HEPATOPROTECTIVE ACTIVITY OF ESSENTIAL OIL OF *CITRUS RETICULATA* AGAINST PARACETAMOL INDUCED HEPATIC DAMAGE IN ALBINO RATS.

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Summary

The objective of the present investigation was to study hepatoprotective activity of essential oil of *Citrus reticulata* in paracetamol induced liver damage model in albino rats. Liver damage in rats was produced by paracetamol (2gm/kg,b.w.,p.o.) in 1% CMC. The essential oil extracted from fruit rind of C. reticulata was administered orally to rats daily for seven days. At the end of the study the biochemical parameters were investigated. Concurrently silymarin was used as standard hepatoprotective agent. The essential oil exhibited significant hepatoprotective effect against paracetamol induced liver damage model in albino rats.

Keywords: *Citrus reticulata;* Paracetamol ; Hepatoprotective.

Introduction

Rapidly growing morbidity and mortality from liver diseases are largely attributable to the increasing number of chemical compounds and environmental pollution, because of livers strategic anatomical location and its large metabolic conversions, is exposed to many kinds of Xenobiotics and therapeutic agents. Unfortunatrly, in the modern era of medicine there is no specific treatment to counter the menacing impact of these dreaded diseases (1).Due to this fact efforts to find suitable palliative and/or curative agents for the treatment of liver diseases in natural products of plants origin is being made. Liver injury induced by paracetamol is the best characterized system of xenobiotics induced hepatotoxicity in human beings. Large doses of paracetamol, a widely used analgesic and antipyretic drug is known to cause hepatotoxicity in man and laboratory animals. The modern medicines have little to offer for alleviation of hepatic ailments, whereas most important representative are of phytoconstituents (2). Several plants have been investigated and reported to possess hepatoprtective activity eg. *Baliospermum montanum* (3),*Ocium sanctum* (4) ,*Tamarindus indica* (5) etc. Similarly *Citrus reticulate* is commonly known as santre.It is one of the essential oil bearing plant belonging to family Rutaceae,it is a perennial bushyt tree with globose fruit.This plant finds its place in indigenous traditional medicinal system. The medicinal properties of this plant being mainly due to the essential oil produced by the secondary metabolism (6).In traditional medicine the essential oil from citrus fruit rind was advised for cuteneous complaints, hemiplagis, snake bite, fever, loss of taste,chronic rheumatism,stomach ache, menorrhagia, spleenomegally, odema and cardiac diseases (7).

However hepatoprotective activity of essential oil of *Citrus reticulata* has not been scientifically investigated. Therefore, in the present study, hepatoprotective effect of essential oil of *Citrus reticulata* has been evaluated in paracetamol induced liver damage in albino rats.

Materials and methods

Plant collection and oil extraction

The fresh mature fruits were collected from Belgaum and were authenticated by Prof.N.A.Jadhav,Dept of Botany B.K.College,Belgaum.Fruit rind was removed and pale yellow colored essential oil waqs extracted by hydrodistillation using Clevenger apparatus.

Animals:

The complete course of the experiment was carried out using healthy male rats of Wistar strain, reared and maintained at the animal house of the institution and were fed on commercial laboratory animal feed (Amrut brand, Sangli) and water *ad libitum*. The rats weighing between 150-250 g were housed for about a week for acclimatization under 12;12 light – dark cycle. The animals were starved overnight with water *ad libitum* prior to the day of experimentation. Ethical clearance was obtained from Institutional Animal Ethics Committee constituted as per CPCSEA guidelines.

Dose determination:

In various groups (n=6, in each) of animals essential oil extracted from fruit rind of *Citrous reticulata* was administered orally at the dose of 200 mg/kg body weight. Silymarin was used as standard. Paracetamol (2gm/kg,b.w.,p.o.) in 1% CMC was administered to all animals except the animals of the normal control group(8).

Methadology

All the treatments were given for seven days. On the 8^{th} day of the start of respective treatment the rats were anaesthetized by ether anesthesia and the blood was withdrawn from retro orbital plexus. It was allowed to coagulate for thirty minutes and serum was separated by centrifugation at 2500 rpm. The serum was used to estimate Serum glutamate pyruvate transaminase (SGPT), Serum glutamate oxaloacetoacetate transaminase (SGOT) , Total protein and billirubin content(9).

Statistical analysis

The results were analysed by ANOVA followed by Dunnet's posthoc test and $p \leq 0.05$ was considered as significant.

Results

Paracetamol intoxication in normal rats elevated the levels of SGPT, SGOT and total protein content and billirubin significantly($p<0.001$), indicating acute centrilobular necrosis. The rats treated with essential oil showed a significant reduction in all the biochemical parameters elevated by paracetamol ($p<0.001$). The percentage reduction of all the three biochemical parameters against hepatotoxin is given in Table 1.

Table 1: Effect of essential oil of *Citrus reticulata* in paracetamol induced Hepatotoxicity

Group	Biochemical parameters mean ± SEM				
	SGOT(IU/L)	SGPT(IU/L)	Total protein (g/dl)	Billirubin (mg/dl)	
				Total	Direct
Normal control	139.7 ± 1.585	89.83 ± 1.249	7.788 ± 0.1703	0.4983 ± 0.023	0.1201 ± 0.068
Negative control (Paracetamol)	214.2 ± 4.438#	168.7 ± 2.472#	4.492 ± 0.1584#	0.900 ± 0.0225#	0.1900 ± 0.0063 #
Essential oil	150.2 ± 4.064 ***	121.5 ± 2.421 ***	5.471 ± 0.1582 ***	0.6227 ± 0.01202 ***	0.1178 ± 0.007032 ***
Standard	149.8 ± 1.452 ***	126.3 ± 1.215 ***	5.605 ± 0.1643 ***	0.7290 ± 0.0171 ***	0.1433 ± 0.006146 ***

# $p<0.001$ considered significant as compared to Normal control group.

*** $p<0.001$ considered significant as compared to Paracetamol control group.

Discussion

Paracetamol, an analgesic and antipyretic, is assumed to be safe in recommended doses; over doses however, produce hepatic necrosis. Small doses are eliminated by conjugation followed by excretion, but when the conjugation enzymes are saturated, the drug is diverted to an alternative metabolic pathway, resulting in the formation of a hydroxylamine derivative, by cytochrome P_{450} enzyme. When the hepatic reserves of glutathione depletes, the hydroxylamine reacts with macromolecules and disrupts their structure and function.

Extensive liver damage by Paracetamol itself decreases its rate of metabolism and other substrates for hepatic microsomal enzymes. Induction of cytochrome P_{450} or depletion of hepatic glutathione is a prerequisite for Paracetamol-induced toxicity. The essential oil of *Citrus reticulata* reduced the elevated levels of all the four biochemical parameters by Paracetamol. Paracetamol induced liver necrosis was inhibited significantly by essential oil of *Citrus reticulata*, which confirms the protective action of the essential oil of *Citrus reticulata* against experimentally induced liver damage in rats. SGPT, SGOT and bilirubin are the most sensitive tests employed in the diagnosis of hepatic disease. It can be concluded from this investigation that essential oil of *Citrus reticulata* possess hepatoprotective activity. Further detailed studies may however confirm the utility profile of this essential oil.

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