Research Article

Protective effect of extracts of *Cocos nucifera* endocarp on Paracetamol induced hepatotoxicity in rats

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Abstract

Background: Hepatotoxicity related to many drugs or their transformation to chemically reactive metabolites that may be influenced by therapeutic, physiological or nutritional factors interfering with drug elimination or formation of a reactive metabolite or their detoxification. It is caused by drug accumulation or may be due to metabolic inhibition by other drugs or liver damage. **Objective:** *Cocos nucifera* (Arecaceae) have variety of ethnic medicinal uses along with antioxidant activity. Objective of present study was to evaluate the hepatoprotective activity with alcoholic (AEECN) and aqueous (AQEECN) extracts of endocarp of *Cocos nucifera*. **Materials and methods**: *Cocos nucifera* fruit endocarp collected from local market of Kanpur were authenticated by NISCAIR, New Delhi and dried in shade at room temperature then subjected to size reduction to a fine powder with the help of mixer grinder. Paracetamol and Silymarin are gift samples from Pharmed, Bangalore, India and Micro Labs- Bangalore respectively. Thiopental sodium was purchased from Neon Laboratories Ltd., Mumbai, India. The following biochemical kits SGPT, SGOT, ALP, BILT and BILD were purchased from Erba Diagnostics Mannheim GmbH, Germany. **Results:** In LD_{50} studies for AEECN and AQEECN up to the maximum dose level of 2000 mg/kg dose no mortality was observed in any of the animals, indicating the practically nontoxic. When compared to toxicant control groups both the extracts have significantly reduced the paracetamol induced elevated levels of serum ALT, AST, ALP, BILT and BILD. The histopathological changes (steatosis), necrosis etc. were partly or fully prevented in animals treated with the two extracts. **Conclusion:** AEECN and AQEECN showed a significant hepatoprotective effect against paracetamol induced hepatic damage. The medium and high doses of AEECN and AQEECN (200 and 400 mg/kg) treated groups showed better hepatoprotective activity when compared to standard drug silymarin (25 mg/kg p.o.) treated group.

Keywords: *Cocos nucifera*, endocarp extracts, paracetamol, silymarin, hepatoprotective activity

Introduction

Cocos nucifera (L.) popularly known as coconut is an important member of the family Arecaceae (palm family), cultivated throughout South India. It is one of the major food crops in tropical countries. The coconut fruit consists of an outer epicarp, a mesocarp, and an inner endocarp. The epicarp, which is the outer skin of the fruit and the mesocarp, which is heavy, fibrous, and tanned when dry, have many industrial uses. The endocarp, also called coconut shell is the hard dark core. Inside is solid white albumen of varied thickness, depending on the age of the fruit, and with an oily pulp consistency and liquid albumen called coconut water that is thick, sweet, and slightly acidic. All parts of the coconut are useful. Traditionally both the green coconut water and solid albumen in ripe fruits are used industrially and in cooking. Additionally, several parts of the fruit and plant have been used by people in different countries for the treatment of various pathological conditions (Lima et al., 2015).

The endocarp of *Cocos nucifera* contains tannins, flavonoids, alkaloids, carbohydrates phenols and phytosterols. The roots are astringent, diuretic and anthelmintic and are useful in uterine disorders, bronchitis, hepatopathy and helminthisis. The cell is cooling, diuretic, and useful in hyperdipsia, strangury and hepatopathy (Warrier et al., 1994).

Coconut shell, also called endocarp is the hardest dark core of

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coconut fruit. It is an agricultural waste and is available in plentiful quantities throughout tropical countries. It is widely used for making charcoal. Though it is considered as a waste, it possess various medicinal activities which include antimicrobial, antioxidant, anti-inflammatory, antitumor, anthelmintic, antidotal, antiseptic, aperients, aphrodisiac, astringent, bactericidal, depurative, diuretic, hemostat, pediculicide, refrigerant, stomachic, styptic, suppurative and vermifuge etc (Singla et al., 2011).

Various published journals and books have revealed that plant based drugs are showing promising hepatoprotective activity and presently except silybon (Micro Labs, Bengaluru) no other allopathic medication is available for the treatment of liver disorders. Some of the plants reported for their hepatoprotective activity are *Andrographis paniculata* (Neha et al., 2000), *Calotropis procer* (Viswanath et al., 2005), *Fumaria indica* (Nimbakar et al., 2000), *Luffa acutangula* (Muna Abid et al., 2005), *Boerhavia diffuse* (Krupavaram et al., 2005) etc. From the literature it was found that *Cocos nucifera* has also been traditionally indicated for treatment of hepatic disorders. Hence endocarp extracts of this plant was select for the study of hepatoprotective activity in PCM induced hepatotoxic rats.

Materials and methods

Plant material

Cocos nucifera fruit endocarp collected from local market of Kanpur were authenticated by NISCAIR, New Delhi and dried in shade at room temperature then subjected to size reduction to a fine powder with the help of mixer grinder.

Chemicals

Paracetamol and Silymarin are gift samples from Pharmed, Bangalore, India and Micro Labs- Bangalore respectivily. Thiopental sodium was purchased from Neon Laboratories Ltd., Mumbai, India. The following biochemical kits SGPT, SGOT, ALP, BILT and BILD were purchased from Erba Diagnostics Mannheim GmbH, Germany.

Animals

Albino rats (Wistar strain) of either sex weighing between 150-200 g were acclimatized for 7 days under standard husbandry condition. i.e. Room temperature, 26 ± 2^0C; Relative humidity, 45-55% and Light/ dark cycle, 12:12 h.

The animals were fed with a synthetic standard diet from Amrut Laboratories & Pranav Agro Industries Ltd. Sangli. Water was allowed *ad libitum* under strict hygienic conditions. All animal studies were performed in accordance to guidelines No. 425 of CPCSEA and Institutional Animal Ethical Committee (IAEC) and all the procedures were followed as per rules and regulations.

Preparation of alcoholic extract

The stem powder was packed in a soxhlet apparatus and extracted with 95% alcohol for 18 h. Appearance of colourless solvent in the siphon tube was taken as the termination of extraction. The extract was then transferred into the previously weighed empty beaker and evaporated to a thick paste on the water bath, maintained at 50°C to get alcoholic extract. The extract was finally air dried thoroughly to remove all traces of the solvent and the percentage yield was calculated (Kokate, 1994).

Preparation of aqueous extract

About 100 g of powder was taken in a round bottom flask (2000 ml) and macerated with 500 ml of distilled water with 10 ml of chloroform (preservative) for 7 days with occasional shaking for every hour in a closed vessel. Then the marc was removed by filtering the extract and then it was concentrated on a water bath maintained at 50°C (Kokate, 1994).

These two extracts were stored in airtight containers in a refrigerator below 10°C. The two extracts were examined for their colour and consistency. Their percentage yield was calculated with reference to air-dried powder sample used for the extraction.

Toxicity studies

The acute toxicity of endocarp of *Cocos nucifera* was determined by using albino rat of either sex (150-200 g), maintained under standard husbandry conditions. The animals were fasted for 3 h prior to the experiment and were administered with single dose of individual extracts of endocarp of *Cocos nucifera* and observed for the mortality upto 48 h study period (Short term toxicity). Based on the short-term toxicity profile, the next dose of the individual extracts was determined as per OECD guidelines No. 425. From the LD_{50} doses 1/20,1/10 and 1/5 doses were selected and considered as low, medium and high dose respectively.

Paracetamol induced hepatotoxicity model (Gupta et al., 2006)

Wistar rats weighing between 150-200 g were divided into 9 groups of 6 rats in each. Group A was administered with vehicle for 14 days and served as normal control, group B (toxicant) with paracetamol (2000 mg/kg, p.o), and group C with silymarin (25 mg/kg, p.o) which was served as standard. Animals in groups D, E, F were treated with three different doses (low, medium and high) of AEECN and groups G, H, I were treated with three different doses (low, medium and high) of AQEECN. Animals of group B, C, D, E, F, G, H and I were intoxicated with paracetamol (2000 mg/kg).

Assessment of hepatoprotective activity: On the 15th day, the animals were anaesthetized and blood was collected from the retro-orbital puncture. Serum was separated after coagulating at 37°C for 30 min and centrifuging at 2000 rpm for 15 min, and estimated for serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), alkaline phosphatase (ALKP) and serum bilirubin (SBLN). The hepatoprotective activity was confirmed through histopathological studies on liver of rats. After collection of blood for biochemical estimation, the rats were sacrificed and the livers were carefully dissected out, cleaned of extraneous tissue, and fixed in 10% formalin for 24 h. Then the paraffin sections were prepared (automatic tissue processor, Autotechnique) and cut into sections of 5 µm thickness, using a rotary microtome. The sections were stained with Haematoxylin-Eosin dye and studied for histopathological changes (Galigher et al., 1971).

Statistical analysis

All the recorded results are expressed as mean ± SEM from 6 animals. Statistical difference in mean was analyzed by using one-way ANOVA (analysis of variance) followed by Post hoc test (Dunnett's 't' test). P< 0.05*, 0.01** and 0.001*** were considered as statistically significant.

Results

In the present study the effect of the AEECN and AQEECN on normal liver functions, was found to be non-toxic in nature. Paracetamol intoxication in normal rats elevated the levels of SGOT, SGPT, ALP, BILD and BILT significantly, indicating acute centrilobular necrosis. The rats treated with AEECN and AQEECN showed a significant reduction in the biochemical parameters elevated by paracetamol (Table 1).

Histopathological examination of liver sections of control group (Figure 1a) showed normal cellular architecture with distinct hepatic cells, sinusoidal spaces and central vein. In the liver sections of the rats intoxicated with paracetamol (Figure 1b), there is disarrangement and degeneration of normal hepatic cells with intense centrilobular necrosis extending to mid-zone and sinusoidal haemorrhages and dilatation.

The liver sections of the rats treated with silymarin and intoxicated with paracetamol (Figure 1c) and rats treated with AEECN and AQEECN (low, medium and high doses) and intoxicated with paracetamol (Figure 1d-1i) showed less vacuole formation, reduced sinusoidal dilation, and less disarrangement and degeneration of hepatocytes, indicating marked regenerative activity. The intensity of centrilobular necrosis was less.

Discussion

Paracetamol, an analgesic and antipyretic, is assumed to be safe in recommended doses; overdoses, 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. The hydroxylamine derivative, a reactive electrophillic agent, reacts non-enzymatically with glutathione and detoxifies. 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}

Table 1. Hepatoprotective effect of different extracts of endocorp of *Cocos nucifera* on PCM induced hepatotoxicity in rats.

Groups Treatment mg/kg	TST (min)	LIV (WT)	LIV (VOL)	ALT (U/L)	AST (U/L)	ALP (U/L)	BILD (g/dl)	BILT (mg/dl)
Normal 10ml/kg (vehicle)	68.67 ±0.95	4.05 ±0.05	5.08 ±0.15	45.06 ±1.32	111.05 ±1.93	113.09±1.54	0.21 ±0.01	0.25 ±0.01
Toxicant 2000 (PCM)	137.16 ±3.85	7.23 ±0.10	6.25 ±0.11	153.78 ±6.93	230.33 ±4.10	243.48±2.40	0.70 ± 0.07	1.44 ± 0.07
Standard 25 (Silymarin)	88.33±1.83**	5.0±0.08**	5.50±0.22**	61.30±1.82**	122.84±1.46**	130.16±0.89**	0.37± 0.01**	0.54±0.01**
AEECN 100	131.33±1.08*	6.74±0.15^{ns}	6.17±0.10^{ns}	143.73± 1.35^{ns}	226.28± 9.96^{ns}	238.22± 1.07^{ns}	0.63± 0.05^{ns}	1.41±0.05^{ns}
AEECN 200	130.16±0.98*	6.09±0.17**	6.00±0.12^{ns}	141.61±1.38*	218.91±4.31^{ns}	235.34±2.73*	0.56±0.01*	1.29± 0.03^{ns}
AEECN 400	94.33±0.71**	5.62±0.16**	5.67±0.21*	78.99±1.42**	142.99±5.48**	161.58±1.24**	0.41± 0.01**	0.70± 0.03*
AQEECN 100	123.33±1.02*	6.71±0.17^{ns}	6.08±0.08^{ns}	136.20±1.51**	207.79±2.70*	235.27± 1.31*	0.60±0.02*	1.27 ± 0.03*
AQEECN 200	96.33±1.49**	5.98±0.13**	5.75±0.11*	80.24±1.12**	187.91± 4.94**	154.83±1.89**	0.42± 0.01**	1.06±0.01**
AQEECN 400	93.50±0.88**	5.41±0.17**	5.25±0.11**	70.18±1.18**	139.45±1.30**	138.24±1.68**	0.43 ±0.01**	0.62 ±0.01**

n = 6, Significant at P< 0.05*, 0.01** and 0.001***, ns = not significant; AEECN- alcoholic extract of endocorp *of Cocos nucifera*, AQEECN- aqueous extract of endocorp of *Cocos nucifera.* TST – Thiopental sodium induced sleeping time.

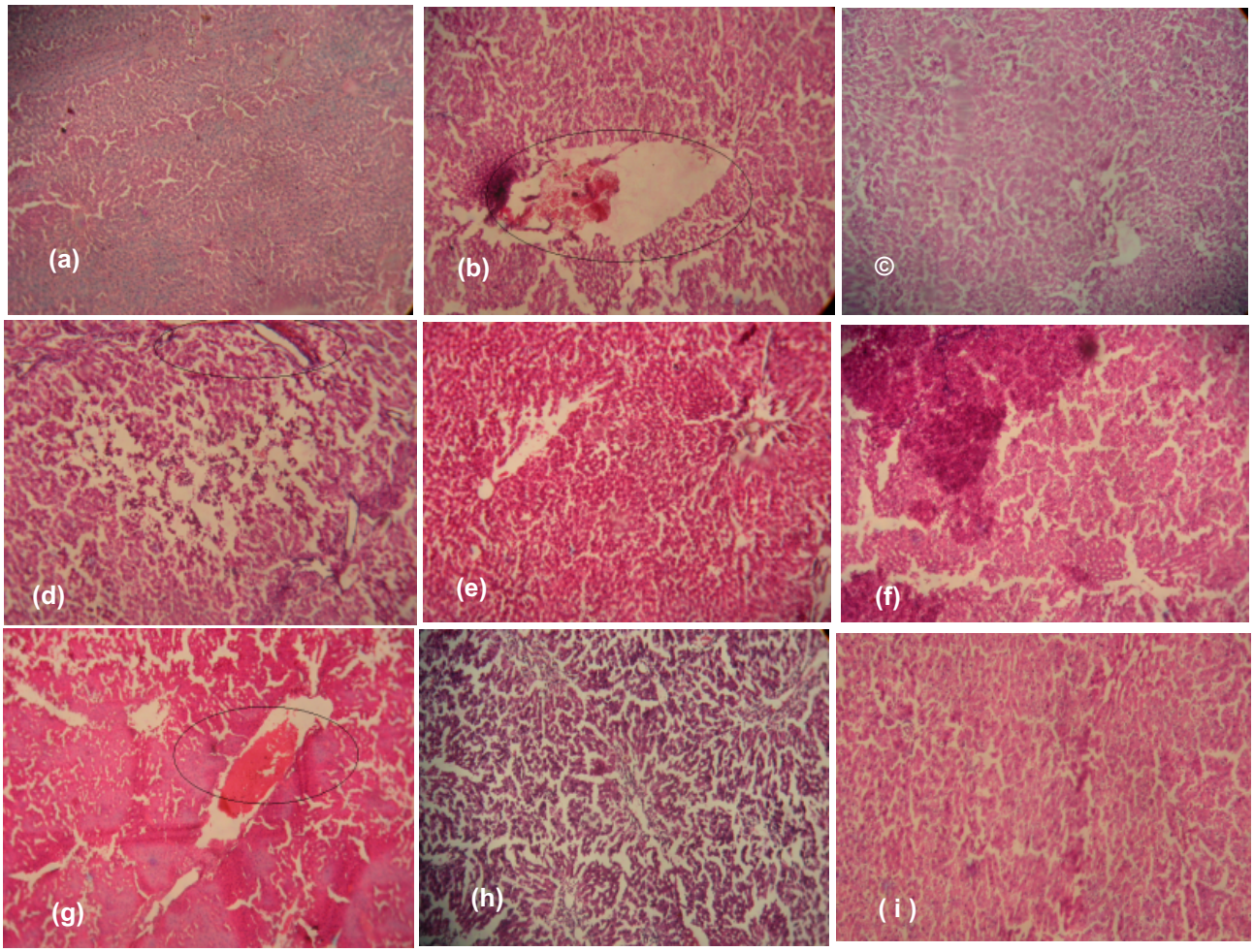

Figure 1. Histological observation of effects of extract on different groups of rat liver. (a) normal hepatic tissue, (b) PCM induced damage in hepatic tissue, (c) Silymarin treated group (d) Effect of AEECN (Low) dose, (e) Effect of AEECN (Med) dose, (f) Effect of AEECN (High) dose, (g) Effect of AQEECN (Low) dose (h) Effect of AQEECN (Med) dose, (i) Treatment with AQEECN (High) dose on PCM induced hepatic damage.

or depletion of hepatic glutathione is a prerequisite for paracetamol-induced toxicity. The alcoholic and aqueous extracts of endocarp of *Cocos nucifera* reduced the elevated levels of biochemical parameters by paracetamol. Paracetamol-induced liver necrosis was inhibited significantly by endocarp extracts of *Cocos nucifera*, which confirms the protective action of the AEECN and AQEECN against experimentally induced liver damage in rats. SGOT, SGPT, ALP, BILD and BILT are the most sensitive tests employed in the diagnosis of hepatic disease. The elevated levels of these parameters were significantly reduced by the treatment of *Cocos nucifera* endocarp extracts. It can be concluded from this investigation that endocarp of *Cocos nucifera* possess hepatoprotective activity.

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References

Galigher AE, Kozloff EN. 1971. Essentials of practical microtechnique. 2nd ed. Philadelphia: Lea and Febiger: 77.

Gupta AK, Misra N. 2006. Hepatoprotective activity of aqueous extracts of *Chamomile capitula* in paracetamol intoxicated rats. American Journal of Pharmacology, 1 (1): 17-20.

Kokate CK.1994. Practical pharmacognosy. 4th ed., Delhi, Vallabh Prakashan, 110-111.

Krupavaram K, Rao NV. 2005. A Study on hepatoprotective activity of root extracts of *B. diffusa*. Indian Drugs, 40: 260.

Lima EBC, Sousa CNS, MenesesLN, Ximenes NC, Santos JAM, Vasconcelos GSG, Vasconcelos S, Lima NBC, Patrocínio MCA, Macedo D, Vasconcelos SMM. 2015. *Cocos nucifera* (L.) (Arecaceae): A phytochemical and pharmacological review. Brazilian Journal of Medical

and Biological Research, 48(11): 953–64.

Muna Abid, Shivraj Gouda T, Venkat Rao N. 2005. Hepatoprotective activity of fruit extracts of *Luffa acutangula* Roxb. Var. Indian Drugs, 40:260.

Neha T, Raval UM. 2000. Hepatoprotective and toxicological evaluation of *Andrographis paniculata* on severe liver damage. Indian Journal of Pharmacology, 32: 288-293.

Nimbakar SR, Juvekar AR, Jogalekar SN. 2000. Hepatoprotective activity of *Fumaria indica* in hepatotoxicity induced by anti-tubercular drugs treatment. Indian Drugs, 37(11):537-542.

OECD guideline for testing of chemicals. 2001. Available at http://www.oecd.org/chemicalsafety/risk-assessment/1948378.pdf

Singla RK, Jaiswal N, Bhat GV, Jagani H. 2011. Antioxidant & antimicrobial activities of *cocos nucifera* linn. (arecaceae) endocarpextracts. Indo Global Journal of Pharmaceutical Sciences, 1(4): 354-61.

Viswanath KM, Quereshi AA, Setty SR. 2005. Hepatoprotective activity of flowers of *Calotropis procera* in Paracetamol induced hepatic injury in rats. Indian Journal of Pharmaceutical Sciences, 44: 261.

Warrier PK, Nambiar VPK, Ramankutty C, Vasudevan Nair R. 1994. Indian Medicinal Plants, Universities Press, Hyderabad, 1st Edition. Volume-I: 126.