HEPATOPROTECTIVE POTENTIAL OF *GARCINIA INDICA* FRUIT RIND EXTRACT AGAINST ACETAMINOPHEN INDUCED HEPATIC DAMAGE IN WISTAR RATS.

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Summary

The aqueous extract of *Garcinia indica* (*GI)* family Clusiaceae, fruit rind was investigated for its hepatoprotective potential against acetaminophen (AAP) induced acute hepatic damage in Wistar rats. The biochemical markers *viz.* Serum Glutamate Oxalate Transaminase (SGOT), Serum Glutamate Pyruvate Transaminase (SGPT), Alkaline phosphatase (ALP), Acid phosphatase (ACP) and serum bilirubin were estimated and compared with both treated and untreated groups. AAP (750mg/kg b.w) has increased the levels of SGOT, SGPT, ALP, ACP and billiribin levels. Pretreatment with aqueous extract of *GI* fruit rind (AEGF) 250mg/kg and 450mg/kg has produced statistically significant hepatoprotective effect by decreasing the activity of biochemical markers and billirubin levels in dose dependent manner. Hence the AEGF could provide a significant protection against AAP induced hepatocellular damage in Wistar rats.

Key words: *Garcinia indica*, Acetaminophen, Silymarin. Hepatoprotective

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Introduction

Liver is the vital organ in the body, responsible for many metabolic functions and detoxification of endogenous and exogenous challenges *viz.* chemotherapeutic agents, xenobiotics, pollutants, viral infections and chronic alcoholism. Liver ailments remain one of the serious health problems. Liver cells are mainly damage by lipid peroxidation and oxidative stress produced by hepatotoxic agents. AAP the most widely used analgesic and antipyretic drug throughout the world, causes severe hepatic damage due to an acute or cumulative over dose[1].

AAP metabolized by hepatic cytochrome P-450 to a minor electrophilic metabolite, N-acetyl-p-benzoquinoneimine (NAPQI) [2], which during AAP overdose depletes glutathione and initiates covalent binding to cellular proteins. These events lead to the disruption of calcium homeostasis, mitochondrial dysfunction, and oxidative stress[3, 4]. In addition serum levels of many biochemical markers like SGOT, SGPT, ALP and billirubin are elevated. The treatments for drug induced hepatic damage involve discontinuation of causative drug and administration of antidotes (such as N-acetyl cystein, in AAP toxicity). However there are several herbs/herbal preparations claimed to have possess beneficial activity in treating hepatic disorder. Plant preparations of *Ocimum sanctum* [6] *, Azadirachta indica*[7]*, etc* have been reported to be hepatoprotectives in AAP induced hepatotoxicity in animal experimental models.

GI (Family-**Clusiaceae**) consist of dried fruits rind known as kokum, distributed in Konkan, Western Ghats, most commonly in the Southern Konkan and Goa. It is an Indian spice used in many parts of the country for making several vegetarian and non-vegetarian dishes.

In Ayurveda it is remedy for dysentery, diarrhea, cardiotonic, ulceration, an appetizer and used as good liver tonic.[8, 9, 10]

GI extracts, especially from its rind, are rich in polyisoprenylated benzophenone derivatives such as Garcinol and its colorless isomer, Isogarcinol. The rind also contains hydroxycitric acid (HCA), hydroxycitric acid lactone, citric acid, and oxalic acid. The fruit also contains other compounds including malic acid, polyphenols, carbohydrates, anthocyanin, pigments, and ascorbic acid. Garcinol shows strong antioxidant activity[11]. Hence antioxidants play an important role in preventing hepatotoxicity[12]. The present study was therefore, planned to investigate hepatoprotective activity of *GI* in male Wistar rats subjected to AAP induced hepatic damage.

Materials & Methods

Plant: The Fruit of *GI* was collected from the Savantwadi, Maharashtra. The fruit was authenticated by Prof R.S. Goudar Department of Botany, RLS institute, Belgaum. A voucher specimen was also deposited in the same section of the department.

Chemicals: Silymarin (microlab. Tamilnadu- India) was used as standard drug. While AAP was obtained from the sunrise Ltd. Ahmadabad. The biochemical estimation kits are procured from coral clinical system Goa.

Preparation of extract: The fruits of *GI* fruits were shed dried , separated from the seed and pulverized to coarse powder, and then the powder was defatted by petroleum ether

(40-60%). About 50 gm of dried defatted fruit rind powder was macerated with chloroform water, I.P. for seven days at room temperature and then the extract was filtered, concentrated by rotary evaporator, and dried in desiccator over sodium sulphite. Then resulting extract was used for hepatoprotective activity.

Animals: Healthy male Wistar rats (180–200 g) were used for this study were kept in standard environmental conditions. After 7 days of acclimatization period, they were randomized indifferent groups, fed with standard rodent diet and with water ad libitum. Ethical clearance was obtained from Institutional Animal Ethical Committee constituted as per CPCSEA guidelines.

Hepatoprotective activity: Animals are divided in to five groups each group having six animals.
Group I served as control was administered normal saline (5 ml/Kg) p.o. single dose daily for seven days.
Groups II served as negative control was similarly treated as group I.
Group III and **IV** were treated with 250 and 400 mg/kg AEGF for seven days respectively.
Group V animals were administered Silymarin 25 mg/kg daily as standard for seven days. On the 8th day rats of group II, III, IV and V challenge with AAP (750mg/kg) P.O single dose to all animals except group I.

Biochemical Studies: After 24 hrs of AAP intoxication blood was obtained from all animals by puncturing retro-orbital plexus under ether anesthesia. The blood samples were allowed to clot for 45 min at room temperature. Serum was separated by centrifugation at 2500 g at 30ºC for 15 minutes and used for the estimation of various biochemical parameters viz. SGOT[13], SGPT[13], ALP[14], ACP[15], serum bilirubin[16].

Statistical Analysis: All values were expressed as Mean ± SEM. The biochemical parameters were analyzed statistically using one-way ANOVA, followed by Dennett's multiple comparisons test (DMRT).The result with $p<0.001$ and $p<0.01$ as considered statically significant.

Results

The result of present study demonstrated hepatoprotective potential of AEGF in AAP-induced hepatocellular damage.Administration of acute toxic dose of AAP (750 mg/kg p.o) increases biomarkers levels viz. SGOT,SGPT,ALP,ACP and serum bilirubin concentration, when compared to normal animals (Table 1).

Oral treatment of AEGF at dose of 250mg/kg and 450mg/kg shows significant ($p<0.001$) decrease in the AAP-induced elevation levels of SGOT, SGPT, ALP, ACP and serum bilirubin in dose dependent manner (Table 1).

Table No.1 Effect of AEGF on various serum biomarkers in control and extract treated animals.

Groups and Design of Treatment	**SGOT (U/L)**	**SGPT (U/L)**	**ALP (U/L)**	**ACP(U/L)**	**Serum Bilirubin (mg/dl)**
Group-I Normal (Normal saline 5ml/kg)	56.83±1.400	42.33±1.453	57.50±0.482	20.12±0.361	0.3083±0.104
Group-II AAP control (750mg/kg)	120.0±1.183$^{\#a}$	133.5±1.455$^{\#a}$	120.0±0.577$^{\#a}$	52.25±0.122$^{\#a}$	1.320±0.067$^{\#a}$
Group-III AEGF (250mg/kg)+AAP (750mg/kg)	108.2±1.195*^{a}	120.07±0.881*^{a}	106.2±1.014^{*a}	25.32±0.410^{*a}	1.055±0.054*^{b}
Group-IV, AEGF (450mg/kg)+AAP (750mg/kg)	92.50±1.979*^{a}	108.2±1.014*^{a}	95.33±0.881*^{a}	23.46±0.657*^{a}	0.890±0.0316*^{a}
Group-V, (Silymarin 25mg/kg)+AAP (750mg/kg)	66.17±0.600*^{a}	55.50±0.7638*^{a}	72.50±0.922*^{a}	21.35±0.214*^{a}	0.530±0.061*^{a}

Values are mean± SEM, 6 animal in each group (n = 6).

compared with normal group, * compared with AAP control group

$^{a}p<0.001$ and $^{b}p<0.01$ values are considered statically significant

Discussion

The increase levels of serum biomarkers and serum bilirubin notice in this study is the outcome of AAP-induced hepatic dysfunction and sign of hepatocellular damage. Oral treatment of AEGF at dose 450mg/kg b.w shows more significant reduction in AAP-induced levels of SGOT, SGPT, ALP, ACP and serum bilirubin than at the dose of 250 mg/kg. These findings could be considered as a functional improvement of hepatocyte.
The silymarin at dose 25mg/kg has better inhibition of the AAP-induced elevated levels of SGOT, SGPT, ALP, ACP and serum bilirubin.
It can be concluded that the AEGF possess good hepatoprotective and antioxidant[17] activities. The significant reduction in the elevated levels of biomarkers is an indication of stabilization of plasma membrane as well as repair of damage tissues caused by AAP. It is not possible to pinpoint the possible hepatoprotective mechanism from the finding of the present study. Further investigation is required to evaluate possible mechanism for the activity.

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