PM 400 mg/kg ($p<0.01$) after 48 hr.

The serum level of biomarkers in PM 400 mg/kg treated group was restored to normal level. The result indicates that treatment with Polyherbal mixture of PM 400 mg/kg protects liver against Rifampicin induced hepatotoxicity.

In the present study, polyherbal mixture 400mg/kg was found to be closer effect as compare to the standard drug Omeprazole in pyloric ligature induced gastric ulcers in experimental animals. On comparing the standard and polyherbal mixture it was seen that polyherbal mixture 400mg/kg and standard drugs afforded more protection against development of ulcers in the pyloric ligature model. This can be explained on the basis of the different mechanisms of ulcer development models.

Conclusion

Polyherbal mixture showed significant Hepatoprotective and Antiulcer activity in experimentally induced hepatotoxicity and ulcer in rat model by decreasing the gastric secretions and increasing /decreasing the biochemical markers level. The Hepatoprotective and Gastro protective activity of polyherbal mixture may be mediated by its present chemical constituent like Flavonoids, Tannins and Terpenoids. Tannins and flavonoids show ulcer healing property and the liver cells are regenerated by presence of chemical like Vitamin A, Vitamin C, Tannins and Quinic acid. Therapeutic mixture of dietary sources /components showed increased potency in preventing and healing gastric ulcer and liver cell regeneration due to presences of antioxidants. Current study thus provides a potential, safer, effective and nontoxic gastro protective and Hepatoprotective tools. Thus the results of the present study substantiate the traditional claims that the use of polyherbal mixture is beneficial in gastric ulcer and liver protection further studies are needed to prepare formulation like suspension.

Abbreviation

PM: Polyherbal mixture
PM 100: Polyherbal mixture-100
PM 200: Polyherbal mixture-200
PM 400: Polyherbal mixture-400
Serum AST: Serum Aspartate aminotransferase
Serum ALP:Serum Alkaline phosphatase
Serum ALT:Serum Alanine aminotransferase
Serum TB: Serum Total Bilurubin
Serum TP: Serum Total proteins
Serum TG: Serum Triglyceride
i.p: Intraperitonial
UI: Ulcer index
PI: Percentage Inhibition
Ml: Milliliter
S.E.M: Standard Error Mean
Gm: Gram
Kg: Kilogram

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