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Phytochemical and Pharmacological Studies on Litchi Chinensis Sonn Fruit

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A B S T R A C T
Medicinal plants play an important role in the development of potent therapeutic agents. The preliminary phytochemical study revealed that the aqueous and ethanolic extracts of *Litchi chinensis* fruit were rich in terms of alkaloids, carbohydrates, mucilage except non-reducing polysaccharides moiety. The cardiac and saponin glycosides were present while the anthraquinone and cynogenetin glycosides were absent. Yet the extracts were rich with steroidal moiety, the fat and oil related phytochemical test does not confirm their presence. As the extracts dose up to the 5000 mg/ kg b.w. did not showed any toxic impact on experimental animals, hence the treatment dose for all experimental purpose was selected as 500 mg /kg. b.w. i.e. $1/10^{th}$ of the maximum safer dose. Concurrently the extracts were analyzed for the therapeutic responses against the induced pain as analgesic, induced inflammation as anti-inflammatory, and for effect on CNS using different animal models. Furthermore the extracts were subjected for estimation of their pharmacological values. Prior that the protocol was prepared submitted and approved with institutional animal ethics committee of CPCSEA. All the procedure was carried out according to standard guideline and as per the approved protocol. The acute toxicity study was carried out as per OECD guidelines to decide dose of the extracts. On the basis of these results we can conclude here that the analgesic, anti-inflammatory and CNS depressant effect of these extracts might be due to present phenolic constituents and more appropriately due to flavonoid contents. Still the research work is required to determine the mechanism of action at molecular level.
Keywords: *Litchi chinensis* fruit, Ethanolic extract, Analgesic, Anti-inflammatory and CNS depressant.

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CONTENTS

1. Introduction

Litchi chinensis fruit: New drugs were introduced in the USA drug market including deserpidine, reseinnamine, reserpine, vinblastine and vincristine which are derived from higher plants. Litchi (*Litchi chinensis Sonn*.) is a subtropical fruit that originated in South-East Asia. In recent years, litchi production has increased steadily around

the world.[1-5] Litchi fruit pericarp (LFP) accounts for approximately 15% by weight of the whole fresh fruit and contains significant amounts of phenolics, among which anthocyanins are the major polyphenols. Anthocyanins play an important pharmacological role against various human diseases, such as cardiovascular disease, cancer, inflammation and allergies [8,14,16,17].

2. Materials and methods

Methodology:

Phyto-chemical Screening of *Litchi chinensis* fruit extracts:

Preliminary phytochemical screening for the presence of alkaloids, carbohydrates, saponins, tannins, flavonoids, steroids, resins, and protein and amino acids were carried out using standard test procedures.

Quantitative analysis of phyto-constituents:

Estimation of total phenolic content of *Litchi chinensis* fruit: The total phenolic content in all the extracts was estimated as Gallic acid equivalent; according to the method described earlier**.** From the stock solution (1 mg/ml) of the extract of fruits, suitable quantity was taken into a 25 ml volumetric flask and mixed with 10 ml of water and 1.5 ml of Folin-ciocalteu's reagent. After 5 min, 4 ml of 20% (w/v) sodium carbonate solution was added and volume was made up to 25 ml with double distilled water. After 30 min, the absorbance was recorded at 765 nm. Percentage of total phenolics was calculated from calibration curve of gallic acid (50-500 µg) plotted by using same procedure and total phenolics were expressed as % equivalent to gallic acid.

Determination of flavonoid content:

The total flavonoid content of all the extracts was determined with aluminium chloride ($AlCl_3$) according to the method described earlier, using rutin as a standard. The fruit extracts (0.1 ml) was added to 0.3 ml distilled water followed by 0.03 ml $NaNO_2$ (5%) and incubated for 5 min at 25°C. Later 0.03 ml $AlCl_3$ (10%) was added and further after 5 min, the reaction mixture was treated with 0.2 ml (1mM) NaOH. Finally, the reaction mixture was diluted to 1 ml with water and the absorbance was measured at 510 nm. The flavonoid content was calculated from a rutin standard curve.

Pharmacological Studies:

Approval of protocol

All the experimental procedure and protocols used in the present study were reviewed and approved by the Institutional Animal Ethics Committee (IAEC) constituted under Committee for the Purpose of control and supervision of experiments on animals (CPCSEA). For this, study protocol (form no- Form B of CPCSEA) was prepared and submitted to the institutional ethics committee for approval to carry out experiments on animals. All the ethical guidelines were strictly followed during all the experiments.

Data Analysis:

Data shall be expressed as a mean ± standard error mean (SEM) and statistical analysis shall be carried out by applying one way analysis of variance (ANOVA) followed by Dunett's multiple comparison test. The results will be considered statically significant and highly significant when $p < 0.05$ and $p < 0.001$ respectively.

Acute toxicity studies:

Acute toxicity studies of fruit extracts were studied in rats according to the guidelines for organization of economic cooperation and development (OECD 423). According to the guidelines, the white male albino rats were used for the test. The animals were given the proper diet and kept in 12 hours light and 12 hours dark cycle. Now the rats were kept on over-night fasting before conducting the experiment. Extracts were administered to the animal in graded doses from 5 mg/kg body weight to 5000 mg/kg body weight. Now the mortality and the toxicity sign were observed continuously for 1 hour and then for 24 hours after administration of extracts**.**

Animals did not exhibit any mortality and toxic sign up to dose of 5000 mg/kg body weight. So $1/10^{th}$ of the maximum safer dose was selected as the test doses. Therefore, 500 mg/kg b.w. dose of the extracts was selected for the pharmacological screening of *Litchi chinensis* fruit extract on the basis of preliminary acute toxicity studies.

Pharmacological screening of extracts:

Evaluation of analgesic activity: Analgesic study was determined in two different models:

Hot plate method: Experimental animals of either sex were randomly selected and divided into 4 groups designated as group-I, group-II, group-III, group-IV consisting of four rats in each group. The animals were positioned on Eddy's hot plate kept at a temperature of 55±0.5 0C. A cut off period of 15 was observed to avoid damage to the paw. Reaction time was recorded when animals licked their fore or hind paws, or jumped prior to and 0, 30, 60 and 90 min after oral administration of the samples.

- **Group I:** Received normal saline (10 ml / kg, b.w.), treated as controlled group.
- **Group II:** Received 500 mg / kg b. w., aqueous extract of *Litchi chinensis* fruit.
- **Group III:** Received 500 mg / kg b. w., ethanolic extract of *Litchi chinensis* fruit.
- **Group IV:** Diclofenac Sodium (10 mg / kg, b. w.), treated as standard.

Acetic acid induced writhing method:

The analgesic activity of the fruit extracts was evaluated using acetic acid induced writhing method in mice. In this method, acetic acid [0.7% v/v acetic acid solution, 0.1 ml/10 g] is administered intra-peritoneally to the experimental animals to create pain sensation. The plant extracts were administered orally. The rats of either sex were divided into six groups each containing four. All the treatments (standard, test and vehicle) were made 30 minutes prior to administration of acetic acid solution.

Group II: Received 500 mg / kg b. w., aqueous extract of *Litchi chinensis* fruit.

Group III: Received 500 mg / kg b. w., ethanolic extract of *Litchi chinensis* fruit.

Group IV: Diclofenac sodium (10 mg / kg, b. w.), treated as standard.

Then the animals were placed on an observation table. Each rat of all groups were observed individually for counting the number of writhing, they made in 15 minutes commencing just 5 minutes after the I.P administration of acetic acid.

Evaluation of anti-inflammatory activity:

The male wistar rats (180-200 g, b.w.) were selected for the study. They were housed in polypropylene cages and were left for two days for acclimatization to animal room maintained under controlled condition of (12 hours light and dark cycle at 22 ± 3 ^{0}C), and were kept on standard pellet diet with water *ad libitum.* Before the study the animals were fasted overnight with free access to water.

Carrageen an induced rat paw edema:

24 healthy male wistar rats were divided into 4 different groups, each containing 6 animals. In this experiment paw edema was induced by injecting 0.1 ml of 1 % carrageen an prepared in 0.9 % normal saline solution into sub plantar tissue of right hind paw. Paw volume was measured with the help of plethysmometer by mercury displacement method at 0 hr, 1 hr, 2 hr, 3 hr, 4 hr, 6 hr and 24 hr.

1 hour before, inducing rat paw edema, all the established six groups were treated orally as follows;

Group I: Received normal saline (2 ml / kg, b.w.), treated as controlled group.

Group II: Received 500 mg / kg b. w., aqueous extract of *Litchi chinensis* fruit.

Group III: Received 500 mg / kg b. w., ethanolic extract of *Litchi chinensis* fruit.

Group IV: Indomethacin (10 mg / kg, b. w.), treated as standard.

Percentage Inhibition = (1 - V_t / V_c) x 100

Where; V_t = Paw volume after drug / extract treatment

V_c = Paw volume in the control

Cotton pellets induced granuloma in rats:

24 healthy male wistar rats were divided into 4 different groups, each containing 6 animals and shaved on the back of neck. Cotton pellets weighing 50 mg each, were sterilized by autoclaving at 120±1 ^{0}C for 1 hour and implanted subcutaneously, one on each side of the subscapular region under the light ether anaesthesia.

Group I: Received normal saline (2 ml / kg, b.w.), treated as controlled group.

Group II: Received 500 mg / kg b. w., aqueous extract of *Litchi chinensis* fruit.

Group III: Received 500 mg / kg b. w., ethanolic extract of *Litchi chinensis* fruit.

Group IV: Indomethacin (10 mg / kg, b. w.), treated as standard.

Evaluation for CNS activity:

Evaluation of loco-motor function using actophotometer:

Twenty four albino rats of either sex are taken and weighing roughly above 150-250 g, and housed in polypropylene cages in a temperature-controlled room (25±2°C) with a 12 h light/12 h dark cycle. All rats will be fed a standard pellet chow diet for 1 week. Food intakes will be monitored daily, and body weights will be measured weekly. All rats will be randomly divided into four groups.

The animals were divided into 4 groups containing 4 animals in each. The normal control group (I), fed with saline water at the dose of 10 ml/kg body weight and treated with diazepam I.P. at the dose of 5 mg/kg. Other four groups (II, IV and III) were treated with the aqueous and ethanol extract of *Litchi chinensis* fruit respectively. The spontaneous locomotor activity of each rat was recorded individually by actophotometer for 10 minutes at the time interval of 30 min, 60 min, 120 min, and 180 min.

Evaluation of muscle relaxant activity using Rotarod method:

This method is used for the testing of muscle relaxant activity in the animals. The animals were divided into 4 group containing 4 animals in each group. The normal control group (I), fed with saline at the dose of 10 ml/kg body weight and treated with diazepam I.P. at the dose of 5 mg/kg. Other four groups (II, IV and III) were treated with the aqueous and ethanol extract of *Litchi chinensis* fruit respectively.

Rotarod apparatus was used in the experiment. The animals were placed at the horizontal rod rotating at 25 rpm and the time to fell down for the animals from the rod is noted down. Experiment was done at the time interval of 30 min, 60 min, 120 min, 180 min and the readings are noted.

Evaluation of anti-anxiety effect using Elevated plus-maze test:

This method is used for the testing of anti-anxiety activity in the animals. The animals are divided into 4 group containing 4 animals in each. The normal control group (I), fed with saline at the dose of 10 ml/kg body weight and treated with diazepam I.P. at the dose of 2 mg/kg. Other four groups (II, IV and III) were treated with the aqueous and ethanol extract of *Litchi chinensis* fruit respectively.

- The number of entries into the open arms (for 5 minutes)
- Average time spent by the rat in the open arms (for 5 minutes)
- (Average time = total time spent in open arms/number of entries in arms).
- Number of entries into close arm (for 5 minutes)
- Average time spent by the rat in the close arm (for 5 minutes)

Evaluation of anti-convulsion effect:

Maximal electric shock model:

Twenty-four healthy Albino rats, of either sex (150-250 g), were divided into 4 groups (n = 4 each). The groups I was given normal saline 10 ml/kg, received diazepam 5 mg/kg i.p., other four groups (II, IV and III) were treated with the aqueous and ethanol extract of *Litchi chinensis* fruit respectively, orally. After 60 minutes of administering the dose, electrical stimulus (150 mA, 0.2 sec duration) was applied through ear-clip electrodes using electro convulsiometer. Electro convulsiometer is used to provide electric shock to rats and then the animals are able to produce hind limb tonic convulsion and time to produce the convulsion is noted down and the % protection was calculated for each group of animals.

Chemically induced convulsion method:

Twenty-four healthy Albino rats, of either sex (150-250 g), were divided into 6 groups (n = 4 each). The groups I was given normal saline 10 ml/kg, Group VI received diazepam 5 mg/kg I.P., other four groups (II, IV and III) were treated with the aqueous and ethanol extract of *Litchi chinensis* fruits respectively, after giving all the treatment of different dosses of standard and test drugs, pentylenetetrazole was administered at the dose of 80 mg/kg body weight I.P. and animals were individually placed in plastic cases and observed immediately after PTZ injection for 30 minutes. The onset and duration of myoclonic jerks/convulsions or hind limb tonic seizures as well as the percentage of protection against mortality were recorded.

3. Result and Discussion

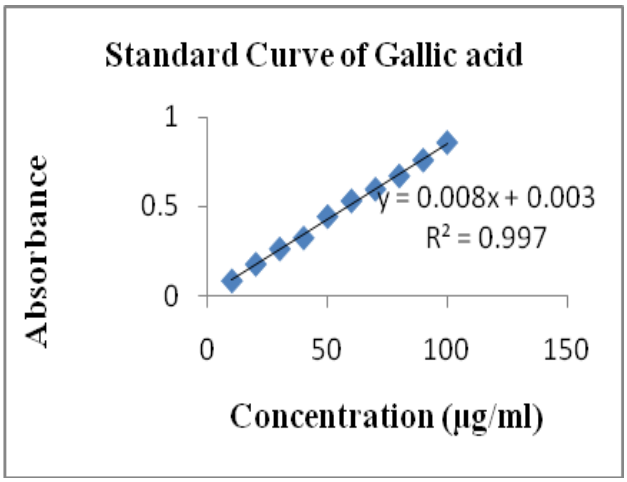

Figure 2: Standard curve of gallic acid

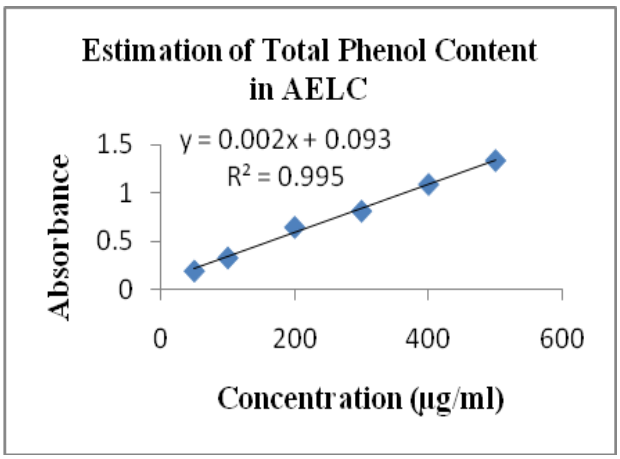

Figure 3: Total Phenol Content in AELC

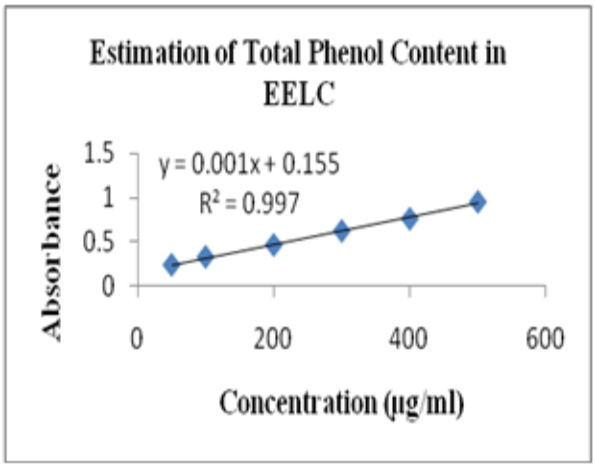

Figure 4: Total Phenol Content in ethanolic extract of *Litchi chinensis*

Table 1: Result of Preliminary phytochemical screening of aqueous and ethanolic extract of *Litchi chinensis fruits*

Phytochemicals	Test	AELC	EELC
Alkaloids	General Test	+	+
Carbohydrates (Monosaccharides, Oligosaccharides and Polysaccharides)	General Test	+	+
	Monosaccharides	+	+
	Pentose Sugars	+	+
	Hexose Sugars	+	+
	Non Reducing Polysaccharides	_	_
	Gums	_	_
	Mucilage	+	+
Proteins and Amino acids	Proteins	_	_
	Amino Acids	_	_
Glycosides	General Test	+	+
	Cardiac Glycosides	+	+
	Anthraquinone Glycosides	_	_
	Saponin Glycosides	+	+
	Cyanogenetic Glycosides	_	_
Flavonoids		+	+
Tannis and Phenolic Compounds	General Test	+	+
Steroids		+	+
Volatile Oils		_	_
Fats and Oils		_	_

Table 2: Total flavonoid content

Sr. No.	Fruit Extract	Total Flavonoid Content (mg/G rutin equivalent)
1.	AELC	15.02
2.	EELC	22.10

Pharmacological screening results:

Analgesic Activity:

Table 3: Effect of aqueous and ethanolic extract of *Litchi chinensis fruits* against acetic acid-induced writhing in rats.

Groups	Dose (Mg/kg) b.w	Mean latency before and after drug administration (S) (Min)				% Inhibition (at Min.)		
		0	30	60	90	30	60	90
Group-I	Vehicle	2.30 ± 0.12	2.50 ± 0.31	2.44 ± 0.61	2.31 ± 0.32	-	-	-
Group-II	AELC (500)	2.38 ± 0.62	3.12 ± 0.71*	5.61 ± 0.42*	4.82 ± 0.32*	19.87	56.51	52.07
Group- III	EELC (500)	2.12 ± 0.71	4.81 ± 0.25**	7.23 ± 0.12**	6.19 ± 0.39**	48.02	66.25	62.68
Group- IV	Diclofenac sodium (10)	2.49 ± 0.19	6.62 ± 0.23**	9.94 ± 0.13**	11.31 ± 0.39**	62.23	75.45	79.58

Acetic acid induced writhing method:

Table 4: Effect of aqueous and ethanolic extract of *Litchi chinensis* fruit against acetic acid-induced writhing in rats.

Groups	Dose (mg/ kg) b.w	No. of Writhing	Percent Inhibition
Group-I	Vehicle	42.91 ±1.29	-
Group-II	AEAD (500)	22.42±1.05*	47.75
Group- III	EEAD (500)	12.31±1.31**	71.31
Group- IV	Diclofenac sodium (10)	10.18±1.19**	76.27

Table 5: Anti-inflammatory effects of aqueous and ethanolic extracts of *Litchi chinensis* fruit on carrageenan-induced rat paw oedema

Group	Treatment / Dose (mg/kg b.w., Oral)	Percentage of hind paw edema at time (h) after Carrageenan administration						
		0h	1h	2h	3h	4h	6h	24h
I	Control 2 ml/kg	101.00 ± 1.18	169.00 ± 2.16	211.50 ± 1.81	217.75 ± 2.07	221.39 ± 3.16	232.06 ± 2.12	215.72 ± 2.14
II	AELC (500)	101.00 ± 2.14	119.42 ± 2.85*	145.22 ± 2.12*	171.16 ± 3.25*	182.44 ± 2.19*	174.16 ± 2.08*	154.17 ± 2.23*
III	EELC (500)	101.00 ± 2.12	109.11 ± 2.25**	121.27 ± 1.41**	131.14 ± 3.21**	139.81 ± 3.19**	117.23 ± 2.16**	119.22 ± 3.42**
IV	Indomethacin (10)	102.00 ± 1.12	104.19 ± 3.21**	109.12 ± 1.21**	107.12 ± 1.24**	104.19 ± 2. 42**	103.01 ± 2.38**	102.62 ± 3.28**

CNS Activity: Evaluation of loco-motor function using acto photometer:

Table 6: Effects of aqueous and ethanolic extracts of *Litchi chinensis* fruit on locomotor activity

Group	Treatment/ Dose (mg / k.g. b.w.)	Time (Min.)				
		0	30	60	120	180
I	Normal Saline (10 ml)	203.11±2.16	212.21±3.14	218.32±2.62	206.09±2.24	209.18±3.11
II	AELC (500)	202.69±3.81	149.36±2.84*	137.42±3.71*	119.32±2.40*	148.13±2.22 *
III	EELC (500)	204.19±3.80	88.91±2.31**	77.51±3.74**	72.18±2.14**	84.12±2.51 **
IV	Diazepam (5)	204.42±4.21	38.5±3.08	21.25±2.31	14.21±1.21	22.13±3.22

Evaluation of effect on muscle relaxant activity by using rotarod apparatus:

Table 7: Effects of aqueous and ethanolic extracts of Litchi *chinensis* fruit on muscle relaxant activity

Group	Treatment/ Dose (Mg / k.g. b.w.)	Time (Min.)				
		0	30	60	120	180
I	Normal Saline (10 ml)	23.25±2.06	24.13±1.70	22.75±2.69	23.24±2.64	22.19±1.25
II	AELC (500)	24.21±1.62	21.37±1.84 *	20.13±0.74 *	17.29±1.24*	21.04±1.46*
III	EELC (500)	22.36±10.72	17.27±0.64 **	14.22±0.62**	10.36±0.28 **	13.45±.49**
IV	Diazepam (5)	23.36±0.87	4.19±0.46**	3.29±0.75**	2.09±0.49**	3.82±0.88**

Assessment of anti-anxiety activity:

Table 8: Anti-anxiety activity of aqueous and ethanolic extracts of Litchi *chinensis* fruits

Group	Treatment /Dose (mg / k.g. b.w.)	Time spent (Sec.) Open arm	No of entries Open arm
I	Normal Saline(5 ml)	20.29±1.01	2.39±1.21
II	AELC (500)	22.94±1.35 *	6.14±1.32 *
III	EELC (500)	36.14±1.32**	12.32±1.12 **
IV	Diazepam (2)	58.54±1.58	18.19±1.64

Assessment of anticonvulsant activity:

Table 9: Anticonvulsant effect of aqueous and ethanolic extracts of *Litchi chinensis* fruits on MES -induced convulsions

Group	Treatment / Dose (mg / k.g. b.w.)	HLTC (sec)	Total duration (sec)	% Protection
I	Normal Saline(5 ml)	23.49 ± 1.31	73.11 ± 1.43	------
II	AELC (500)	20.11 ± 1.06 *	56.28 ± 1.12 *	23.02
III	EELC (500)	14.39 ± 1.11 **	32.18 ± 1.42 **	55.98
IV	Diazepam (5)	4.61 ± 1.62**	13.21 ± 1.96**	81.93

Effect on PTZ induced convulsion:

Table 10: Anticonvulsant effect of aqueous and ethanolic extracts of *Litchi chinensis fruit* on PTZ -induced convulsions

Group	Treatment/ Dose (mg / k.g. b.w.)	Onset of convulsion (sec)	Total duration (sec)	HTLC (sec)	Protection (%)
I	Normal Saline(5ml)	46.21± 1.21	148.32 ± 1.06	17.31 ± 1.22	-----
II	AELC (500)	55.18 ±1.23**	101.11±1.41**	14.72± 1.09	31.83
III	EELC (500)	74.08 ±1.54**	69.14±1.50**	6.91± 1.35	53.38
IV	Diazepam (5)	113.41 ± 1.07	22.24±1.21	3.86± 1.01	85.00

4. Conclusion

The objective of present study was to evaluate the effect of aqueous and ethanolic extract of *Litchi chinensis* fruit for anti-inflammatory, analgesic and CNS activities. The plant parts were subjected for the qualitative phytochemical screening yet the fruits were collected from pollutant free and hygienic area. Furthermore the extracts were subjected for estimation of their pharmacological values. Prior that the protocol was prepared submitted and approved with institutional animal ethics committee of CPCSEA. All the procedure was carried out according to standard guideline and as per the approved protocol. The acute toxicity study was carried out as per OECD guidelines to decide dose of the extracts. As the extracts dose upto the 5000 mg/ kg b.w. did not showed any toxic impact on experimental animals, hence the treatment dose for all experimental purpose was selected as 500 mg /kg. b.w. i.e. $1/10^{th}$ of the maximum safer dose. EEAD, EELC.

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