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EFFECT OF *SAPINDUS MUKOROSSI* SAPONIN FRACTION AND ANTHRAQUINONE FRACTION OF *RHEUM EMODI* ON CCL_4 INDUCED CYTOCHROME P_{450} DAMAGE IN WISTAR RATS

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ABSTRACT

The cytochrome P_{450} protective activity of *Sapindus mukorossi* and *Rheum emodi* were evaluated against CCl_4 induced prolongation of pentobarbitone sleep time in Wister rats. The fractions, at a dose of 0.2 g/kg,0.4g/kg p.o., Sapindus mukorossi, Rheum emodi significantly prevents the CCl_4 induced prolongation of pentobarbitone sleep time ($P<0.01$), indicating the cytochrome P_{450} protection activity. However, when treated alone, the fractions (0.2 g/kg and 0.4 g/kg, p.o.) shows a significant prolongation of pentobarbitone sleep time ($P<0.01$), indicating the intrinsic cytochrome P_{450} inhibition activity. In addition, the extracts show neither stimulant nor depressant effect on the CNS, as evident from locomotor activity test. In conclusion, the results indicate that, the Sapindus mukorossi saponin fraction and anthraquinone fraction of rheum emodi at an oral dose of 0.2 g/kg and 0.4 g/kg, p.o., shows significant protective effects against CCl_4 induced cytochrome P_{450} damage and also show a significant intrinsic cytochrome P_{450} inhibition activity.

KEYWORDS: **Cytochrome P_{450}, Carbon tetrachloride, *Sapindus mukorossi* saponin fraction, Anthraquinone fraction of *Rheum emodi***

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INTRODUCTION

Hepatic microsomal cytochrome P450 (CYP450) enzymes, a unique family of proteins, catalyze the oxidation of almost all the compounds of our environment, i.e., xenobiotics[1]. These are mainly metabolized in liver and there are three stages for their detoxification, termed first, second and third phases. Cytochrome P450 1A2 (CYP1A2) is a major enzyme responsible for first stage detoxification reactions[2]. Xenobiotics are the drugs, food additives, pollutants etc. to which humans are exposed in this modern age. They also include chemical carcinogens such as polychlorinated biphenyls (PCBs) and certain insecticides. More than 200,000 manufactured environmental chemicals exist. In some cases their products are mutagenic or carcinogenic[3]. Cytochrome P450 3A (CYP3A) is involved in the biotransformation of more than half of all drugs currently available[4]. Liver contains highest amounts of CYP450 among all the tissues of the body. Hepatic microsomal P450 enzymes have shown the great diversity of sizes, shapes, and modes of binding of the substrate binding sites of these mammalian cytochromes P450 (mainly from human liver). These active sites are conformationally very flexible that can adapt themselves to the xenobiotics structure for the best possible efficacy of substrate oxidation catalysis. They may also change nonreactive substance to a reactive substance which may be harmful, e.g., CCl4 to its reactive species which damages the liver[5, 6]. Cytochrome P3A2 is one of the most abundantly expressed cytochrome P-450s in the rat liver and almost identical to and functionally equivalent to human Cytochrome P3A4, which metabolizes numerous drugs including barbiturates which also induce them[7]. Pretreatment with the extract an herbal plant Ginkobiloba potentiated acetaminophen toxicity in cultured rat hepatocytes by inducing CYP3[8]. Gama amino butyric acid (GABA) is the principal inhibitory neurotransmitter in the mammalian central nervous system (CNS). Barbiturates like pentobarbital potentiate the effect of GABA by binding at the GABAA receptor thereby inducing sleep[9].

A number of medicinal plants are used in traditional system of medicinal for the management of liver disorders. Nature has given us a large number of medicinal plants, some of which are yet to be explored and validated for their medicinal value. The 21st century has seen a paradigm shift toward therapeutic evaluation of herbal products in liver diseases, carefully synergizing the strengths of traditional medicine with the modern concept of evidence based medical evaluation, standardization and randomized placebo controlled clinical trials to support clinical efficacy. Several herbs are known to possess antioxidant properties and may be useful as liver protective agents[10]. Plant saponins and anthraquinones are widely distributed amongst plants and have a wide range of biological properties. The more recent investigations and findings on plant saponins and anthraquinones have reported many biological activities. In view of these reports plant containing saponins and anthraquinones was selected to screen against ccl4 induced cytochrome p450 damage in rats. The genus *Sapindus* belongs to the family Sapindaceae, which has about 2000 species. Most of the species of the *Sapindus* genus are in use for the treatment of several diseases and other commercial purposes. *Sapindus mukorossi*, commonly known as a Reetha, is a deciduous tree. The tree is indigenous to northern and central India and is widely distributed in the Himalayan region, Haryana, Uttar Pradesh, and Chhattisgarh[11]. Traditionally; it is used in the treatment of asthma, snakebite, tooth disorders, piles, dermatological disorders, and hepatic disorders. It is a rich source of potential biological activities[12–15]. A survey of the chemical literature reveals that a great deal of phytochemical work on different parts – fruit, pericarp, seeds, leaves, ripe fruit, roots, and stems – of *S. mukorossi, S. saponaira, S. trifoliatus*, etc, has been carried out[16,17].

Chemically, the fruit of *S. mukorossi* is valued for the saponins. *Rheum emodi* Wall. ex Meissn. (Polygonaceae) is a leafy perennial herb distributed in altitudes ranging from 2800 to 3800m in the temperate and subtropical regions of Himalayas from Kashmir to Sikkim in India[18]. Roots of R. emodi are reported to have antibacterial and antifungal activities[19-20]. In addition several other biological activities such as laxative, diuretic, and in vivo inhibitory effect towards P388 leukemia in mice are also reported .It has the property of purgative, hemostatic, antipyretic, antihelmintic, laxative, atonic indigestion, constipation , diarrhea, dysentery, jaundice, liver disorder, antibacterial, antitumor, antifungal, diuretic, hemostatic, cholagogue, antihypertensive, lowers serum cholesterol, anti-inflammatory and antioxidant activity[21-23]. In the present study, both Cytochrome P450 (CYPs) inhibition and protection activity of the Sapindus mukorossi and Rheum emodi were evaluated. The intrinsic CYPs inhibition activity was evaluated by studying the ability of the plant extract to potentiate the pentobarbitone sleep time in rats. The CYPs protection activity was evaluated by studying the ability of the plant extract to prevent the CCl4 induced prolongation of sleep time in rats.

MATERIALS AND METHODS

Extraction, isolation and standardization of saponins and Total anthraquinone glycosides

One kilogram (1 kg) of dried powder of the fruit was extracted with cold ethanol (70%) by maceration for seven days and solvent was removed under reduced pressure. Ethanolic extract gives positive test for reducing sugar, tannins, flavonoids, alkaloids, triterpenoidal saponin and absence of glycoside and fixed oil. The crude ethanolic extract was resuspended in water and chloroform in HCl (50% v/v) was added to carry out acidic hydrolysis of saponin to isolate sapogenin. Chloroform phase was separated and concentrated under 40°C up to 1/3 of the original volume. Chloroform phase was exhaustively extracted three times with water-saturated n-butanol and solvent was removed under reduced pressure. Brown coloured dried powder with 2.41% of yield represents the crude sapogenin mixture and was designated as *S. mukorossi* sapogenin fraction (SMSF). It showed positive results for Salkowski and Noller's test indicating the presence of triterpenoids in the saponins fraction [24]. The isolated saponins fraction was standardized qualitatively by TLC profile using precoated silica gel plates as stationary phase, Ethyl acetate: Methanol: Water (81:11:8) and anisaldehyde-Sulphuric acid as spray reagent. The qualitative separation of saponins by TLC revealed the presence of 11 spots in S. Mukorossi. Compound with Rf value 0.27 confirmed the presence of terpenoid saponins. TLC profile of this investigation was similar to that reported in literature. Total saponins fraction was quantified by Vanillin- Sulphuric acid assay following procedure of Hiai et al (1976). In brief to 0.5 mL of aqueous solution of the sample, 0.5 mL vanillin solution of 8% (w/v) and then 5.0 mL of sulfuric acid of 72% (w/v) were added and thoroughly mixed in an ice water bath. The mixture was then warmed in a bath at 60 ˚C for 10 minutes then cooled in ice–cold water. Absorbance at 535 nm was recorded against the blank with the reagents using a Jasco V–530 UV/VIS spectrophotometer (Jasco, Tokyo Japan) [25].

Rheum emodi rhizome powder 500 g was extracted with 3x 3 L of 70% ethanol using a soxhlet apparatus. The extract was evaporated to dryness to yield a crude ethanol extract. A portion of the crude ethanol extract (75 g) was further extracted by adding 170 ml of water and 30 ml of 70% ethanol. After 10.5% w/v ferric chloride hexahydrate solution (50 ml) was added, the mixture was refluxed for 30 min before adding 20 ml concentrated hydrochloric acid and refluxed for another 30 min. When the mixture was cooled down, it was filtered and the filtrate was extracted with 5 x 200 ml of chloroform. The collected extracts from chloroform layer were evaporated after aqueous layer was added with 0.1 g of sodium

bicarbonate to adjust to neutral pH. The solution was then centrifuged at 4000 rpm for 20 min. The supernatant which contained anthraquinone glycoside was evaporated finally brown dried power with 2.25% of yield represented total anthraquinone glycoside mixture and was designated as a total anthraquinone glycoside fraction of *Rheum emodi* (TAGF of *Rheum emodi*).It showed positive results for Borntragers test, indicating the presence of anthraquinone glycoside in the *Rheum emodi Rhizome* extract. The isolated anthraquinone glycoside fraction was standardized by thin layer chromatography (TLC) profile using precoated silica gel plates (60 F254) the stationary phase, Petroleum ether : Ethyl acetate : Formic acid (75 :25 : 1), and 10% methnolic KOH as the spray reagent. The standardization of total anthraquinone glycoside by TLC revealed the presence of four spots in *Rheum emodi* extract. Compounds with R_f values of 0.52, 0.38, 0.27 and 0.84 confirmed the presence of anthraquinone glycosides The TLC profile of this investigation was similar to that reported in the literature.

Experimental animals

Wistar albino rats 8 weeks old of both sexes, weighing 150–250 g, were acclimatized to the experimental room at 22.2°C, controlled humidity conditions (55%) and 12/12-hour light/dark cycle. They were caged, with a maximum of two animals in one polypropylene cage, were fed with standard food pellets, and water was provided ad libitum supplied by Hindustan Unilever (Mumbai, India). All the studies conducted were approved by the Institutional Animal Ethical Committee of Nizam Institute Pharmacy and Research Institute according to prescribed guidelines of the Indian government's Committee for the Purpose of Control and Supervision on Experiments on Animals.

Chemicals

Carbon tetrachloride was obtained from Merck Ltd., Mumbai, India. The other chemicals and reagents used were of analytical grade.

Cytochrome P450 protection activity

Rats were divided into three groups of six each. Group 1 received vehicle (10 ml/kg, p.o.) Served as control, group 2 received total anthraquinone glycoside fraction of *Rheum emodi* (TAGF of *Rheum emodi*: 0.4 g/kg, p.o.), group 3 received *S. mukorossi* sapogenin fraction (SMSF: 0.2 g/kg, p.o.). A week before the study, normal sleep time of all the animals was determined using pentobarbitone sodium (25 mg/kg, i.p.). The sleeping times were measured as the duration of loss of righting reflex and the rats were considered asleep unless they could right themselves 3 times in a minute. The time between loss of righting reflex and its recovery was recorded for each animal[26-27]. A week later, all the animals were given their assigned treatments 24 h before CCl4 administration. The pentobarbitone sleep time was determined after 2 h of CCl4 administration.

Cytochrome P450 inhibition activity

Rats were divided into two groups of six each. Group 1 received vehicle (10 ml/kg, p.o.) and served as control, group 2 received total anthraquinone glycoside fraction of *Rheum emodi* (TAGF of *Rheum emodi*: 0.4 g/kg, p.o.), group 3 received *S. mukorossi* sapogenin fraction (SMSF: 0.2 g/kg, p.o.). A week before the study, normal sleep time of all the animals was determined as mentioned previously. A week later, 1 h after the assigned treatments, pentobarbitone sleep time was once again determined to ascertain the cytochrome P450 inhibition activity.

Locomotor activity

Rats were divided into four groups of six each. Group 1 received vehicle (10 ml/kg, p.o.) and served as control, group 2 received standard, caffeine (20 mg/kg, i.p.) and group 3 received total anthraquinone glycoside fraction of *Rheum emodi* (TAGF of *Rheum emodi*: 0.4 g/kg, p.o.), group 4 received *S. mukorossi* sapogenin fraction (SMSF: 0.2 g/kg, p.o.). The basal activity scores of all the animals were recorded 2 days before the start of study using

a photoactometer. On the day of the study all rats were given their assigned treatments, 30 minutes after the treatment each rat was retested for activity scores for 10 min and the difference in the activity scores were compared with the control scores.

Statistical analysis

The results were expressed in mean ± SEM, statistical significance was determined by using students't' test.P< 0.01was considered statically significant.

RESULTS

Cytochrome P450 protection activity

The effect of *Sapindus mukorossi* saponin fraction and anthraquinone fraction of *rheum emodi* on cytochrome p_{450} protection activity results was given in table 1. The results reveal that oral dose of total anthraquinone fraction of *Rheum emodi* 0.4 g/kg and *Sapindus mukorossi* saponin fraction 0.2 g/kg, significantly prevents the CCl4 induced prolongation of pentobarbitone sleep time when compared to the control (P<0.01).

Table 1

Effect of Sapindus mukorossi saponin fraction and anthraquinone fraction of rheum emodi on cytochrome p_{450} protection activity in rats.

Group	Treatment	Pentobarbitone sleep time (in minutes)	
		Before CCl4 Administration	After CCl4 Administration
01	Vehicle 10 ml/kg,p.o.	100.6 ± 2.3	186.93 ± 6.1
02	TAGF of *Rheum emodi*: 0.4 g/kg, p.o.	98.1 ± 2.5	148.0 ± 8.2*
03	SMSF: 0.2 g/kg, p.o.	96.2 ± 1.2	142.2 ± 4.1*

Values are mean ± SEM, n = 6, *P< 0.01.

Abbreviations: CCl_4, Carbon tetrachloride; TAGF of *Rheumemodi*, Total anthraquinone glycoside fraction of *Rheum emodi*; SMSF, *S. mukorossi* sapogenin fraction.

Cytochrome P450 inhibition activity

The effect of *Sapindus mukorossi* saponin fraction and anthraquinone fraction of *rheum emodi* on cytochrome p_{450} inhibition activity results was given in Table 2. The results reveal that oral dose of total anthraquinone fraction of *Rheum emodi* 0.4 g/kg and *Sapindus mukorossi* saponin fraction 0.2 g/kg, significantly increases the pentobarbitone sleep time when compared to control (P<0.01).

Table 2

Effect of Sapindus mukorossi saponin fraction and anthraquinone fraction of rheum emodi on cytochrome p_{450} Inhibition activity in rats.

Group	Treatment	Pentobarbitone sleep time (in minutes)	
		Before treatment	After treatment
01	Vehicle 10 ml/kg,p.o.	95.6 ± 3.8	96.8 ± 5.0
02	TAGF of *Rheum emodi*: 0.4 g/kg, p.o.	98.9 ± 3.6	152.0 ± 8.6*
03	SMSF: 0.2 g/kg, p.o.	96.7± 1.8	149.8± 4.3*

Values are mean ± SEM, n = 6, *P< 0.01.

Abbreviations: CCl_4, Carbon tetrachloride; TAGF of *Rheumemodi*, Total anthraquinone glycoside fraction of *Rheum emodi*; SMSF, *S. mukorossi* sapogenin fraction.

Locomotor activity

The effect of *Sapindus mukorossi* saponin fraction and anthraquinone fraction of *rheum emodi* on locomotor activity results was given in Table 3. The results reveal that oral dose of total anthraquinone fraction of *Rheum emodi* 0.4 g/kg and *Sapindus mukorossi* saponin fraction 0.2 g/kg, shows no significant effect on the locomotor activity. Standard, caffeine, at a dose of 20 mg/kg, i.p., however, shows significant increase in the locomotor activity as compared to control (P<0.01).

Table 3
Effect of Sapindus mukorossi saponin fraction and anthraquinone fraction of rheum emodi on locomotor activity in rats

Group	Treatment	Locomotor activity score	
		Before treatment	After treatment
01	Vehicle 10 ml/kg,p.o.	510±10.7	518±11.7
02	Caffeine 20 mg/kg, i.p.	506±12.2	641± 18.2*
03	TAGF of *Rheum emodi*: 0.4 g/kg, p.o.	513±9.4	486±23.2
04	SMSF: 0.2 g/kg, p.o.	511±18.2	482±46.3

Values are mean ± SEM, n = 6, *P< 0.01.

Abbreviations: TAGF of *Rheumemodi*, Total anthraquinone glycoside fraction of *Rheum emodi*; SMSF, *S. mukorossi* sapogenin fraction.

DISCUSSION

It is a well known fact that the hepatic cytochrome P450 enzymes are subjected to induction and inhibition by exposure to a wide variety of xenobiotics. These enzymes are affected by multiple active constituents of medicinal plants or herbs. Many compounds isolated from herbs have been identified as substrates, inhibitors, and/or inducers of various CYP enzymes. Some flavonoid compounds have recently been isolated from plants like hops, Humuluslupulus. Their chemical structures are similar to other plant-derived compounds, many present in the human diet, that have been proved to have cancer chemo preventive properties due to inhibition of cytochrome P450 enzymes that activate carcinogens[28]. The duration of pentobarbital-induced sleep in intact animals is considered as an index for the activity of hepatic CYPs[29]. Most xenobiotics cause hepatotoxicity by getting metabolized to active metabolites through CYPs. These toxic metabolites cause damage to different cellular organelles, including CYPs. Drugs with potential ability to inhibit and protect CYPs can, therefore, prevent the formation of toxic metabolites and their damaging effects on the liver. The duration of pentobarbitone induced sleep time in intact animals is considered as an index for the activity of hepatic CYPs. Drugs with potential CYPs protection activity are likely to prevent the CCl4 induced prolongation of pentobarbitone sleep time and drugs with inhibition activity on CYPs are likely to potentiate the pentobarbitone sleep time. Further, CCl4 prolongs the pentobarbitone sleep time through destruction of CYPs. The destruction of CYPs is known to be mediated by free radical species generated during the metabolism of CCl_4[30]. Drugs having CYPs protection ability will prevent the prolongation of sleep time induced by CCl4. In the present study animals treated with total anthraquinone fraction of *Rheum emodi* and *Sapindus mukorossi* saponin fraction .show a significant decrease in the CCl4 induced prolongation of pentobarbitone sleep time, as compared to CCl4 alone treated control animals. These

observations indicate the CYPs protection activity of *Rheum emodi* and *Sapindus mukorossi*. Further, animals pretreated with extract of *Rheum emodi* and *Sapindus mukorossi.* Show a significant potentiation in the pentobarbitone sleep time when compared to the control. The above findings suggest that the potentiation of pentobarbitone sleep time may be due to inhibition of CYPs by the extract of *Rheum emodi* and *Sapindus mukorossi.* However, variation in pentobarbitone sleep time can also be achieved by CNS drugs without altering the CYPs enzyme activity. The results obtained from the locomotor activity test reveal that *Rheum emodi* and *Sapindus mukorossi* have neither CNS stimulant nor depressant activity, thus indicating that the potentiation of pentobarbitone sleep time is exclusively due to the inhibition of CYPs by the extract of *Rheum emodi* and *Sapindus mukorossi.* In conclusion, the above results indicate that total anthraquinone fraction of *Rheum emodi* 0.4 g/kg and *Sapindus mukorossi* saponin fraction 0.2 g/kg have a significant protective effect against CCl4 induced cytochrome P450 damage and also show significant intrinsic cytochrome P450 inhibition activity.

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