

Original Research Article

Evaluation of the Fertility Indices of Female Mice Exposed to Dimethoate and Beetroot Extract (*Beta vulgaris*)

Abstract

*Aim: The aim of this study was to evaluate of the fertility indices of female mice exposed to Dimethoate and Beetroot Extract (*Beta vulgaris*).*

Experimental Design: A completely randomized experimental design using standard methods for analysis.

Location and Duration of Study: This study was carried out in the Animal house, Department of Animal and Environmental Biology of Rivers State University, Nkpolu-Oroworukwo Port Harcourt, Nigeria. GPS 4°47'50"N 6°58'49"E. This study lasted for 21 days.

*Methodology: Thirty (30) sexually matured female mice were randomly divided into 6 groups (A-F) with 5 mice each. They were exposed for a period of 21 days to Dimethoate and 100% extract of *Beta vulgaris*. Group A was orally administered distilled water and rodent pellet only and acted as control. Group B was administered 20mg/kg/day of Dimethoate only. Group C was administered 5mg/kg/day of Dimethoate + 250mg/kg/day of 100% fresh extract of *Beta vulgaris*. Group D was administered 10mg/kg/day of Dimethoate + 250mg/kg/day of 100% fresh extract of *Beta vulgaris*. Group E was administered 15mg/kg/day of Dimethoate + 250mg/kg/day of 100% fresh extract of *Beta vulgaris*. Group F was administered 20mg/kg/day of Dimethoate + 250mg/kg/day of 100% fresh extract of *Beta vulgaris*. At the end of the 21 days exposure the final weight of the mice in each group was taken and blood collected by ocular puncture for hormonal profiling. Hormones such as Prolactin, Progesterone, estradiol, Follicle Stimulating Hormone (FSH) and luteinizing hormone (LH) were analyzed based on the manufacturer's instruction using Randox Monza assay kit. The females were then allowed to mate with fifteen unexposed males in **ratio 2:1**. At the end of 24 days and following successful parturition essential data were collected from the F₁ animals.*

*Result: Results showed significant increase in body weight for groups that were co-administered *Beta vulgaris* as compared to Group B that was administered Dimethoate only. Litter size gradually decrease significantly from seven in the control to two in group B which received Dimethoate only. Reproductive hormones significantly increased in groups coadministered *Beta vulgaris* and Dimethoate compared with group B administered Dimethoate only.*

It is thus deduced that B. vulgaris might increase fertility in experimental animals, while Dimethoate showed significant impact on the reproduction in female experimental animals by reducing the fertility indices, such as reproduction hormones, reduction in fetal implantation leading to low number of offspring (Litter size).

Keywords: Beetroot, Dimethoate, Fertility indices, Hormones, Parturition,

1.0 Introduction

Pesticides constitute a major group of harmful chemicals released into the environment. A suspected mechanism of the disruption of female reproductive function is the induction of hormonal imbalance reported to be brought about by pesticides.[1]

The increased use of pesticides all over the world is alarming and this has drawn the attention of researchers due to the adverse effects they have on non-target organisms [2].

Pesticides are used in agriculture to enhance food production by eradicating unwanted pests and controlling disease vectors. Organophosphorus compounds are the group of pesticides widely used in agriculture [3]. Dimethoate is an organophosphate insecticide with numerous uses on both agricultural and non-agricultural site [4, 5]. In human, the main groups at risk of exposure to higher doses of dimethoate are producers and workers at pesticides factories and farm owners. Indeed, majority of population are exposed to lower doses of dimethoate through food contamination, drinking water or by application of household insecticides containing Dimethoate [6]. Several physiological and behavioral dysfunctions occur in animals after exposure to low doses of organophosphate pesticides, hence it is possible that dimethoate could affect humans and wildlife in their natural habitat. According to [7] dimethoate has been reported to cause developmental toxicity as well as reproductive failures in organisms upon repeated exposures. Developmental and Reproductive toxicity of this pesticide on adult rodents of both sexes has been demonstrated including decrease number of implantation and live fetuses, incidences of resorptions and decrease fetal body weights, Irregularities of estrous cycle and altered levels of serum gonadotrophins in females while impairment of fertility, suppressed libido low semen quality and altered testosterone levels and testicular degeneration have been reported in males [1, 8].

Dimethoate at 28mg/kg was reported to be associated with a significant decreased of sperm count, motility and viability, and significantly increased percentage of morphologically abnormal spermatozoa compared with the controls [9].

The primary site of action of organophosphate pesticides in mammals is the central and peripheral nervous system and it's by inhibiting acetylcholinesterase, a biochemical event that result in accumulation of endogenous acetylcholine at the nerve ending [10].

Beta vulgaris is a plant in the Chenopodiaceous family. It is the well-known in its numerous cultivated varieties, the most well-known is the purple root vegetable known as beetroot or table garden beet.

Beetroot can be eaten raw, used for juice extraction baked or boiled. Beets have used in traditional medicine for hundreds of years to treat constipation, gut and joint pain, dandruff [11].

It was shown by modern pharmacology that red beets extract exhibit anti-anemic, anti-ischemic, anti-inflammatory, antioxidant, anticarcinogenic, antihypertensive and hypoglycemic activities [12]. Not only does the beetroot have nutritional value, its leaf also does but they are rarely eaten. However, the uniqueness of red beets does not reside in their nutritional value.

Beta vulgaris has been ranked among the most potent antioxidant vegetables [13]. As a known antioxidant vegetables and fruits nutrient are important to maintain human health and neutralize a negative effect of oxidative stress in human. Oxidative stress is indicated in many disease including atherosclerosis neuro degenerative disease, Myocardial infarction, cancer as well as ageing [14].

Beside considerable antioxidant and anti-inflammatory activity, *Beta vulgaris* phytochemicals provide an anti-proliferative effect in breast, liver, colon and bladder cancer cell lines through the induction of both intrinsic and extrinsic apoptotic pathway [15]. However, it is important to note that Red beet belongs to oxalate-accumulating plants. Oxalic acid is one of the compounds present in beetroot at high concentration and may have some side effects. As a result of this, regular consumption of red beet is contraindicated for people with urolithiasis.

Pesticides are made for the eradication of pest as the name implies which are the target species but still affect non-target species such as humans and animals. In developing nations such as Nigeria, women play an important role in agriculture irrespective of their reproductive status.

During pregnancy, the fetus can be exposed to pesticides during farming thereby endangering the health of the unborn child. Since the use of pesticides cannot completely be avoided, this study was undertaken to evaluate the role of Beetroot as a potential antioxidant in mitigating the effect of this pesticides in human.

2.0 Materials and methods

2.1 Experimental Location

The experiment was carried out in the animal house of the Department of Animal and Environmental Biology, Rivers State University, Port Harcourt. (Coordinates 4°48'14"N 6°59'12"E)

2.2 Experimental Animals

Thirty sexually matured female mice (mean weight 23.0 ± 2.24 g) were used in the study. The mice were housed individually in plastic cages under standard conditions (12hL: 12hD) room temperature 26 ± 2°C relative humidity 54%) and was acclimatized for 14 days before commencement of experimentation. The animals had free access to feed (rodent pellet) and cool clean water. All experiments were conducted according to the institutional animal care protocols at the Rivers State University Port Harcourt, Nigeria and follow guidelines for the ethical treatment of experimental animals.

2.3 Chemical

Dimethoate an organophosphate insecticide was obtained from a reputable agrochemical outlet in Port Harcourt, Rivers State.

2.4 Plant Extract

Beetroot which belongs to the family of Chenopodiaceae was bought from mile 1 market in Port Harcourt and washed thoroughly to remove dirt. 100% of Beetroot extract was extracted using a juice extractor (model Bender ST-002).

2.5 Experimental Design and Procedure

Thirty (30) adult mice were randomly divided into 6 groups (A-F) with 5 mice each. They were exposed for a period of 21 days to Dimethoate and 100% extract of *Beta vulgaris*.

Group A was orally administered distilled water and rodent pellet only and acted as the control. Group B was administered 20mg/kg/day of Dimethoate only. Group C was administered 5mg/kg/day of Dimethoate

+ 250mg/kg/day of 100% fresh extract of *Beta vulgaris*. Group D was administered 10mg/kg/day of Dimethoate + 250mg/kg/day of 100% fresh extract of *Beta vulgaris*. Group E was administered 15mg/kg/day of Dimethoate + 250mg/kg/day of 100% fresh extract of *Beta vulgaris*. Group F was administered 20mg/kg/day of Dimethoate + 250mg/kg/day of 100% fresh extract of *Beta vulgaris*. At the end of the 21 days exposure the final weight of the mice in each group was taken.

All animals were observed daily for behavioral changes and mortality as well as food and water intake throughout the experimental duration. Animals were weighed and the weight recorded daily to the nearest 0.01gram. For hormonal profile analysis, blood was collected in a plain venipuncture tube by ocular puncture. The blood was allowed to clot and centrifuged at 3000g to separate the serum from the cells. Samples were stored at 4⁰C till analysis. Hormones such as Prolactin, Progesterone, estradiol, Follicle Stimulating Hormone (FSH) and luteinizing hormone (LH) were analyzed based on the manufacturer's instruction using Randox Monza assay kit

2.6 Mating Procedures

At the end of the twenty-one (21) days administration, fifteen (15) males that were not previously exposed to the pesticide were introduced to the 30 females of the same age and species. They were paired in the ratio 1:2 for mating purposes. Twenty-four hours after introduction of males to the females, vaginal swap was taken and examined under the microscope to ensure that mating had taken place. The gestation length was fixed at 21- 24days. The gestation length, body weight, litter size were determined and pups were checked for deformities. At the end of 24 days and following successful parturition essential data were collected from the F₁ animals.

Statistical Analysis: Data were subjected to analysis of variance using the SPSS 20 software for determination of Mean and Standard deviation. Mean separation where significant difference was observed was carried out using Turkey's pairwise comparison test.

3.0 RESULTS

3.1 Visual observation

There were no visible changes in the physical appearances of mice during the period of study. However, animals showed signs of restlessness, itching of paws and increased feeding pattern especially in the group that received Dimethoate only without coadministration of *Beta vulgaris*.

3.2 Effect of *Beta vulgaris* on the body weight of Swiss mice

The effect of oral administration of Dimethoate and *Beta vulgaris* on bodyweight in Swiss mice is shown in Figure 1A-F.

Fig 1A shows the effect of oral administration of Dimethoate and *Beta vulgaris* on the bodyweight of animals in the group A for 21 days. The initial body weight increased from 26.34 ± 2.56 g to 28.84 ± 2.34 g in week 1 and gradually increased to 30.52 ± 1.99 g in week 2. A steady increase was also observed in week 3 with 31.18 ± 2.04 g. By week 3, a statistically significant increase equivalent of 20% weight gain was observed. In the group B animals administered Dimethoate only the body increased steadily from 26.65 ± 2.04 g in week 1 to 27.85 ± 3.03 g approximately 4.80% increase in week 2 followed by 29.31 ± 1.92 g in week 2 (5.90%) and 31.89 ± 1.21 g (9.20%) in week 3 respectively. This was approximately 0.5% reduction in weight gain considered statistically non-significant compared to the control group

Fig 2c shows the effect of oral administration of Dimethoate and *B. vulgaris* on the body weight of animals in the group C exposed to 5mg/kg/bw/day and 250mg/kg/bw/day of 100% fresh extract of *B. vulgaris*. The initial body weight of 26.53 ± 2.35 g increased to 29.69 ± 2.12 g in week 1 and 32.63 ± 2.04 g, 33.89 ± 1.09 g in week 2 and week 3 respectively. There was significant ($p < 0.05$) increase 27.74% in body weight gain with administration of *B. vulgaris*.

Group D animals also showed a gradual body weight increase from initial weight of 26.63 ± 1.81 g to 28.86 ± 1.56 g in week 1, 29.91 ± 2.11 g, 30.35 ± 1.23 g in week 2 and week 3 respectively indicating 13.97% weight gain.

Group E and Group F also showed a gradual increase in body weight throughout the duration of experiment. However, the increase recorded in all groups was not statistically different from group A that

was not exposed to any treatment. Feed intake was also not adversely affected by any of the regime of treatments.

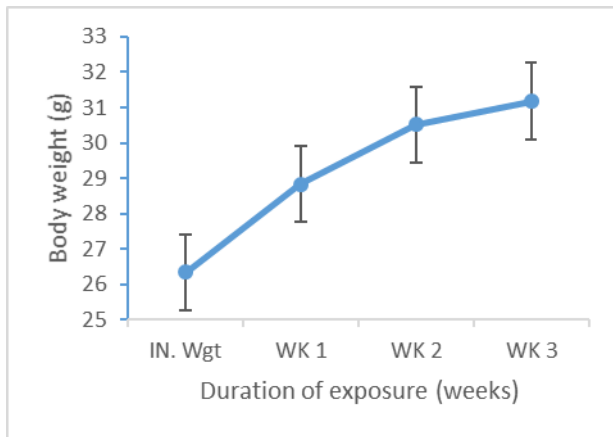


Fig 1a. Bodyweight of group A animals

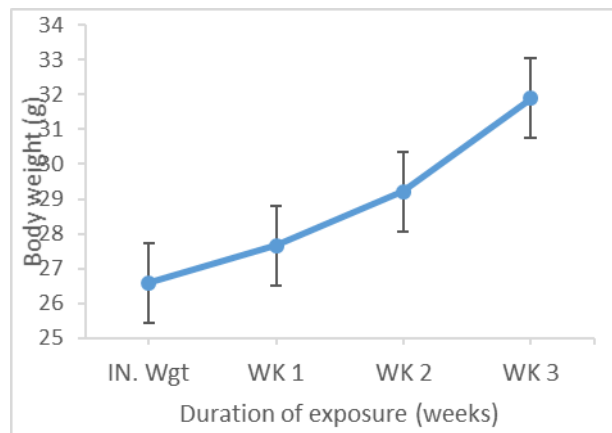


Fig 1b. Bodyweight of group B animals

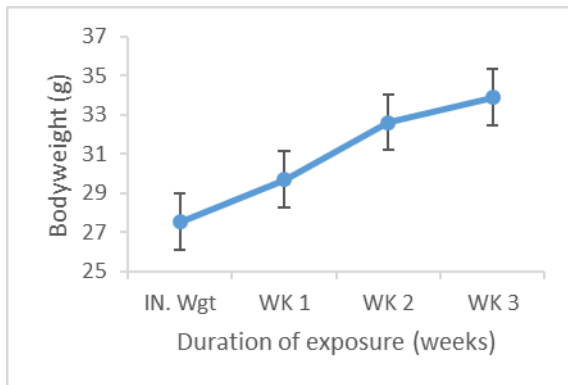


Fig 1c. Bodyweight of group C animals

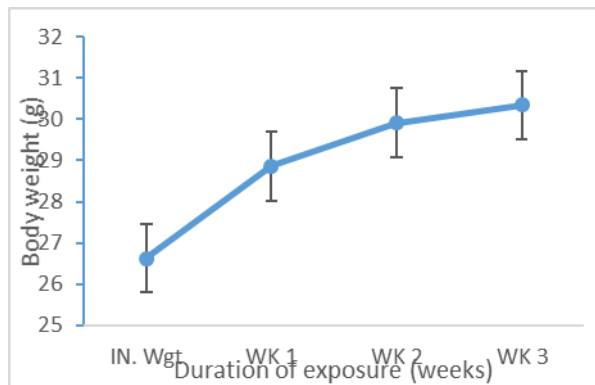


Fig 1d. Bodyweight of group D animals

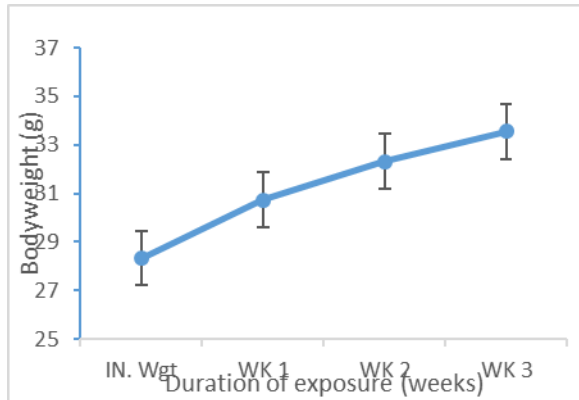


Fig 1e. Bodyweight of group E animals

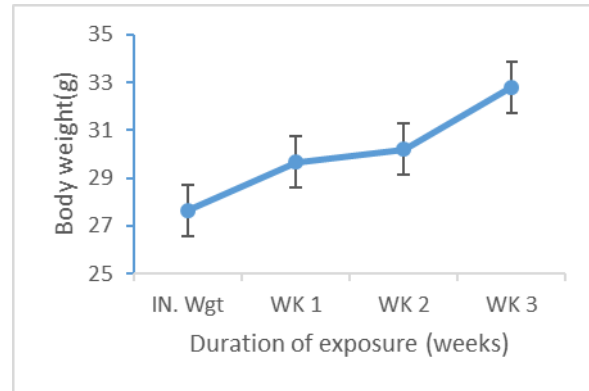


Fig 1f. Bodyweight of group F animals

3.3 Effect of varying concentrations of Dimethoate and coadministration of *Beta vulgaris* on Hormonal file of the Female Swiss Mice.

The concentrations of the hormone Prolactin was not significantly different from the control animal in groups A, C-F. However, Prolactin level was significantly ($p < 0.01$) elevated in group B (Fig.2A, 1.40ng/ml) which received 20mg/kg/bw/day of Dimethoate only.

Fig. 2B shows the effect of coadministration of Dimethoate and *Beta vulgaris* in Swiss mice Estradiol .The level of Estradiol was significantly (< 0.05) reduced in group B as compared to the control. Other treatment groups C-F coadministered 250mg/kg/bw/day had elevated Estradiol from 51pg/ml in C treated with 5mg/kgbw/day along with 250mg/kgbw/day to D administered 10mg/kgbw/day and coadministered 250mg/kgbw/day followed by 52 and 50mg/kgbw/day respectively.

Comparison of the concentration of Progesterone level between the control group and the treatment groups showed significantly (< 0.05) reduction (7.23 vs 12.67ng/ml) in group B that did not receive extract of *Beta vulgaris* and control respectively. There was increased concentration in the D,E,F groups that received 250mg/kgbw/day of *B. vulgaris* extract (Fig.2C)

Influence of varying concentrations of Dimethoate and coadministration of *B. vulgaris* on the concentration of Luteinizing hormone is shown in Fig.2D. There was gradual elevation of hormone from (1.42 iu/L) in control group to 2.35, 1.64, 2.30, 1.70 iu/L) for groups C-F respectively. The lowest concentration was recorded in group B which received 20mg/kgbw/day of Dimethoate only.

The impact of Dimethoate and coadministration of *B.vulgaris* on concentration of Follicle Stimulating Hormone is shown in Fig. 2E. The control group had a concentration of 0.30 iu/ml. The value of the hormone increased significantly ($p < 0.05$) to 0.62 iu/ml and thereafter gradually decreased non-significantly from groups D, E, F respectively. The lowest value of the hormone significant ($p < 0.05$) was observed from group B that was administered 20mg/kgbw/day only for the duration of the study.

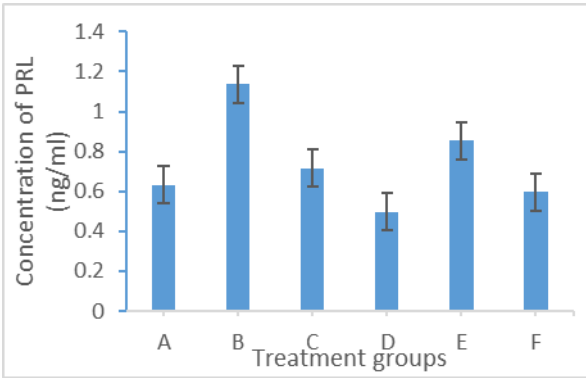


Fig.2A: Effect of Dimethoate and *Beta vulgaris* on Prolactin in female Swiss mice

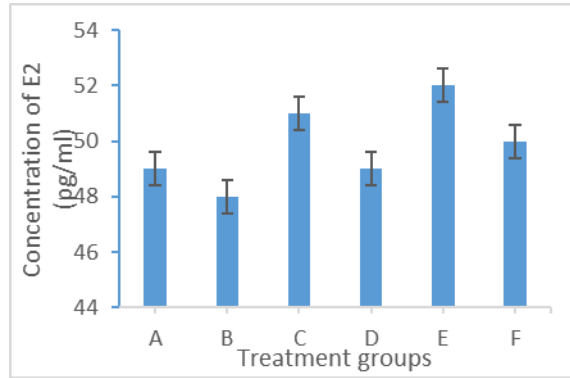


Fig.2B: Effect of Dimethoate and *Beta vulgaris* on Estradiol in female Swiss mice

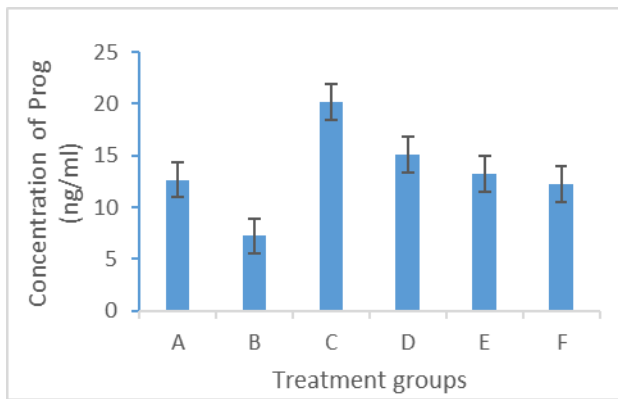


Fig.2C: Effect of Dimethoate and *Beta vulgaris* on Progesterone in female Swiss mice

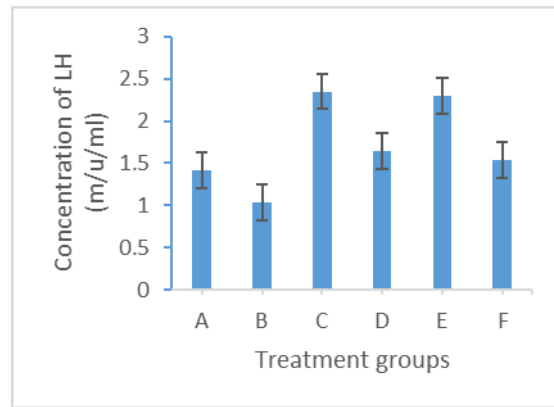


Fig.2D: Effect of Dimethoate and *Beta vulgaris* on Luteinizing hormone in female Swiss mice

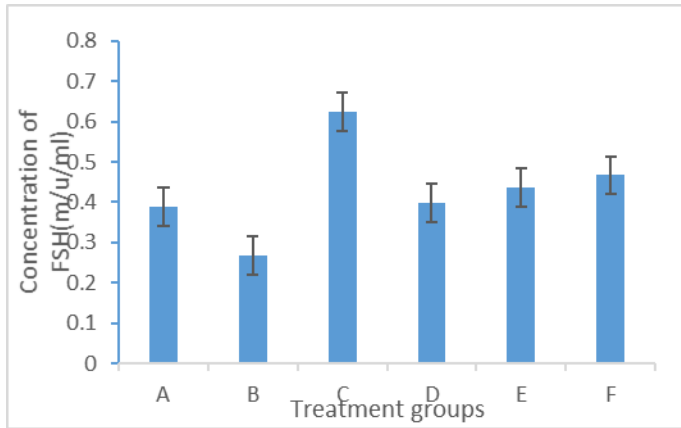


Fig.2E: Effect of Dimethoate and *Beta vulgaris* on Follicle stimulating hormone in female Swiss mice

3.4 Impact of Dimethoate and *Beta vulgaris* on Litter size of Female Swiss Mice.

Litter size gradually decrease non significantly from seven in the control and in group C which received 5mg/kgbw/day of Dimethoate along with 250mg/kgbw/day of 100% extract of *B.vulgaris*. Groups D, E, F had litter size of six, five, five respectively. The lowest litter size of two pups was obtained from group B which received 20mg/kg/bw/day of Dimethoate only as treatment for the duration of the experiment

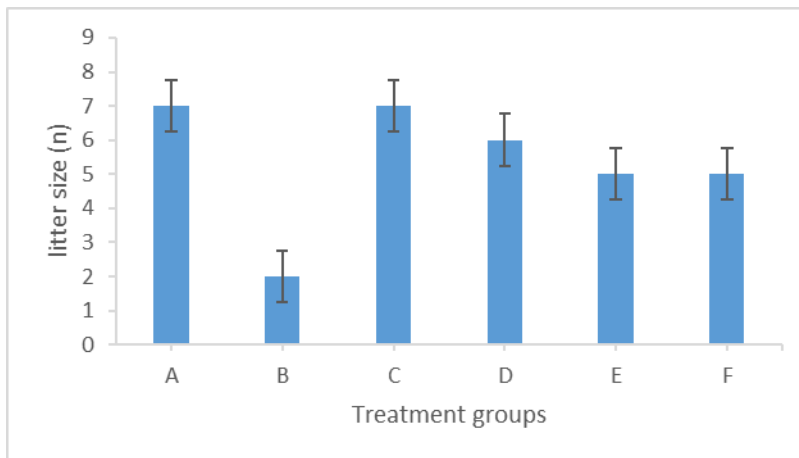


Fig.3: Effect of varying concentrations Dimethoate and *Beta vulgaris* on Litter size in female Swiss mice

4.0 Discussion

Endocrine toxicity of pesticides has been well documented and many studies have reported their adverse effect on the reproductive axis of both males [16, 17, 18, 19] and females [20]. Secondary infertility has become a common issue for which medical and herbal interventions are sought. *Beta vulgaris* is a common vegetable eaten raw, cooked and taken as juice.

Evaluation of the effect of *Beta vulgaris* on fertility indices of female mice exposed to Dimethoate showed significant increase in the reproductive hormones assayed, progesterone, estradiol, follicle stimulating hormone and luteinizing hormone compared to the group of animals administered Dimethoate only. This is in agreement with the report of [18,21]. The increase in reproductive hormone in the female mice used is indicative of the capacity of *B. vulgaris* extract to impact positively on fetal implantation, maintenance of pregnancy to full term and perhaps increase in litter size observed in the current study as well as conferring protection of the pups against abnormalities. This can be attributed to its antioxidant and anti-inflammatory property as also observed by [22].

The capacity to increase the concentration of estradiol is attributed to the presence of the element Boron which enhances fetal implantation in female mice. This agrees with [23] who reported a negative impact on early pregnancy associated with Boron deficient diet in rats while [23, 24] observed a negative effect on early embryonic development and the capacity to counteract genotoxicity according to [24]. These earlier studies validate the present observation that *B. vulgaris* which among other components contains Boron could lead to increase in litter size and production of pups without abnormalities. Moreover, elevation of estradiol and progesterone shows its ability to maintain healthy pregnancy as these hormones play vital role in the physiology, anatomy and perhaps molecular balance of uterine *milieu* for implantation and growth.

Conclusion

It is thus deduced that *B. vulgaris* might increase fertility in experimental animals, as well as, enhance the development of the brain in such animals. Dimethoate on the other hand showed significant impact on the

reproduction in female experimental animals by reducing the fertility indices, such as reproduction hormones, reduction in fetal implantation leading to low number of offspring (Litter size), inhibition of fertilization and ovulation and embryonic growth, increase in fetal mortality rate and delayed parturition.

Recommendation

Beta vulgaris contains vitamins, minerals and element and has potential as an aphrodisiac, the high content of vitamin C, Folic acid and the element boron confer on it a high anti-oxidant activity. It is recommended as refreshing juice for both males and females as it enhances production of reproductive hormones.

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