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Effect of different doses of lyophilized beetroot on fertility and reproductive hormones

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Abstract: Infertility has recently been a common issue for which multiple medicinal as well as surgical treatments are available. *Beta vulgaris* commonly referred to as garden beet is one of the most utilized vegetable especially in salad dressings. The Present study was conducted to evaluate the effect of lyophilized beet root on fertility and reproductive hormones. The study was carried out in January 2017 on two different animal models. For fertility purpose albino mice of both genders weighing 20-25gm were divided into 5 pairs for each group, First group was control given distilled water, next was treated I being given beetroot 500mg/kg and last one was given 1000mg/kg beetroot. Similarly 5 pairs were made of buck and doe's weighing 1.75gm-2.5gm and labelled as Control, Treated I and Treated II and were administered distilled water, 500mg/kg and 1000mg/kg beetroot respectively. The pups were again mated after they grew up and in this way F_1 generation was also observed. The kits were observed for presence of anomaly. The estrogen, progesterone and testosterone levels were evaluated at baseline, during pregnancy and post pregnancy in rabbit model. Improved fertility with no anomalies in the pups and kits were observed. The reproductive hormones were also highly significantly increased ($p<0.001$) as compared to baseline values. The study suggests that beetroot would be very beneficial in improving fertility and maintaining hormonal level during fertility hence it would prove to be very beneficial in maintaining pregnancy as well as treating infertility.

Keywords: Beetroot, fertility, pregnancy, reproductive hormones.

INTRODUCTION

Since traditional times plants have been used by man for different purposes such as source of food, clothing, shelter, fragrance as well as for medicinal purpose. Ayurvedic, Unani and Chinese traditional systems are based on using plants as a source of medicine which has led to the development of a number of drugs (Gurib-Fakim, 2006). Plants possess significant pharmacological activities, although herbal therapy possesses minimal side effects chances of drug-drug interactions are there (Pribitkin, 2005). Around 80% of the world's population in developed countries depends on herbs for primary health care according to a report published by WHO (World Health Organization) (Mohamed *et al*., 2016). Traditional medicine has been defined by WHO as " knowledge, beliefs, health practices and approaches about animal, plants and mineral based medicines applied alone or in combination to diagnose, prevent or treat patients" (WHO, 2008).

Since ancient times infertility has been well known. However since last century tremendous advancements have been made in infertility treatment. A number of new techniques and medications have been designed for infertility. However with increase in hormonal knowledge there are many challenges such as multiple pregnancies, emotions and monetary cost to the family (Beall and Decherney, 2012). According to literature studies the most commonly used herbal preparations used during pregnancy are cranberry, *Rubus idaeus*, *Mentha piperita*, *Zingiber officinale*, *Matricaria chamomilla, Leonurus cardiac, Linum usitatissimum* and *Echinacea purpurea* (Holst *et al*., 2011). Besides that *Actaea racemosa and Hydrastis Canadensis have been used to treat uterine infections (Lans et al., 2009).*

Beta vulgaris is a vegetable belonging to family Chenopodiaceae that has been used as a source of nutrition since ancient times. Garden beet (fig. 1) is a common name used for it. Mostly the beetroot is red colored. The plant has a height of 1-2 inch with leafy stems (Carmen, 2008). Since traditional times roots and leaves of beet root have been used for different ailments. It was used by ancient Romans for treating pyrexia and constipation (De Azeredo *et al*., 2009 a). It has been used for wound healing by Hippocrates. Beet juice was considered as aphrodisiac in middle ages. Various systemic disorders of blood, liver, heart, lung, GI (gastro-intestinal) and CNS (central nervous system) have been treated using beetroot (De Azeredo, 2009 b).

Beet root provides a good source of micronutrients such as Sodium (Na) 66gm, Potassium (K) 380gm, Magnesium (Mg^{+2}) 11 gm, Phosphorous (P) 51 gm, Iron (Fe) 1gm, Zinc (Zn) 0.4gm, folic acid 150 mg, carotene 20 mg, ascorbic acid 25mg, pantothenate varies between 0.12-0.10mg, pyridoxine between 0.03-0.04 mg, thiamine between 0.01 mg-0.02 mg, riboflavin between 0.01

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mg0.03 mg and niacin 0.1mg. These constituents are present in 100 gm of raw beet root (Heinerman, 1994).

The red color of beetroot is due to its main constituent i.e. betalains (Lechner *et al*., 2010). By high performance liquid chromatography (HPLC) it was found that *Beta vulgaris* contains Ferulic acid, Pcoumaric acid, Beta vulgarin, flavonoids, L-tryptophan, cyclo-DOPA (Dihydroxyphenylalanine), 5,5,6,6-tetrahydroxy-3,3-biindolyl and phenolic amides (Neelwarne , 2012).

Current studies have highlighted the use of lyophilized beet root as a diuretic (Sarfaraz and Najam, 2018), as an anti-inflammatory agent (Sarfaraz and Najam, 2017) and as an analgesic (Sarfaraz and Najam, 2019). It also possesses nephroprotective effect (Sarfaraz and Najam, 2019). Literature studies have also shown its use in different doses to increase high density lipoprotein (HDL) and decrease cholesterol (Al-Dosari *et al*., 2011). Betaine in dose of 6gm OD has been shown to influence release of nitric oxide in human volunteers (Iqbal *et al*., 2006). Literature studies have also shown it to inhibit calcium oxalate crystal formation (Saranya and Geetha, 2014). The current study was designed to evaluate the effect of different doses of lyophilized beetroot on fertility and reproductive hormones.

MATERIALS AND METHODS

Beet root Lyophilized powder

Beta vulgaris lyophilized powder of root was purchased from Sun Rise Nutra Group, Qingdao, China having Lot No. CTC 2015 0320. As the powder was photosensitive so it was stored in zip log plastic bag which was further enclosed in aluminum foil.

Animal Selection, Grouping and Dosing Protocol

The testing was carried out on two different animal models. Rodent group (20-25gm albino mice of both sexes were selected) and grouped randomly into 15 pairs. Monogamous breeding was followed (one male and one female pair). During the selection of female mice it was ensured that they were non-parturient and non-gravid. The animals were housed in the animal house of Department of Pharmacology, Faculty of Pharmacy, University of Karachi under 25±2^{o}C room temperature. They were fed standard diet and water at their will.

The First 5 pairs were termed as Control and given 0.1ml distill water, the next 5 pairs were termed as Treated I and administered oral lyophilized beetroot 500mg/kg/day. The remaining pairs were marked as Treated II and given 1000mg/kg lyophilized beetroot daily by oral route. Stock solution was prepared by dissolving 100gm beet root in 200ml distilled water and adjusted based on individual weight of animals.

When the females were near delivery they were switched to another cage. The pups delivered from all the groups were observed for behavioral changes as well as other anomalies. The number of pups delivered by all three groups were counted separately and this generation was labelled as F_0 (Riaz *et al*., 2010). When the pups grew up into mice, randomly again 5 males and females were selected from above specified groups and mated. There pups were called F_1 off springs.

The other model selected was of albino rabbits weighing 1.75gm-2.5gm. 15 pairs of the buck and doe's were made as in mice model. Label control was given to first 5 pairs and administered distilled water, Treated I were administered 500mg/kg lyophilized *Beta vulgaris* and the remaining 5 pairs were labelled Treated II and given 1000mg/kg *Beta vulgaris* orally daily. The kits delivered were observed for normal behavior or presence of any anomaly.

Ethical Approval

The study was conducted in January 2017 approved by Karachi University Board of Advanced Studies and Research vide Resolution No10 (P) 18. For the handling of animals the specification provided by Hubrecht and Kirkwood 2010, were followed and for breeding protocol National Research Council 2010 was consulted.

Determination of Reproductive Steroids

All the Doe's were selected for evaluation of reproductive steroids. By cardiac puncture 5ml blood sample was withdrawn in a test tube. It was then centrifuged for 10 minutes in Humax 14 K (Germany) at 3000rpm for plasma separation. It was then stored at -20^{o} C till further process.

Determination of Progesterone and Estradiol

For evaluation of female reproductive steroids Radioimmunoassay methodology by Abraham and his colleagues was followed (Arora *et al*., 2019).

Determination of Testosterone

For evaluation of testosterone R-35 antisera was used following method mentioned by Stone (Stone *et al*., 1971).

STATISTICAL ANALYSIS

SPSS 20 was used for statistical evaluation. Mean ± S.D (n=10) was used for presenting the data. Statistical analysis was performed using Two-way Anova (analysis of variance) followed by Post hoc Tukey's test and multiple pair wise comparisons.

RESULTS

Table 1 shows significant ($p<0.001$) increase in number of pups delivered by both doses of beet root as compared to control. High dose (1000mg/kg) showed significant effect as compared to low dose 500mg/kg.

Table 1: Effect of beetroot on Number of Pups at different Doses

Drugs	F0 Generation (10 pregnancies)	F1 Generation (10 pregnancies)
Control 0.1ml Distill water	79 ±1.5	82 ± 1.3
Treated I *Beta vulgaris* 500mg/kg	113 ±1.8***	117 ±1.0 ***
Treated II *Beta vulgaris* 1000mg/kg	118 ±1.3***!!!	126 ±0.98 ***!!!

Table 2: Effect of different doses of beet root on Reproductive steroids in Rabbit Fertility model

Reproductive Steroids	Estrogen		Progesterone		Testosterone	
Drugs	*Beta vulgaris* 500mg/kg	*Beta vulgaris* 1000mg/kg	*Beta vulgaris* 500mg/kg	*Beta vulgaris* 1000mg/kg	*Beta vulgaris* 500mg/kg	*Beta vulgaris* 1000mg/kg
Baseline	28.4 ±0.20	28.3 ±0.19	0.4 ±0.01	0.41 ±0.02	1.4 ±0.11	1.3 ±0.13
During Pregnancy	41.9 ±0.28 ***	35.2 ±0.21***!!!	7.8 ±0.04 ***	9.5±0.06***!!!	3.7 ±0.19 ***	3.8 ±0.60***
Post Pregnancy	28.0 ±0.15	23.3 ±0.17	0.1 ±0.03	0.33 ± 0.02	1.6 ±0.12	1.4 ±0.14

By taking mean of all treated values they are compared with mean and standard deviation of control readings by applying two ways ANOVA (analysis of variance). Multiple comparisons were done by applying post hoc Tukeys test. The P values were considered significant as follows

***p≤0.001 = significant as compared to control

!!!p≤0.001 = significant when compared among treated groups

Table 2 shows significant (p≤0.001) elevation in estrogen, progesterone and testosterone level by beet root at both doses in comparison with their baseline readings. When compared during pregnancy 1000mg/kg beetroot showed significant (p≤0.001) reduction in estrogen level as compared to 500mg/kg. However at 1000mg/kg during pregnancy the progesterone level was significantly (p≤0.001) increased as compared to 500mg/kg. When comparison among the groups was done there was insignificant difference between the two doses during pregnancy.

Fig. 2 labelled as (a) (b) and (c) shows the effect of 500mg/kg beetroot dosing during gestation on delivered pups of swiss albino mice. The pups delivered were light pink in color and healthy. They did not show any behavioral, development or birth defect. The last fig. 2(c) shows all pups matured into healthy adult mice.

Fig. labeled 3 (a) and (b) shows the effect of 1000mg/kg beet root dosing on delivered pups of swiss albino mice. The pups were all light pink in color and healthy. No birth defects were observed. There wasn't any presence of developmental or behavioral anomaly.

DISCUSSION

A study was designed to evaluate the effect of lyophilized beetroot on virility. Our results showed that beetroot increased the number of pup's dose dependently. Literature studies show that beetroot has been considered as an aphrodisiac since traditional times (El-Gamal *et al*., 2014). Beetroot contains the micronutrient boron and studies have shown that boron metal increases both estradiol and free testosterone levels and boron supplementation is considered very beneficial in increasing sexual hormones (Naghi *et al*., 2011). Previous literature search validates this claim and reports a good relation between boron metal and increased testosterone hormone concentration, improved semen quality and increased libido in male rabbits (Kamil *et al*., 2009).

Fig. 1: Beetroot

Our hormonal test results conducted on rabbits show increased levels of all three hormones (estrogen, progesterone and testosterone) during pregnancy when treated with different doses of beetroot. Gestation is considered as a physiological state that is comprised of multiple mechanisms to promote nutritional needs of the growing fetus. There are significant hormone related adjustments involved during this phase (Osol *et al*., 2019). Normally it is thought that estrogen and progesterone elevation plays an important role in physiological, anatomical and molecular adjustment for creating a suitable environment for fetal growth in the uterus (Rosenfeld *et al*., 2002).

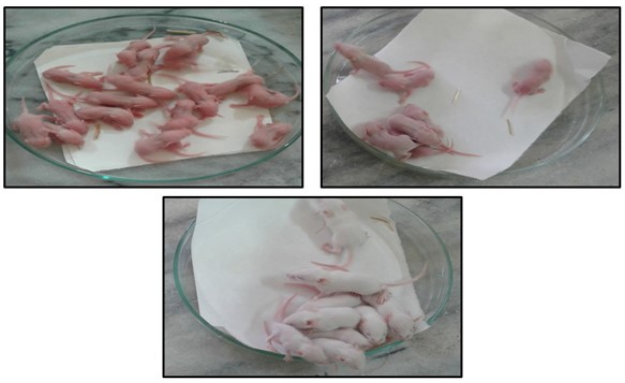

Fig. 2 (a) (b) and (c): Swiss albino mice pups delivered and matured administered with 500mg/kg beetroot

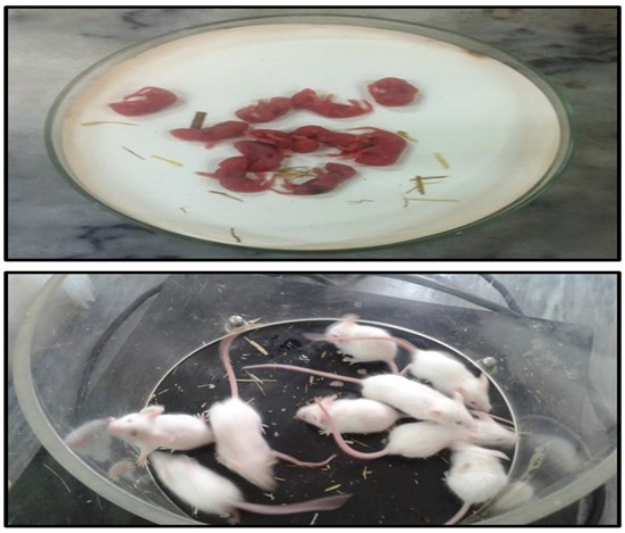

Fig. 3 (a) (b) and (c) : Swiss albino mice pups delivered and matured administered with 1000mg/kg beetroot

Increase in vascular surface area occurs during angiogenesis process, besides that vascular reactivity is also changed during pregnancy. During the different trimesters of pregnancy there is about 40-fold increase in uterine blood flow (UBF) which occurs in 3 phases mostly. During first phase uterine artery becomes vasodilated and causes increase in microvascular volume to create suitable environment for implantation of embryo. Next phase involves remodeling and angiogenesis of uterine vascular bed. Finally there is marked increase in uterine blood flow due to increase vasodilation for provision of nutrients to the fetus through placenta (Grazul-Bilska *et al*., 2019).

Previous Literature studies have highlighted the role of NO (nitric oxide) as one of the important dilator which stimulates uterine vasodilation by estrogen induction (Lechuga *et al*., 2019). A recent study has shown that beet root is a rich source of nitrates that is absorbed in proximal intestine and leads to increase in nitric oxide production (Mirmiran *et al*., 2020). This can also be considered as one of the factors that led to increased fertility observed by beetroot (Gallardo and Coggan, 2018).

Besides this beetroot also contains folic acid, which is considered important not only for red blood cell production, leading to increased hemoglobin, improved oxygen capacity, which improves blood flow but also plays a critical role in fetal brain development (Moser *et al*., 2019; Chen *et al*., 2019; http://ndb.nal.usda.gov/ndb/foods/list.). The pups observed in our study were all healthy with no anomalies. These results justify the above statement that beetroot not only possesses aphrodisiac properties, but is also an important dietary fertility agent that plays a role in normal fetal development too.

Our results showed that at 1000mg/kg the increase in estrogen was less as compared to progesterone and testosterone. It is already known that betaine present in *Beta vulgaris* reduces the concentration of homocysteine by converting it into methionine by re-methylation pathway without involving the folate derivatives. The methionine is eliminated after incorporation into body protein. If methionine is in increased quantity it gets converted into S-adenosyl methionine which leads to formation of homocysteine again (Bakker and Brandjes, 1997). Literature studies have shown an inverse relation between homocysteine and estrogen. It has been suggested that increased homocysteine would lead the estrogen to stimulate methionine synthase in the kidney leading to transamination pathway and reduction in homocysteine concentration (Morris *et al*., 2000). Hence although the estrogen concentration highly significantly increases in comparison to baseline, it is not highly significantly increased compared to 500mg/kg beetroot.

CONCLUSION

From above study we came to the conclusion that beetroot can be beneficial in inducing fertility and maintaining reproductive hormones during pregnancy thus maintaining pregnancy. There was no harmful effects on the delivered pups/ kits showing it was not teratogenic. However further models and studies need to be conducted to evaluate effects on humans at 500mg/kg and 1000mg/kg doses, since it would prove to be very beneficial for infertility and associated problems.

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