

## **Acute and sub-chronic toxicity study of *Cocos nucifera* leaf extracts in mice**

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### **ABSTRACT**

In the present study, the safety profile of *Cocos nucifera* L. (Arecaceae) leaf was evaluated by acute and sub-chronic toxicity study of the petroleum ether, chloroform and methanol extracts of *C. nucifera* leaf in Swiss albino mice. In acute toxicity study, each extract up to 2000 mg/kg body weight orally did not produce any toxic effect or death. In sub-chronic toxicity study, the three extracts were administered at the single daily dose of 200 mg/kg body weight orally for 28 consecutive days and at the 29th day, the hematological, histological, serum and hepatic biochemical parameters were evaluated by sacrificing the animals. No mortality was observed during the course of whole study period. No detectable alterations were found in hematological, biochemical and histological parameters in treated groups when compared to vehicle control group after 28 days. The results of the present study therefore indicated that *C. nucifera* leaf is safe in adult male albino mice demonstrating no noticeable toxicity.

**Keyword:** Acute toxicity, biochemical, *Cocos nucifera*, leaf, sub-chronic toxicity.

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### **INTRODUCTION**

*Cocos nucifera* Linn. belonging to the family Arecaceae, is commonly known as *Narkel* in Bengali, *Nariyel* in Hindi and Coconut in English. It is a large palm of about 60 to 90 feet height found across the sea levels of the tropical and subtropical world. It is found throughout India mainly at the costal regions. The coconut is known for its great versatility as observed in the many edible, domestic, commercial and industrial uses of its different parts. [1] It has certain traditional medicinal uses also. Coconut water (coconut liquid endosperm) is a refreshing beverage consumed worldwide as it is nutritious and beneficial for health. It is used in local application in small pox to avoid the burning sensation. It helps in avoiding the spots left after it and also a good way to

avoid dehydration. The fixed oil from fruit is used in nourishing hair and the fruit is regarded as an aphrodisiac agent. [2] The present study aimed to establish the acute and sub-chronic toxicity profile of petroleum ether, chloroform and methanol extracts of *C. nucifera* leaf in adult Swiss albino mice.

## **MATERIALS AND METHODS**

### **Plant material:**

The mature leaf of the plant *Cocos nucifera* L. (Arecaceae) was collected in the month of August 2011 from North 24 Parganas, West Bengal, India. The plant material was taxonomically identified at the Central National Herbarium, Botanical Survey of India, Howrah, West Bengal, India. The voucher specimen (CNH/95/2011/Tech II/591) was maintained in our research laboratory for future reference. The leaves were collected and the midrib of each leaf was separated. Then plant material was shade-dried with occasional shifting and then powdered with mechanical grinder, passing through sieve no. 40, and stored in an air-tight container.

### **Preparation of extract:**

The powdered plant material was extracted with petroleum ether, chloroform and methanol successively using simple percolation technique. The solvent were almost removed from the extracts in a hot water bath leaving the semisolid masses (yields: PECN – 1.79%, CECN – 2.17%, MECN – 2.17%) and stored in conical flasks wrapped with aluminum foil. Preliminary phytochemical screening of these extracts exhibited the presence of triterpenoids and steroids in PECN; triterpenoids in CECN; steroids, saponins, tannins and glycosides in MECN. [3]

### **Drugs and chemicals:**

Bovine serum albumin from Sigma Chemical Co., St. Louis, Mo, USA; Trichloroacetic acid (TCA) from Merck Ltd., Mumbai, India; Thiobarbituric acid (TBA), 5,5'-dithio bis-2-nitro benzoic acid (DTNB), phenazonium methosulphate (PMS), reduced nicotinamide adenine dinucleotide (NADH) and reduced glutathione (GSH) from SISCO Research Laboratory, Mumbai, India; potassium dichromate and glacial acetic acid from Ranbaxy, Mumbai, India. All the other reagents used were of analytical reagent grade obtained commercially.

**Animals:**

Adult Swiss albino mice of either sex weighing 18-25 g were used for the present investigation. They were housed in clean polypropylene cages and maintained under standard laboratory conditions (temperature  $25 \pm 2^\circ\text{C}$  with dark/light cycle 14/10 h). They were fed with standard pellet diet (Hindustan Lever, Kolkata, India) and water *ad libitum*. The animals were acclimatized to laboratory conditions for one week prior to experiment. All experimental procedures were reviewed and approved by the Institutional Animal Ethics Committee.

**Acute toxicity:**

The acute toxicity of PECN, CECN and MECN in Swiss albino mice was studied as reported method. [4] Each extract were given to four groups ( $n = 6$ ) of mice at 50, 500, 1500 and 2000 mg/kg body weight, p.o. The treated animals were kept under observation for 3 days, for mortality and general behaviour. No death was observed till the end of the study.

**Sub-chronic toxicity:**

The adult Swiss albino mice were divided into four groups containing 6 animals per group. The first group received normal saline (5 ml/kg body weight, p.o.) and the other three groups received the three extracts each at 200 mg/kg body weight p.o., respectively daily for 28 consecutive days. Food and water intake of animals were observed during this period. Twenty four hours after the last dose (i.e., at the 29th day), blood was collected from overnight fasted mice of each group by cardiac puncture for estimation of hematological and serum biochemical parameters. Then the mice were sacrificed by cervical dislocation for the study of liver biochemical parameters and organ weights. [5]

**Body weight and organ weights:**

The body weight of mice of each group were measured just before and 28 days after the extract treatment, respectively. Heart, lung, liver and kidney weights of all mice were measured immediately after post treatment sacrifice.

**Hematological studies:**

Collected blood was used for the estimation of hemoglobin (Hb) content; red blood cell count (RBC) and white blood cell counts (WBC) by standard recommended procedures [6, 7].

**Estimation of serum biochemical parameters:**

Collected blood was used for the estimation of serum biochemical parameters viz. serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), serum alkaline phosphatase (SALP), serum cholesterol and total protein contents by using commercially available reagent kits (Span Diagnostics, Surat, India).

**Estimation of liver biochemical parameters:**

Lipid peroxidation i.e., thiobarbituric acid reactive substances (TBARS) was estimated by the previously reported method and expressed as mM/100 g of liver tissue. [8] Reduced glutathione (GSH) was determined by the reported method and was expressed as mg/100 g of liver tissue. [9] Catalase (CAT) activity was assayed according the method described by standard method and expressed as  $\mu$ moles of  $H_2O_2$  consumed/min/mg of liver tissue. [10]

**Statistical analysis:**

The all experimental data were expressed as mean  $\pm$  standard error of mean (SEM).

**RESULTS:**

In acute toxicity study, PECN, CECN, MECN up to 2000 mg/kg body weight orally exhibited no toxic effect or death in mice. There were no significant changes in body weights and organ weights of mice of extract treated groups (after 28 days) from saline control group (Table 1). No mortality was evident from the experimental results in mice. The food and water intake of treated group was found comparable to the control group without showing significant alteration in body weight and growth rate. From the present study it was seen that there was no significant changes in the counts of WBC, RBC and hemoglobin content in the treated group compared to normal control group (Table 2).

After 28 days of treatment no significant alterations were observed in all serum and hepatic biochemical parameters in animals of extract treated group when compared to those of normal control group (Tables 3, 4).

**Table 1:** Effect of *C. nucifera* leaf extracts on body weight and weight of organs in mice.

Treatments	Initial body wt (g)	Final body wt (g)	Final Heart wt (g)	Final Lung wt (g)	Final Liver wt (g)	Final Kidney wt (g)	Final Pancreas wt (g)
Normal control (0.9% NaCl)	19±0.13	26±1.13	0.12±0.05	0.14±0.08	1.19±1.13	0.29±0.95	0.19±0.09
PECN (200 mg/kg)	20±0.19	24±1.29	0.11±0.06	0.12±0.09	1.16±1.05	0.27±0.68	0.17±0.08
CECN (200 mg/kg)	21±0.29	25±1.28	0.12±0.05	0.13±0.07	1.17±1.18	0.28±0.79	0.18±0.06
MECN (200 mg/kg)	20±0.91	26±1.09	0.13±0.03	0.14±0.06	1.18±1.25	0.29±0.98	0.19±0.03

Values are expressed as mean ± SEM ( $n = 6$ ).

## **DISCUSSION:**

The present study was aimed to investigate the possible toxic effects of the pet. ether, chloroform and methanol extract of *C. nucifera* leaf (PECN, CECN, MECN) in adult Swiss albino mice. The results of acute toxicity study revealed that the extracts may be safe in Swiss albino mice. Various parameters were thoroughly studied in the sub-chronic toxicity study. The body weights, food and water intakes were found to be unaltered during the 28 days treatment period when compared to control group. Similarly there were no significant changes in different vital organ weights also. No mortality was observed during this period. Also in the study of hematological parameters there was no alteration of the normal levels of RBC, WBC and hemoglobin compared with the extract treated groups. Therefore, the test extracts had no toxic effect to the blood and haematopoietic system of mice.

**Table 2:** Effect of *C. nucifera* leaf extracts on hematological parameters in mice.

<b>Treatments</b>	<b>Hemoglobin (g/dl)</b>	<b>RBC (10<sup>6</sup> cells/ml)</b>	<b>WBC (10<sup>3</sup> cells/ml)</b>
Normal control (0.9% NaCl)	13.96 ± 0.85	6.63 ± 0.54	3.15 ± 0.42
PECN (200 mg/kg)	11.28 ± 0.36	5.83 ± 0.34	4.36 ± 0.83
CECN (200 mg/kg)	12.73 ± 0.65	6.03 ± 0.56	3.96 ± 0.33
MECN (200 mg/kg)	13.18 ± 0.16	6.23 ± 0.76	3.36 ± 0.69

Values are expressed as mean ± SEM (n = 6).

**Table 3:** Effect of *C. nucifera* leaf extracts on serum biochemical parameters in mice

<b>Treatments</b>	<b>SGOT (IU/dl)</b>	<b>SGPT (IU/dl)</b>	<b>SALP (IU/dl)</b>	<b>Bilirubin (mg/dl)</b>	<b>Cholesterol (mg/dl)</b>	<b>Total protein (mg/dl)</b>	<b>Urea (mg/dl)</b>	<b>Uric acid (mg/dl)</b>	<b>Creatinine (mg/ml)</b>
Normal control (0.9% NaCl)	42.51 ±1.29	35.99 ±1.27	83.29 ±1.89	0.91 ±0.15	151.33 ±9.6	7.22 ±1.7	42.15 ±1.13	6.92 ±1.89	0.95 ±0.13
PECN (200 mg/kg)	48.86 ±1.19	38.63 ±1.63	89.18 ±1.91	1.06 ±0.29	155.69 ±10.6	5.98 ±1.8	45.63 ±1.29	8.05 ±1.63	2.04 ±0.29
CECN (200 mg/kg)	45.92 ±1.78	37.98 ±1.33	88.72 ±1.29	0.99 ±0.18	154.15 ±10.7	6.52 ±1.9	44.23 ±1.27	7.95 ±1.65	1.78 ±0.27
MECN (200 mg/kg)	43.16 ±1.68	36.25 ±1.83	86.28 ±1.19	0.93 ±0.45	153.33 ±10.6	6.92 ±1.5	43.68 ±1.19	7.15 ±1.33	1.18 ±0.19

Values are expressed as mean ± SEM (n = 6).

**Table 4:** Effect of *C. nucifera* leaf extracts on liver biochemical parameters in mice.

<b>Treatments</b>	<b>TBARS (mM/100 g of wet liver tissue)</b>	<b>GSH (mg/ 100 g of wet liver tissue)</b>	<b>CAT (μmoles of H<sub>2</sub>O<sub>2</sub> consumed/min/mg of wet liver tissue)</b>
Normal control (0.9% NaCl)	1.15±0.5	45.54±1.8	83.27±3.3
PECN (200 mg/kg)	1.19±0.6	40.27±1.9	80.18±3.6
CECN (200 mg/kg)	1.18±0.2	41.29±1.8	81.15±3.8
MECN (200 mg/kg)	1.17±0.7	42.17±1.5	82.13±3.1

Values are expressed as mean ± SEM (n = 6).

The serum biochemical parameters were studied to evaluate the possible alterations in hepatic and renal functions influenced by the extracts. Serum biochemical parameters related to hepatic function namely SGPT, SGOT, SALP, bilirubin and cholesterol contents exhibited no significant alterations as compared to the control mice. It is well known that almost all drugs, chemicals and xenobiotics are eliminated through renal excretion hence it was found necessary to estimate the effects of the extracts on kidney functions. [11] Serum biochemical parameters related to kidney functions viz. urea, uric acid creatinine and total protein demonstrated no significant differences with respect to control group animals. Therefore, it can be inferred that all the three extracts did not affect the normal hepatic and renal functions on 28 days treatment.

Free radicals or reactive oxygen species (ROS) are regarded to be involved in the pathogenesis of several degenerative diseases. Antioxidants can retard or stop the uncontrolled generation of ROS, thus help to reduce oxidative stress-induced diseases. [12] In the present study, liver antioxidant parameters viz. lipid peroxidation (TBARS), reduced glutathione (GSH) and catalase activity (CAT) were estimated to ascertain the functioning of normal liver antioxidant defense systems, and it was found that no alterations in these parameters took place thereby implying maintenance of normal hepatic non-enzymatic and enzymatic antioxidant mechanisms during treatment by the test extracts.

From the present investigation, it can be concluded that PECN, CECN and MECN exhibited excellent safety profile in acute and sub-chronic toxicity studies in mice. The present study establishes the reliable safety profile of *C. nucifera* leaf extracts in adult male Swiss albino mice offering no obvious toxicity.

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