Panacea Research Library

Journal Homepage: www.prlpublisher.com/pharmacy/pjpps

Panacea Journal of Pharmacy and Pharmaceutical Sciences
ISSN: 2349 7025

Original Research Article

Pharmacological evaluation of nanoemulsion of the seed extract of *Carica papaya* for male oral contraceptive action in mice

Meenakshi Sinha*[1], K. Balamurgan[1], N. Ganesh[2]

[1]*Department of Pharmacy, Faculty of Engineering and Technology, Annamalai University, Annamalai Nagar, Chidambaram - 608002, Tamil Nadu, India;* [2]*Jawaharlal Nehru Cancer Hospital and research Centre, Idgah Hills, Bhopal – 462001, Madhya Pradesh, India*

Article Info

Article history:
Received: 18 Sep 2014
Received in revised form: 15 Sep 2014
Accepted: 18 Sep 2014
Available online: 20 Oct 2014

Keywords:
Carica papaya
Contraceptives
Evaluation
Nanoemulsion

Abstract

Carica papaya Linn. is the most widely cultivated and best known species serve as contraceptive as reported in ancient literature. In aim of the present study is to demonstrate the male oral contraceptive efficacy of seed oil of *Carica papaya* Linn. *In vivo* evaluation and comparison between crude extract and nanoemulsion for oral male contraceptive activity were carried out on Swiss albino mice including MTT cell cytotoxicity assay. From this study it has been demonstrated that *Carica papaya* seed oil possesses reversible male contraceptive potential, when administered orally. Even though the crude seed oil is more effective contraceptive the nanoemulsion can be considered a better contraceptive as it does not cause any behavior changes, morbidity and tissue toxicity as well as easily administered and delivered. Hence papaya seed oil can be utilized for delivery of contraceptive action in males.

Introduction

Oral contraceptives are among the most widely used agents used throughout the world and reported to have a revolutionary impact on global society. For the first time in history, they provide a convenient affordable and completely reliable means of contraception for family planning and avoidance of unplanned pregnancies. The population explosion is one of the major problems of present era in the world. The increment of population raises so many sufferings like lack of food, water, energy and raw material supply, decline in mortality etc. before human beings and it also has increased the life expectancy. In view of above discussion, scientists have started to tackle this serious problem by developing the effective contraceptives. Papaya tree belongs to a small family *Caricaceae* having four genera in the world. The genus *Carica* Linn. is represented by four species in India, of which *Carica papaya* Linn. is the most widely cultivated and best known species. Among the other species, *C. cauliflora*, *C. pubescens* Lenne and *C. quercifolia* are possible sources of breeding material for inducing frost and virus resistance in cultivated papaya. The fruits, leaves and latex obtained from papaya plant are used medicinally and for various other purposes. Papain, a major chemical compound extracted from fruit and stem latex is used in brewing and wine making and in the textile and tanning industries.

Carica papaya Linn. is the most widely cultivated and best known species serve as contraceptive as reported in ancient literature. The aim of the present study is to evaluate an herbal formulation based on papaya seed oil (nanoemulsion, prepared previously in our lab and data has been already published) for oral male contraceptive potential in comparison with its crude extract in Swiss albino mice.

Experimental

Plant collection

Carica papaya fruit were collected from Bhopal fruit market in June 2013. Plant was authenticated by Dr.

***Corresponding author:** Meenakshi Sinha. E-mail: msinha1612@gmail.com
These authors have no conflict of interest to declare.
Copyright © 2014, Panacea Journal of Pharmacy and Pharmaceutical Sciences. All rights reserved

Patil at Government M.L.B. Postgraduate Autonomous College, Bhopal, India. A sample was preserved in our college herbarium with sheet number 309.

Animals

Swiss albino mice (25-30 g) were provided from Jawaharlal Nehru Cancer Hospital and Research Centre, Bhopal, India. They were maintained at a temperature of 25 ± 1 °C and relative humidity of 45 ± 5% under 12 hr light/12 h dark cycle. The animals have free access to feeds (894/8 Mehrauli, New Delhi, India) and water *ad libitum*. The animal studies were approved by the IAEC constituted As per CPCSEA guidelines (CPCSEA registration number: 2004/EC/2010-18.11.2010).

Extraction

Seeds were isolated from the fruits, washed with double distilled water. Seeds were shade dried and powdered with the help of mixer grinder. Weight of powdered seeds was noted down and was extracted successively with chloroform and methanol in Soxhlet extraction apparatus. The extraction was carried out at 55 °C, 8 h for 10 days. About 200 g of seed powder was extracted. The extract was filtered and the resultant filtrate was distilled in order to remove the solvent completely. The volume of the oil extracted with chloroform was measured. Semisolid content that was extracted with methanol was weighed and percentage yield was calculated.

Cell cytotoxicity assay by MTT

Blood was obtained from heart of mice. Five milliliters of heparinized blood was taken and layered over 2 ml of Hi-Sep solution gently in a centrifuge tubes. Tubes were centrifuged for 1 h at 3500 rpm. The buffy coat layer at the interface between plasma and RBCs sediment was collected and the cells were washed with Hi-sep media in centrifuge tubes. The cells were counted with help of Nauebauer chamber. RPMI-1640 media (5 ml) and 200 µl of phytoheamagglutinin (PHA) were added to isolated cells. The culture was incubated in carbon dioxide incubator at 37 °C for 48 h. Now culture were transferred to centrifuge tubes and centrifuged at 1500 rpm for 15 min. The cells were again counted with the help of Nauebauer chamber. After designated incubation of culture flasks, the cells were counted using a haemocytometer and cell viability was determined by trypan blue exclusion. A 3×10^4 cells in 100µl of MEM media were plated per well in a 96 well plate. Media alone was taken as blank, whereas, cells with media as control. Now 25 µl of various test samples were plated as per scheme in their assigned wells. Cells were also incubated with standard drugs (cyclophosphamide, paclitexal, cisplatin and oxaliplatin] and vehicle control as 5% DMSO in a few number of wells. Well plates (96) were incubated for 24 h in a humidified incubator at 37 ºC with 5% CO_2. After completion of 24 h, 5 mg/ml of 20 µl MTT prepared in PBS (pH 7.4) was plated in each well and the plate was placed on a shaking table for 5 min, in order to mix the MTT into the media. The 96 well plates were incubated at 37 ºC with 5% CO_2 for 3 h. After incubation, 100 µl of media is discarded from each well and are dried. The formazan produced is re-suspended in a 100 µl DMSO and placed on a shaking table for 5 min to thoroughly mix the formazan into the solvent. The plate cover was removed and absorbance was measured in each well including the blank, control, positive and vehicle control at 630 nm in microplate reader. The average values were determined from triplicate readings. The percentage cell survival and percentage cell were calculated by following formula:

Percentage cell viability = (OD drug mean/OD blank mean) × 100

In vivo male contraceptive study

The amount of test drugs administrated was calculated depending on the body weight of animal. Chloroform extract and nanaoemulsion was given in 25 and 45 mg/kg body weight. The drug was then administrated orally to each animal with help of metal canuula. All animal were divided into 14 groups of 3 animals each. The groups were treated as follows:

Group I (control group): Animals were treated with vehicle (9:1 tween 80 and span 80 blends) orally for 10 days.

Group II: Animals treated with normal feed.

Group III: Animals were treated with single dose of chloroform extract (25 g/kg) orally for 10 days and after dosing period mice were sacrificed.

Group IV: Animals were treated with single dose of chloroform extract (25 g/kg) orally10 days and after dosing animal were kept for mating with female mice.

Group V: Animals were treated with single dose of chloroform extract (45 g/kg) orally for 10 days and after dosing period mice were sacrificed.

Group VI: Animals were treated with single dose of chloroform extract (45g/kg) orally for 10 days and after dosing period mice were kept for mating with female mice.

Group VII: Animals were treated with single dose of nanoemulsion prepared by chloroform extract of seed oil (25 mg/kg) orally for 10 days after dosing mice were sacrificed.

Group VIII: Animals were treated with single dose of nanoemulsion prepared by chloroform extract of seed oil (25 mg/kg) orally for 10 days after dosing mice were kept for mating with female mice.

Group IX: Animals were treated with single dose of nanoemulsion prepared by chloroform extract of seed oil (45 mg/kg) orally for 10 days after dosing mice were sacrificed.

Group X: Animals were treated with single dose of nanoemulsion prepared by chloroform extract of seed oil (45 mg/kg) orally for 10 days after dosing mice were kept for mating with female mice.

Group XI: Animals (female mice) with normal feed and kept for mating with male mice which were treated with chloroform extract (25 mg/kg).
Group XII: Animals (female mice) with normal feed and kept for mating with male mice which were treated with chloroform extract (45 mg/kg).
Group XIII: Animals (female mice) with normal feed and kept for mating with male mice which were treated with nanoemulsion (25 mg/kg).
Group XIV: Animals (female mice) with normal feed and kept for mating with male mice which were treated with nanoemulsion (45 mg/kg).
Sperm count: Epididymides from each mouse were removed. Epididymmal spermatozoa were quickly obtained by punctures of both caput and cauda epididymes with a disposable hypodermic needle (gauge 21). Samples of approximately 1:2 of fluid from the epididymes lumens of caput and cauda were tend to a 20-fold dilution with a spermicidal solution containing 5 g of sodium bicarbonate and 1 ml of 35% formaldehyde in 100 ml physiological saline. The sperms were counted using a Neubauer heamocytometer.

Statistical analysis

Data were expressed as mean ± S.D. and statistical analysis was carried out using Tucky-Kramer multiple comparisons test. The values were calculated and analyzed using GraphPad Instant 3.06 software run on windows XP (Microsoft Corporation).

Results and Discussion

A total of 185 gm of dried seed powder was subjected to this method of extraction, yielding around 42.50 ml of seed oil.

In vitro cell cytotoxicity assay

In order to determine the toxicity of the oil to cells and tissue, MTT Assay was carried out. Lymphocytes from blood were isolated and around 4800-5000 cells were feed per well in a 96 well plate. The oil at 50% (in chloroform) and 100% concentrations was added to the wells. A vehicle control (chloroform) was also included. The plate was incubated in a CO_2 incubator at 37^{o}C for about 20-22 h, followed by addition of yellow MTT dye (5 mg/ml of PBS) and further incubation at 37^{o}C for 4-8 h. The viable cells were able to take up the tetrazolium salt and by the action of mitochondrial enzyme, succinate dehydrogenase, reduce it to purple formazan crystals. These crystals being insoluble precipitate out and can be solubilised in DMSO. The absorbance was read at 630 nm. The percentage viability of cells incubated with various oil concentrations was calculated (Table 1). The papaya seed oil at both 50% and 100% concentrations exhibited no toxicity to the cells with percent viability being greater than 99.9%. Chloroform exhibited some level of toxicity with the cell viability being reduced to around 84%. Its toxic effect was however not observed when used as a vehicle.

Table 1: Cell viability assay.

Group	Absorbance at 630 nm	Mean A_{630}	% Viability*
Media only	0.519 0.567 0.569	0.55	-
Media + cells	0.562 0.635 0.612 0.705 0.638 0.613	0.61	100
Vehicle control (chloroform)	0.558 0.566 0.593 0.539 0.500 0.551 0.477	0.51	84.02
50% Papaya seed oil	0.910 0.838 0.941	0.89	>99.9
100% Papaya seed oil	- 1.197 1.084 1.040 0.911	1.05	>99.9

**(Absorbance of test sample/absorbance of cells) × 100*

In vivo studies to determine male oral contraceptive potential

Studies to determine the contraceptive potential of the papaya seed oil were carried out *in vivo* in male Swiss albino mice. Two experimental groups were taken, each comprising of three Swiss albino males. Group 1 was administered with 50 µl of prepared drug and Group 2 with 100µl of the same. The dose was administered orally for the duration of six days (Table 2).
All the male mice were kept each in separate cages and away from female mice for three days prior to mating. Twelve healthy female Swiss albino mice, 6-8 weeks old, were selected for mating. Their initial body weights were measured. Also, vaginal smears (stained with methylene blue/giemsa) were prepared to determine their respective oestrous phases. (Table 3)

Table 2: Initial weights of male Swiss albino mice.

Group		Body Weight (g)
1	a	26.60
	b	28.55
	c	27.00
2	A	30.00
	B	27.65
	C	27.00

Table 3: Body weights and oestrous cycle phases of female Swiss albino mice on the day mating initiation.

Female code	Body weight (g)	Oestrous cycle phase
F1	21.10	Diestrous
F2	19.50	Diestrous
F3	21.95	Diestrous (late)
F4	22.55	Metestrous
F5	22.50	Proestrous
F6	21.40	Proestrous
F7	25.25	Proestrous
F8	25.70	Diestrous
F9	25.10	Metestrous
F10	18.90	Metestrous
F11	23.90	Diestrous
F12	17.70	Proestrous

Following three days of abstinence, each male Swiss albino was kept for mating with two female Swiss albino mice for duration of five days. During these five days body weights, presence/absence of vaginal plug and mounting frequency were monitored. No vaginal plug was observed during these 5 days. Mounting at a very low frequency, occurred only for the initial three days (Table 4) and was mostly observed to occur in the evening hours (4-6 pm). The weights of all mice, especially females, during mating period and for a few days thereafter were monitored to determine occurrence of pregnancy (Table 5). However, no significant variation in weights was observed indicating that pregnancy was not induced.

Table 4: Mounting frequencies of male Swiss albino mice during the mating period.

Day	Male mice					
	G1a	G1b	G1c	G2a	G2b	G2c
1	-	1	-	1	2	1
2	-	-	2	1	-	-
3	-	-	-	1	-	-
5	-	-	-	-	-	-
6	-	-	-	-	-	-

Table 5: Body weights (g) of mice over a period of 40 days (measured intermittently).

Mouse code	Day													
	1	2	3	5	6	14	15	16	17	19	20	21	22	23
G1a	25.50	26.10	26.80	26.50	28.65	27.55	27.60	28.80	28.90	28.75	28.80	26.30	28.65	28.60
F1	20.80	20.75	21.25	20.50	22.15	20.60	20.70	21.80	21.75	22.35	21.55	20.20	21.25	22.00
F2	18.45	18.70	19.60	18.90	19.30	18.70	19.00	20.05	20.25	18.60	18.90	18.45	20.10	19.10
G1b	29.50	28.55	29.35	28.70	29.40	27.50	28.50	30.30	29.80	30.30	29.45	29.40	29.90	30.75
F3	22.30	22.00	23.30	22.60	21.90	23.30	24.50	24.50	24.60	25.00	25.00	24.70	24.55	24.00
F4	22.85	22.90	23.10	23.65	23.95	20.50	21.40	20.60	21.05	20.20	16.80	16.30	17.60	17.55
G1c	25.70	25.60	25.90	25.30	23.85	22.40	22.60	22.95	23.35	23.70	23.80	23.15	24.00	24.10
F5	22.90	22.30	22.80	22.60	22.40	22.40	22.40	22.50	22.75	23.15	23.55	22.75	23.10	23.30
F6	23.35	23.00	23.60	22.35	22.45	22.30	21.40	22.40	22.65	23.40	22.80	22.95	22.85	23.15
G2a	29.10	29.50	29.00	30.30	31.70	24.85	31.45	31.40	30.75	31.30	32.00	29.90	31.45	31.35
F7	24.65	24.90	24.55	27.15	26.75	24.45	25.00	25.25	25.50	25.90	26.50	25.20	26.50	26.30
F8	24.30	24.80	24.65	26.20	26.20	30.70	25.00	24.50	25.10	25.30	26.15	23.80	24.85	25.95
G2b	26.00	26.35	25.90	25.05	25.35	20.60	20.85	20.40	20.40	20.55	20.50	20.40	20.70	24.45
F9	24.80	24.00	24.00	24.25	25.00	22.85	23.00	23.30	23.80	24.10	24.00	23.40	24.45	25.55
F10	19.40	19.80	19.70	21.50	22.10	20.60	21.00	20.80	20.60	21.30	21.35	21.40	21.70	20.45
G2c	26.60	26.80	26.50	26.30	27.10	24.80	24.10	24.65	24.30	24.40	24.50	24.95	24.95	20.35
F11	23.50	23.60	24.00	24.20	25.25	25.00	25.35	25.40	27.05	27.20	25.45	25.30	26.20	25.10
F12	18.60	18.50	18.75	18.30	19.15	19.70	19.95	20.70	21.60	21.00	20.15	20.65	20.25	21.60

Table 6: Sperm count.

Group/mouse code	Sperm count in all four corner squares of Neubaur's chamber	Total sperm count/ml
G1a	364	182,000
G1b	384	192,000
G1c	283	188,666
G2a	347	173,500
G2b*	-	-
G2c	368	184,000

**succumbed to infection*

After forty days, the male mice were sacrificed. Their sperm count was recorded by first dissolving the semen sample (obtained from vas deferens) in 500 µl of normal saline, followed by determining their viability using trypan blue exclusion assay (2:1, sample and trypan blue) (Table 6). Also, a smear of diluted sample was prepared, stained with eosin and DPX mounted for further analysis. Blood from each of the mice was drawn and serum separated and stored at 4 ^{o}C for further studies. Tissue samples of testis and jejunum section of intestine were obtained and fixed in 10% formalin solution for histopathological analysis.

Conclusion

From this study we can conclude that *the Carica papaya* seed oil possess reversible male contraceptive potential, when administered orally. Even though the crude seed oil is more effective contraceptive the nanoemulsion can be considered a better contraceptive

as it does not cause any behavior changes, morbidity and tissue toxicity as well as easily administered and delivered. Hence papaya seed oil can be utilized for delivery of contraceptive action in males.

References

1. Bagros M. Research on the coagulation of cervical secretion envisaged as contraceptive process. Therapie 1979;34:523-527.
2. Bingel AS, Benoit PS. Oral contraceptives: therapeutics versus adverse reactions, with an outlook for the future I. J Pharm Sci 1973;62:179-200.
3. Chinoy NJ, George SM. Induction of functional sterility in male rats by low dose Carica papaya seed extract treatment. Acta Eur Fert1983;14:425-429.
4. Chinoy NJ, D'Souza JM, Padman P. Contraceptive efficacy of *Carica papaya* seed extract in male mice (Mus musculus). Phytother Res 1995;9:30-36.
5. Chopra RN, Nayar SL, Chopra IC. *Glossary of Indian Medicinal Plants*. New Delhi: Publications and Information Directorate, Council of Scientific and Industrial Research; 1956, p. 256-257.
6. Deshpande VY, Mendulkar KN, Sadre NL. Male antifertility activity of *Azadirachta Indica* in mice. J Postgrad Med 198026:167-169.
7. Dhawan BN, Dubey MP, Mehrotra BN, Tandon JS. Screening of Indian plants for biological activity: Part IX. Indian J Exp Biol 1982;18:594-602.
8. Lohiya NK, Goyal RB, Jayaprakash D, Ansari AS, Sharma S. Antifertility effects of aqueous extract of *Carica papaya* seeds in male rats. Planta Med 1994;60:400-405.
9. Lohiya NK, Pathak N, Mishra PK, Manivannan B. Contraceptive evaluation and toxicological study of aqueous extract of the seeds of *Carica papaya* in male rabbits. J Ethnopharmacol 2000;70:17-27.