ejpmr, 2021,8(4), 519-523

SJIF Impact Factor 6.222

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

SYNERGISTIC EFFECT OF SOME PLANT SEED EXTRACTS ON BODY AND ORGAN WEIGHT OF FEMALE WISTAR RATS- A CONTRACEPTIVE APPROACH

***[1]Asha B. Kadam and [2]B. M. Gaykar**

[1]Department of Botany, Dada Patil Mahavidyalaya, Karjar, Ahmednagar, India.
[2]Department of Botany, Ahmednagar College, Ahmednagar, India.

***Corresponding Author: Asha B. Kadam**

Department of Botany, Dada Patil Mahavidyalaya, Karjar, Ahmednagar, India.

Article Received on 07/02/2021 **Article Revised on 27/02/2021** **Article Accepted on 17/03/2021**

ABSTRACT

Synergistic effect of seeds extract of *Ricinus communis* L., *Moringa oleifera* Lam., *Sapindus emarginatus* Vahl. *Crotalaria juncea* L. and *Trigonella foenum-graecum* L. on body and organ weight of female Wistar rats was considered to evaluate contraceptive activity. There was no substantial significant gain or loss in body weight due to oral contraceptive Mala-N 0.15 mg Levonorgesral + 0.03 mg Ethenyl estradiol) or even by synergistic formulation of various plant seeds extract. The gain or loss in vital organs corresponds to the intake of energy in the form of diet, type of oral contraceptive and synergistic formulation.

***KEYWORDS**: Synergistic effect, organ weight; Wistar rats, contraceptive.*

I. INTRODUCTION

In many pockets of India the plants are traditionally used by tribal and ethnic people for contraception. Individual plant part or formulation of various plant parts was found to be effective for contraceptive activity. Various plants were used as anti-fertility agents (Kadam and Gaykar 2018). These are termed as contraceptive plants. The fertility control can be achieved by various natural contraceptive plant products under biological and pharmacological functions. The objective of this study was to evaluate the potential contraceptive activity of the synergistic formulation made from the seeds extract of *Ricinus communis* L., *Moringa oleifera* Lam., *Sapindus emarginatus* Vahl. *Crotalaria juncea* L. and *Trigonella foenum-graecum* L. The contraceptive activity was evaluated by using Wistar female rats as experimental animals. The synergistic formulation extracts were administered orally to sexually mature, healthy virgin female Wistar rats. The parameters such as body weight, food consumption, organ weight, were considered to evaluate contraceptive activity.

Different types of hormonal and non hormonal contraceptives have been developed for female but the safe herbal based contraceptive is still waiting by most of the women (Kadam et al., 2013). Development and formulation of new herbal contraceptives for the female depends on a thorough knowledge of the anatomy and physiology of the reproductive tract. The female has five major phases in reproduction. These phases are developing an ovum; receiving the spermatozoa and transporting them from the vagina to oviducts; supplying a suitable environment for fertilization; directing the fertilized ovum across the fallopian tubes to the uterus and providing suitable hormones for implantation and further development of fetuses.

During these phases of reproduction, if any of the phases is undulating or disturbed by the external or internal factor then the fertilization will not be successful. So the parameters such as body weight and organ weight were considered to evaluate contraceptive activity.

II. MATERIAL AND METHODS

A) Collection of plant material

On the basis of literature and survey of trible people (Gaykar et al., 2006) following plants were selected for the scientific study of spermicidal activity. The selected plants were *Trigonalla foenum-graecum* L. (Fabaceae). *Sapindus trifoliatus* L. *Moringa oleifera* Lam. (Moringaceae), *Crotalaria juncea* L., (Fabaceae), (Sapindaceae), *Ricinus communis* L. (Euphorbiaceae).

The plant and seed samples were collected from various areas of Ahmednagar District, India in their natural habitat. They were identified from Botanical Survey of India, Western Circle, Pune. The voucher specimens were deposited in the Herbarium, BSI, Pune as well as Herbarium of Department of Botany, New Arts, Commerce and Science College, Ahmednagar. (ABK 001, ABK 002, ABK 003, ABK 004, ABK 005).

B) Extraction of Plant Material

Collected seeds were cleaned, dried, and finely powdered in a grinding machine. 250 gm of each seed samples were powdered and was extracted in Petroleum ether

using Soxhlet extraction or hot continuous extraction (James et al., 2014, Azwanida, 2015). In this method, finely ground sample was placed in a porous bag and placed in thimble chamber of the Soxhlet apparatus. Petroleum ether solvent was heated in the bottom flask, vaporizes into the sample thimble, and condenses in the condenser and drip back. It is continuous process. Once the process has finished, the solvent was evaporated using a rotary evaporator, leaving a small yield of extracted plant material (about 10 to 15 ml) in the glass bottom flask. The advantage of this method is that large amounts of drug can be extracted with a much smaller quantity of solvent. The extract was preserved at 4^0 C till the acute oral treatment. Aqueous extract was prepared at the time of dosing the animals. It was prepared with distilled water. Powdered seed samples were extracted separately.

C) Synergistic Formulation and COCPs **Solution**

Aqueous extracts of selected plants seeds were mixed together in 1:1:1:1:1 ratio. Similarly Petroleum ether extracts were also mixed together in equal quantity and it was formulated in the same way by mixing together. This was termed as "Synergistic formulation of plant extract". The acute toxicity of this formulation was studied by Kadam and Gaykar (2017). COCPs (Mala N) tablets contain ethinyl estradiol and levonorgestrel were purchased from local pharmacy, the tablets were crushed into powder form. 10 mg of the powder was dissolved in 1 ml of propylene glycol at 37°C (Hill et al 2016).

D) Ethical Approval

Authors hereby declared that the experimental protocol was approved by the Institutional Animal Ethics committee (RP31/1516). The work was carried out at Apt Research Foundation, Pune. Each animal was used only once. For ethical reason, all animals were sacrificed at the end of the study. Experimental protocol was followed according to Guidelines for Care and Use of Laboratory Animals.

E) Experimental Animals

Thirty six virgin female and 12 male Wistar rats with an average weight of 180 g were bred in the animal house were used for this study. They were screened and observed to exhibit regular estrous cycle. The rats were maintained on a 12 h light and 12 h dark cycle, provided with pelleted rat chow and water *ad libitum*.

F) Treatment protocol

The animals were equally divided into six treatment groups (6 animals / group).

Group I- Control- received normal diet,

Group II received standard (Mala-N - 0.15 mg Levonorgesral + 0.03 mg Ethenyl estradiol). 1.4 mg/kg body weight.

Group III-received aqueous extracts 500 mg /kg body weight,

Group IV-received aqueous extracts 1000 mg /kg body weight,

Group V-received pet ether extracts 500 mg /kg body weight,

Group VI-received pet ether extracts 1000 mg /kg body weight.

Total 6 groups of 36 animals were used to check contraceptive activity. Plant extracts were further subjected to animals to check contraceptive activity *in vivo*. All treatments were given for 30 days to cover six regular estrous cycles. Treated female rats were mated with fresh male rats (3:1). The females were examined for signs of mating by vaginal smear technique, phases of estrous cycle, body weight and food consumption. The rats were sacrificed for study of organ weight.

G) Body and organ weights

Final body weights of the animals were recorded a day after the last dose administration. Vital organs were excised, cleared of supporting tissues and weighed. The heart, kidney and liver were removed and cleared of adherent tissues before they were weighed immediately with an electronic table top balance. All the control and experimental groups of female rats were evaluated for any changes in their body weight as well as for their reproductive organ weight by the method of Amini and Kamkar (2005).

H) Statistical analysis

Data were expressed as Mean ± S.E.M. Statistical analysis was carried out by one-way analysis of variance (ANOVA) with significance expressed as $P< 0.05$.

III. RESULTS AND DISCUSSION

A) Body weight

The body weight of treated and control rats were measured at the intervals of 7 days and the measurement was taken up to 56 days. There was non significant increase ($P>0.05$) in the body weight of rats of control, Standard (mala-N), and 1000 mg/kg dose of aqueous and petroleum ether extract. Body weight was significantly increased ($P<0.05$) at 500 mg/kg b.w of both aqueous and petroleum ether extracts (**Figure 1**). There are some individual reports of plant parts for the correlation of body weight and anti-fertility effects. Aqueous Leaf extract of Moringa oleifera at dose of 175 mg/kg BW showed gain in body weight of Charles foster strain albino rats which was less in the treatment group as compared to the control group (Sethi et al 1988). The data revealed that the body weights of rats were not found altered following treatment of seed extract of Trigonella foenum-graecum in Female Rats as compared to control (Sharma and Bhinda 2005). Administration of ethanol extract fraction of Crotalaria juncea seeds to female rats showed no change in the body weight. (Vijaykumar et al 2007). The data revealed that the body weights of rats treated with steroidal Extract of Trigonella foenum-graecum (seeds) in Female rats were not found altered (Sharma and Bhinda 2005). Oral administration of contraceptive steroid hormone does not significantly alter the body weight of diabetic rats

(Adeghate, 2000). There were no significant differences (P > 0.05) in the mean body weight of methanol extract of Ricinus communis seed treated rats, before and during the treatment period compared with the vehicle treated control group (Raji et al 2006). In the present experiment it was found that the mean body weight of rats administered with oral contraceptive standard Mala N (0.15 mg Levonorgesral + 0.03 mg Ethenyl estradiol) was increased by 51.34 gm. These finding may be due to 56 days of age. The longitudinal study (Prema and Madhavapeddi 1982) results showed tendencies to gain or lose weight in different women, but no significant difference in the mean body weight with oral contraceptive use in different durations. The findings of (Ekhator and Osifo, 2012) reported that Levonorgestreal and Ethenyl estradiol containing combined oral contraceptive pills elicit anti-obesity properties and potential weight management in rabbits. The concluding remarks of the present study is that there was no substantial significant gain or loss in body weight due to oral contraceptive Mala N 0.15 mg Levonorgesral + 0.03 mg Ethenyl estradiol) or even by synergistic formulation of various plant seeds extract. The presence of Pregnane - A Parent of Progesterone from *Trigonella foenum - graecum* Linn is one of the chemical constituents of synergistic formulation (Kadam and Gaykar, 2017).

B) Vital Organs weight:

The orally treated Wistar rats were sacrificed and dissected after 56 days on completion of the experiment. The gain or loss in organs weight is one of the important aspects from the point of Fertility index and fertility or contraceptive activity. The gain or loss in vital organs corresponds to the intake of energy in the form of diet, type of oral contraceptive and synergistic formulation.

The vital organs are bodily organs that are essential for life. These include Adrenal, Kidneys, Heart, Liver, Spleen, Lungs, Brain, and Pancreas etc. The administration of aqueous and Petroleum ether synergistic formulation at the dose of Mala N, 500 and 1000 mg/kg, caused gain or loss in weight, but no significantly, when initial and final body weight were compared (Table 1 and 2)

a) Adrenal

The adrenal glands are endocrine glands that produce a variety of hormones including adrenaline and the steroids aldosterone and cortisol (Santulli 2015). Adrenal showed no significant (P>0.05) increased weight in all treated animals over control except the rats treated with 500 mg synergistic formulation of plant extract made in petroleum ether. The weight of adrenal gland was measured 0.40 mg in (500mg) Pet ether extract. The increased weight in this treatment was four times more than control. The increased weight of Adrenal gland must have secreted functional sex hormones in the gonads and other target organs. It can be correlated with the fertility index in the same treated rats. So the concluding remark can be attributed as increasing Adrenal weight may decreases fertility.

b) Kidney

The weight loss in kidney was significant (P=0.003<0.005). The results obtained of Kidney were significantly different in all treated animals over control and the animals treated with oral contraceptive Mala N (0.15 mg Levonorgesral + 0.03 mg Ethenyl estradiol). The weight of kidney in control was 1.80a ± 0.10 gm while in other treatment it was decreased up to 1.03b ± 0.22 gm. The previous report on the effect of combined oral contraceptive pills (COCP) on kidney function showed that usage of COCP did not have impact on kidney function (Ekhator et al., 2014). The present investigation also showed that there was no any side effect on kidney due to oral contraceptive as well as the synergistic formulation of plant extracts used as oral contraceptive.

c) In other vital organs there was no significantly (P>0.005) decreasing weight in Heart, Kidney, liver, Spleen, Lungs, Brain, and Pancreas etc. No marked variation was seen in the weights as compared to control. The weight loss was observed in spleen, lungs, brain and pancreas. Similar results were reported in Trigonella. The weights of the heart, spleen, liver and kidney did not significantly increase with the oral administration of Trigonalla foenum Graecum (Fenugreek) seeds extract. No marked variation was seen in the weights as compared to control (Effraim et al 1999).The weight of liver was decreased in declined trend over control. It was decreased up to 4.87 mg in rats treated with 1000mg synergistic formulation extract prepared in petroleum ether while it was 10.03 mg in control. These results showed the positive response to oral contraceptive Mala N as well as synergistic formulation plant extract. The data revealed that the synergistic formulation is effective without any side effects on vital organs. Our results were supported by the some previous work. The report of GM 4 contraceptive efficacy revealed that GM-4 formulation is safe and significantly more effective than N-9 in preventing conception without any statistically significant differences in absolute and relative organ weights (brain, thymus, heart, lung, liver, kidney, pancreas, spleen, and reproductive organs) of test vs. control mice at the conclusion of the 13-week study. (Osmond et al 2001, Kadam and Gaykar 2019).

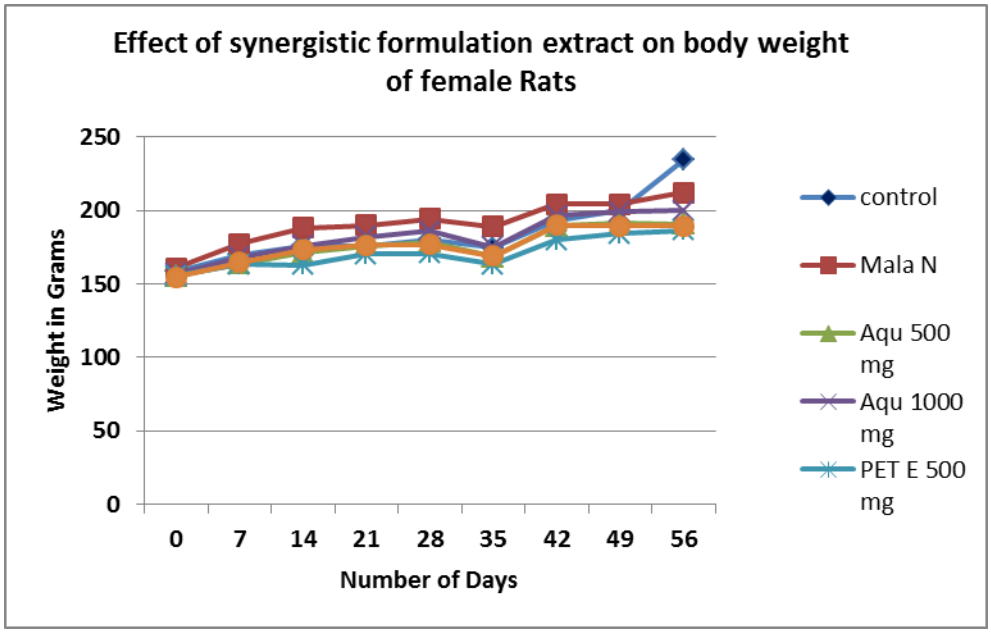

Figure 1: **Effect of synergistic formulation plant seed extract on body weight of female Rats.**

Table No 1 : Synergistic effect of plant seed extracts on organ weight (g) of female Wister rats.

Organ	Adrenal	Heart	Kidneys	Liver
Control	0.091a ± 0.004	0.77a ± 0.034	1.80a ± 0.10	10.03a ±0.24
Mala N	0.075ab ±0.011	0.66a ±0.037	1.47a ±0.08	7.16b ± 0.48
Aqua 500mg	0.16a ± 0.11	0.74a ± 0.06	1.26b ± 0.05	6.46b ±0.29
Aqua 1000 mg	0.10a ±0.01	0.72a ±0.04	1.30b ± 0.03	6.48b ± 0.42
PET E 500 mg	0.40ac ± 0.23	0.54ab ± 0.11	1.03b ± 0.21	5.03bc ± 1.04
PET E 1000 mg	0.12a ± 0.07	0.52ac ± 0.11	1.03b ± 0.22	4.87bc ± 0.99
CD at 0.05%	0.29	0.2	0.37	1.78
P-value at 0.05%	0.28	0.099	0.003	6.47E-05

Where data presented are means of six replicates; values within the same column with different letters a,b,c) are significantly different at 0.05% P- level by Single factor ANOVA test followed by CD & Turkeys' HSD test; ± Standard error of means.

Table No 2: Synergistic effect of plant seed extracts on organ weight (g) of female Wistar rats.

Organ	Spleen	Lungs	Brain	Pancreas
Control	0.71a ±0.035	1.42a ± 0.11	1.56a ± 0.05	0.77a ±0.12
Mala N	0.67a ±0.05	1.48a ±0.19	1.68ab ± 0.03	0.81a ±0.12
Aqua 500mg	0.79a ± 0.10	1.25a ±0.05	1.59a ±0.06	0.68a ±0.02
Aqua 1000 mg	0.78a ±0.24	1.47a ±0.07	1.65 a ± 0.04	0.54a ±0.04
PET E 500 mg	0.51a ±0.10	1.18a ±0.25	1.34a ±0.27	0.82a ± 0.28
PET E 1000 mg	0.51a ± 0.11	1.09a ± 0.25	1.25ac ± 0.25	0.51a ± 0.13
CD at 0.05%	0.34	0.46	0.42	0.38
P-value at 0.05%	0.43	0.48	0.31	0.48

Where data presented are means of six replicates; values within the same column with different letters a,b,c) are significantly different at 0.05% P- level by Single factor ANOVA test followed by CD & Turkeys' HSD test; ± Standard error of means.

IV. CONCLUSION

There was no substantial significant gain or loss in body weight due to oral contraceptive Mala-N 0.15 mg Levonorgesral + 0.03 mg Ethenyl estradiol) or even by synergistic formulation of various plant seeds extract. The gain or loss in vital organs corresponds to the intake of energy in the form of diet, type of oral contraceptive and synergistic formulation.

V. ACKNOWLEDGEMENT

Authors are thankful to Principal and Head, Department of Botany Dada Patil Mahavidyalaya, Karjat for seed money support during the study. Also the authors are

thankful to Principal, Ahmednagar College, Ahmednagar.

VI. REFERENCES

1. Adeghate A. Effect of oral contraceptive steroid hormone on metabolic parameters of streptozotocia-induced diabetic rat. Contraception, 2000; 62: 327-329.
2. Amini A. and Kamkar F. The effects of gossypol on spermatogenesis in NMRI mice, Iranian J Sci and Technol. Trans, 2005; 29: 123-133.
3. Azwanida N.N. A Review on the Extraction Methods Use in Medicinal Plants, Principle, Strength and Limitation. Med. Aromat Plants, 2015; 4: 196.
4. Effraim K.D., Salami H.A and Nwafor P.A. The effect of aqueous seed extract of *Trigonalla foenum graecum* (Fenugreek) on haematological parameters in Albino Rats. Afr. J. Biomed. Res, 1999; 2: 47-51.
5. Ekhator C.N., and Osifo U. C. The effect of Oral contraceptive pills (OCP) on body weight: A call for further studies. Int. J. Basic Applied Innov. Res., 2012; 1: 155-160.
6. Ekhator C.N., Omorogiuwa A. and Akpamu U. Effect of Combined Oral Contraceptive Pills (COCP) Containing Levonorgestrel and Ethinylestradiol on Kidney Function. *Trends in Applied Sciences Research*, 2014; 9: 269-274.
7. Elizabeth V.M., Kigondu, *et al*. In vivo Antifertility and Safety Profiles of Kenyan *Moringa oleifera* Lam. (Moringaceae) extracts. Afr. J. Pharmacol. Ther., 2016; 5(4): 212-220.
8. Gaykar B.M., Kulkarni A. A, and G.B Borkar Ethnobotanical plants of tribal areas of Ahmednagar Dist. (M.S) Jour. Drug Res. Aurveda and Sidhha, vol XXVII No, 2006; 1-2: 73-61.
9. James R., Malcolm K., Dariel B. and Joanna V. Using Soxhelet Ethanol Extraction to Produce and Test Plant Material (Essential Oils) for Their Antimicrobial Properties. Journal of Microbiology & Biology Education, 2014; 15(1): 45-46.
10. Kadam A. B., Gaykar B.M. and Bedarkar P. Systematic study on understanding present and prospective contraceptives. Flora and Fauna, 2013; 19(1): 51-57.
11. Kadam A. B. and B. M Gaykar Assessment Of Acute Oral Toxicity Of Synergistic Formulation Extract Of Traditional Contraceptive Plants. International Journal of Pharmacognosy and phytochemical Research, 2017; 9(3).
12. Kadam A. B. and B. M. Gaykar Pregnane - A Parent of Progesterone from *Trigonella foenum - graecum* Linn. International Journal of Pharmaceutical Sciences and Research, 2017; 8(12): 5194-5198.
13. Kadam A. B. and B. M. Gaykar Immobilization Effect of Traditional Contraceptive Plants on Human Sperm. Indo Global Jour of Pharmaceutical Sciences, 2018; 8(1): 21-25.
14. Kadam Asha and Balasaheb Gaykar Development of Molecular Condom by Using Anti-Fertility Phytochemical. Indo Global Jour of Pharmaceutical Sciences, 2019; 9(2): 63-68
15. Osmond J. D., Seang H. Yiv, Barbara W., and Fatih M. U. Contraceptive efficacy and safety studies of a novel microemulsion-based lipophilic vaginal spermicide. Fertility and sterility, 2001; 75(1): 115-124.
16. Prema K. and Madhavapeddi R. Effect of oral contraceptives on dietary intakes and nutritional status. Indian Journal of Medical Research, 1982; 75: 682-6.
17. Santulli G. M. *Adrenal Glands: From Pathophysiology to Clinical Evidence*. Nova Science Publishers, New York, NY, 2015.
18. Sethi N., Nath D., Shukla S. C. and Dyal R. Abortifacient activity of a medicinal plant *Moringa oleifera* in Rats. Ancient Science of Life, 1988; 7(4): 172 – 174.
19. Sharma J.D. and Bhinda Anjula Antifertility Activity of Steroidal Extract of *Trigonella foenum-graecum* (seeds) in Female Rats. Asian J. Exp. Sci., 2005; 19(1): 115-120.
20. Vijaykumar B., Malashetty and Saraswati B.P. Effect of chromatographic fractions of ethanolic extract of *Crotalaria juncea* seeds on ovarian follicular kinetics and estrous cycle in albino rats. Iranian Journal of Pharmacology and Therapeutics, 2007; 6(2): 159-163.