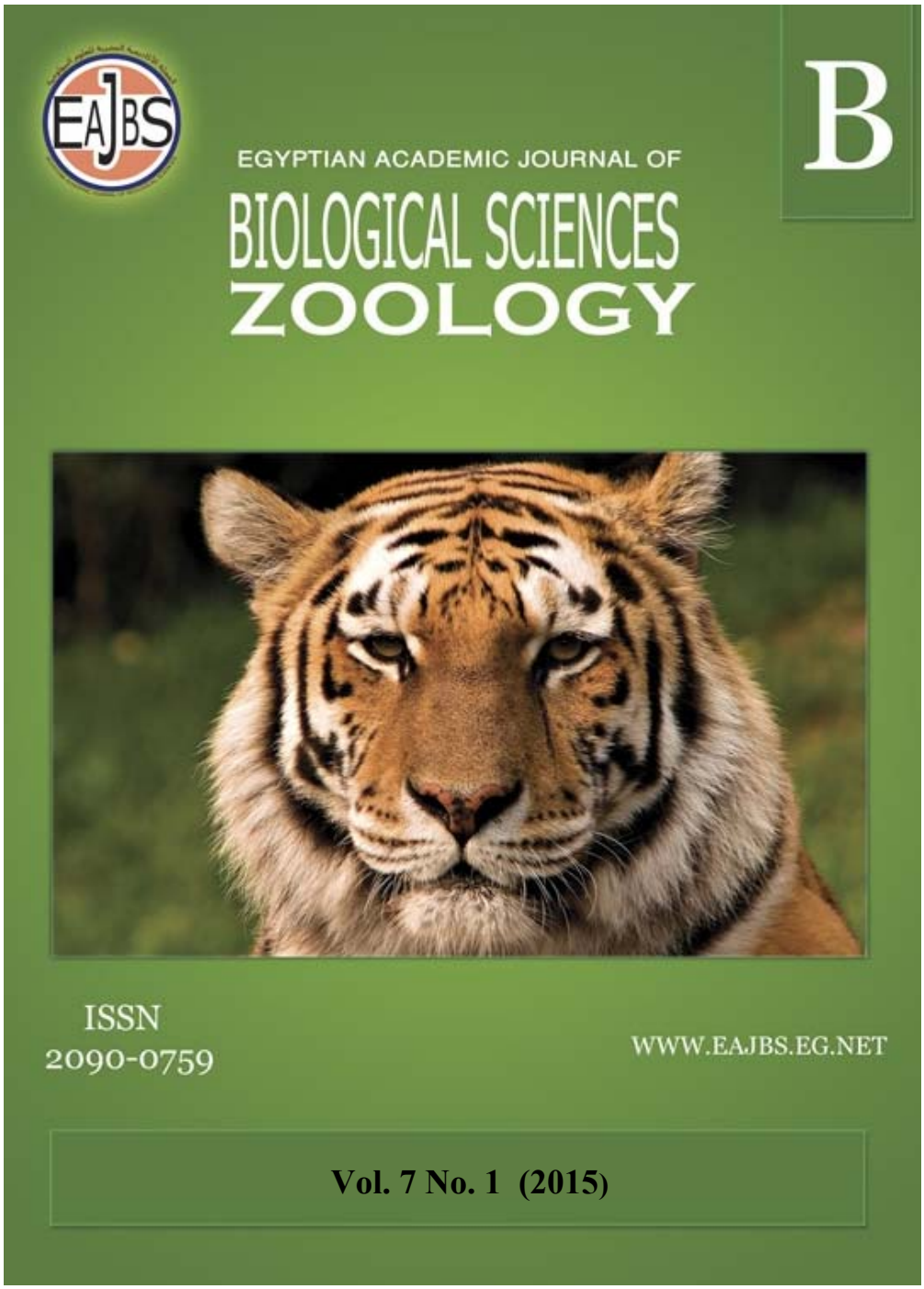

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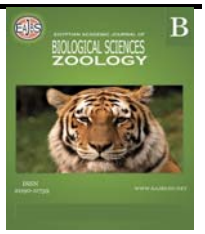
www.eajbs.eg.net

Citation :*Egypt.Acad.J.Biolog.Sci. (B.Zoology) Vol.7(1)pp1-12 (2015)*

Egypt. Acad. J. Biolog. Sci., 7(1): 1- 12 (2015)

Egypt. Acad. J. Biolog. Sci.
B. Zoology
ISSN: 2090 – 0759
www.eajbs.eg.net



Effect of zearalenone (Mycoestrogen) on morphometrics of female mice and ameliorative role of saffron

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ARTICLE INFO

Article History
Received: 2/1/2015
Accepted: 5/2/2015

Key words:
Zearalenone
Mycotoxicity
Teats
Vagina Prolapse
Saffron

ABSTRACT

The *Fusarium* species mycotoxin metabolite Zearalenone (ZEA) mimics the animal body production of estrogen and interferes with conception, ovulation, reproductive organ development and fetal development in farm animals as well as in humans. This research paper gives an overview about acute and chronic toxicity of ZEA and ameliorative role of saffron on interior and exterior morphology of female mice (*Mus musculus*). The morphological changes were seen, when six animals of each experimental group were administrated with ZEA intraperitonially (IP) (2.5mg/kg. b.w) in dimethyl sulphoxide (DMSO) and oral administration of saffron (50mg/kg. b.w) for 30, 60 and 90 days. Post of each experiment, we observed that estrogenic mycotoxin reduces body weight and increases reproductive organ weight (P-value < 0.01) followed by swelled ovaries, uterus, enlarged teats, and prolapsed vagina. However, animals treated with ZEA+saffron exhibits almost normal morphology of mice. Besides this, mice treated with saffron alone, reveals normal architecture of teats, vagina and reproductive organs.

Conclusion: The experimental investigation indicates that ZEA produces various morphological changes, while promising approach of using saffron to protect and enhancing the function of reproductive system and its associated organs has been proved benificial.

INTRODUCTION

A non-steroidal estrogenic mycotoxin Zearalenone (ZEA) is produced by *Fusarium* species. This mycoestrogen is found in contaminated cereal crops, interferes with basic metabolic system of animals. Zearalenone was isolated in 1962 by Stob (1962) and its structure was elucidated by Urry (1966). The leading targets of ZEA were those tissues enriched in the estrogen receptors, ovary, uterus, liver, kidney and immune systems (Abbes *et al.,* 2006).

Apart from interfering with estrogen function, it causes tissue oxidative stress (Hou *et al.,* 2013), so it can lead to carcinogenicity, cytotoxicity, immunotoxicity,

DNA damage and chromosomal aberration in rodents and humans (NTP 1982; Frag *et al.* 2010).

The toxin produces agonistic as well as antagonistic effect on the estrogen receptor (17β-estradiol) and thus exhibits distinct estrogenic properties with varying effect on reproductive system in several species of animals. Ingestion of ZEA and its other derivatives by humans might contribute to decreased resistance to infectious agents and neoplasm, and these compounds may function as unrecognized etiological factor of immune dysfunction diseases (Pestka *et al.* 1994). The mycotoxin causes hepatocellular adenomas in female mice and pituitary adenomas in both male and female mice. while as, in gilts it showed cytoplasmic degeneration, vacuolization and atrophy in liver, kidney and spleen (NTP 1982; Visconti *et al.* 2008; Jiang *et al.* 2011). Moreover, ZEA drastically reduced the number and motility of live spermatozoa in adult male albino mice and also affects the reproduction of animals at the level of changes in the function of the reproductive organs, even at the level of gametes-oocytes and spermatozoa (Kim *et al.* 2003;Sambuu *et al.* 2013) In addition to this in females, ZEA caused an increased size of the uterus and mammary glands, swelling of the vulva and hatching the birth canal in rats, mice and guinea pigs (Ruddick *et al*.1967). Reduction in fertility, damage to the reproductive tract, vaginal prolapse, increased embryonic re-absorption, changes in weight of adrenal, thyroid, pituitary glands, changes in serum levels of progesterone and 17β-estradiol were also reported by various workers (WHO 2000; Croubels and De Backer 2009 and Anmar *et al*., 2014).

Various pharmacological studies have shown that saffron extract and its active compounds have anticonvulsant, antidepressant, anti-inflammatory and anti-tumor activities (Karimi *et al.* 2001; Hosseinzadeh *et al.* 2007). Saffron is derived from dried stigmas of *Crocus sativus* (L.), a member of family Iridaceae. The main components of saffron are saffranal, crocin, crocetin, picrocrocin and zeaxanthin etc. In indigeneous system of medicine, it is used to cure chronic diseases such as asthma, arthritis, cold, coughs, acne and several skin diseases (Katariya *et al.* 2011). Traditionally, people believed that saffron is essential for the reproductive organs and therefore, necessary for healthy and complete pregnancy, because during pregnancy low dose of saffron intake helps to maintain the body temperature in females (Bisset 1994). The main components of saffron such as crocetin and saffranal have a wide range of various beneficial biological activities with no toxic side effects (Asai *et al.* 2005). In recent findings, saffron and its constituents may help to prevent or treat cancer, Parkinson's disease, reduce cholesterol level, protect against toxicants, enhance mental function and improve memory (Nair*et al.* 1991; Salomi *et al.* 1991; Escribano *et al.* 1996; Verma and Bordia 1998; Abe and Saito 2000; Ahmad *et al.* 2005; Nandan 2005; Premkumar *et al.* 2006; Alireza *et al.* 2011). The benefits of saffron have not yet ended, it also reduce inflammation, disorders of brain, kidney and acts as a diuretic (Bilal *et al.* 2011). Besides this, the herbal remedy maintains integrity of walls of blood vessels which reduces the blood pressure of body and prevents blockage or bursting (Xuan 1999; Fatehi *et al.* 2003; Katariya *et al.* 2011). Saffron not only attenuates the oxidative stress but also may serve as an adjunctive therapy in delaying the progression of ischemic heart disease (Jaspreet *et al.* 2012). In reproductive part; saffron or its constituents enhance ovarian function that ease the menstrual flow as well as regularizes menstrual cycles and acts as antispasmodic. It may enhance pituitary-ovary axis activities; boost the levels of FSH, LH and estradiol in addition to stimulating folliculogenesis in humans females (Mokhtar *et al.* 2010).

In males saffron rejuvenates reproductive system, which corrects the conditions like erectile dysfunction, premature ejaculation, low sperm count and low sperm motility (Szafranska *et al.* 2002). Thus, the aim of current study is to investigate the possible ameliorative role of saffron against ZEA induced morphological alterations of reproductive tract and its associate organs in female mice *Mus musculus*.

MATERIAL AND METHODS

Experimental animals*:*

The experimental investigation of long term exposure of ZEA and saffron was carried out at the Laboratory of Reproductive Endocrinology, Department of Bioscience Barkatullah University Bhopal. Twenty four clinically healthy mice (*Mus musculus*) aged eight weeks were taken with an initial body weight approximately 25± 5g. All animals were kept in individual polypropylene cages in a group of six each. They were fed standard diet with *ad libitum* water. Temperature was maintained at (25 ± 2 ^{0}C) with a 12 hours light/dark cycle.

Chemical and Antidote:

Zearalenone was purchased from company Sigma Aldrich (Z2125) whereas, saffron (stigma) was brought from South Kashmir Pompore, District Pulwama (Jammu and Kashmir, India) and was identified at Department of food Technology, Islamic University (Kashmir).

Dose and Duration:

An Alternate dose of ZEA (2.5mg kg/b.w) was administrated intraperitonially (IP) to female mice for 30, 60 and 90 days. Powder form of ZEA was dissolved in dimethyl sulphoxide (1% DMSO) and stored in 4^{0}C for stock solution. While as crude form of Saffron was dissolved in water and administrated orally for the same duration.

Experimental Design:

All animals were divided into four groups of six each.

Group I

This group served as control, received normal diet.

Group II

Animals of such group were administered with ZEA 2.5 mg /kg bwt for 30, 60 and 90 days.

Group III

Animals of this group were administered 2.5 mg ZEA/kg bwt along with 50mg saffron/ kg bwt for 30, 60 and 90 days.

Group IV

This group of animals were provided only saffron (50mg /kg bwt.) for 30, 60 and 90 days.

Before sacrifice the animals at different intervals, body weight and photographs of teats and vaginal opening were taken out externally and then animals were sacrified and the reproductive organs i,e utreus and ovary dissected out quickly,cleaned,weighted and photographs were ceased in a camera.

Statistical analysis:

The significance of difference between groups was tested using Two-way ANOVA. The value $p<0.01$ was considered significant.

RESULTS

Morphology:

Morphological analysis of control group of ZEA and saffron shown in (Figs. 1-A, 2-A, 3-A), revealed a normal archetecture of vagina, teats, exterior structure of ovary and uterus. However, ZEA treated animals for 30, 60 and 90 days reveled prolapsed vagina, enlarged teats, thick, swelled as well as short sized uterus (Figs.1-B, 2-B, 3-B). However, when the morphology of animals treated with ZEA along with saffron were assessed after 30, 60 and 90 days, the results were quite interseting. The animals exhibited almost normal architecture of vagina, teats, ovaries and uterus as compared to animals exposed with ZEA (Figs. 1-C, 2-C, 3-C), while, saffron administered animals for similar durations also exhibited normal features of reproductive and associate organs (Figs. 1-D, 2-D, 3-D). Food intake capcity were found reduced in case of ZEA treated group than control and saffron treated animals. No depression or other neurological manifestations were observed.

Body weight and Reproductive organ weight:

The body weight and reproductive organ weights were also monitored for each group before and after the experimentations. The animals treated with ZEA for all three durations revealed low body weight gain, but it was significantly reduced ($p<0.01$) after 90 days when compared to untreated and saffron administered mice and even its initial recorded weight at zero day of experiment. Along with the above, the animals also showed no body weight gain as compared to the group treated with ZEA for 60 days (Table 1). However, the mice administered with ZEA+saffron did not show any noticeable difference in their body weight gain as compared to control and other experimental groups. Whereas, animals given saffron extract exhibited healthy and significantly increased body weight similar to control.

Table 1:Effect of Zearalenone and Saffron on body weight (g) of female mice (*Mus musculus*)

Group→	Control		ZEA		ZEA+Saffron		Saffron	
Duration↓	*Before Treatment*	*After Treatment*	*Before Treatment*	*After Treatment*	*Before Treatment*	*After Treatment*	*Before Treatment*	*After Treatment*
30	26.54±0.45	28.97±0.46	27.18±0.47	24.32±0.64[a]	26.67±0.65	27.4±0.83	23.58±0.53	25.48±0.60
60	26.918±1.17	30.02±0.59	27.87±0.59	23.26±0.97[a]	23.65±0.86	25.85±0.79	28.17±0.54	31.05±0.66
90	27.09±0.87	34.038±0.99	28.08±0.89	21.026±0.81[ab]	24.76±0.73	25.18±0.60	25.10±0.57	33.47±0.76

n=06 The data are expressed as mean ± SE. Significant differences at $P < 0.01$.
[a]Significantly variant from control ($P < 0.01$).[b]Significantly variant from saffron ($P < 0.01$).

Besides this, we also found an increment in weight of uterus and ovaries of ZEA exposed animals in all durations, once again the long duration animals (60 and 90 days) were found more affected and displayed a significant ($p<0.01$) increment in their reproductive organ weight as compared to control (Table 2).

Table 2: Effect of Zearalenone and Saffron on weight (mg) of female reproductive organ (ovary + uterus)

Group/ Duration→ ↓	**30 Days**	**60 Days**	**90 Days**
Control	0.206±0.011	0.241±0.021	0.231±0.014
ZEA	0.261±014[a]	0.320±0.010[ab]	0.327±0.126[ab]
ZEA+Saffron	0.200±0.018	0.218±0.011	0.228±0.014
Saffron	0.220±0.014	0.258±0.017	0.259±0.012

n=06 The data are expressed as mean ± SE. Significant differences at $p < 0.01$.
[a] Significantly variant from control ($P < 0.01$) [b]Significantly variant from saffron ($P < 0.01$).

However, animals administered with ZEA+saffron exihibited insignificant change in their reproductive weight as compared to ZEA exposed animals (Table 2), but those animals administered with saffron only revealed slightly increased but statistically insignificant reproductive weight gain (Table 2).

DISCUSSION

The experimental facts presented herein support the hypothesis between non-steroidal elements and endogenous estrogenic receptor activity in animals. The function of ovaries and development of other associate organs directly depends on estrogen and its receptors (Knapczyk *et al.* 2008; Mayr *et al.* 1992). After the formation of a complex between estrogenic components and receptors, ZEA produces structural changes in the receptor that lead to binding with estrogen-responsive elements of DNA. Consequently, the ZEA induced transcription of genes sensitive to estrogens (Mueller *et al.* 2004). The binding affinity of ZEA to estrogen receptor in target tissues is 10% in comparison with 17estradiol (Klopman *et al.* 2003). Our morphological results confirmed the previous literature that ZEA is an estrogenic chemical or an endocrine disrupter that leads to deteriorating changes in reproductive and its associated organs (Morrison *et al.* 2003). Fallout of our experiment also revealed that increased weight of ovary and uterus may be hyperestrogenic response leads to uterine RNA synthesis as well as an increase in RNA polymerase activity, resulting in the synthesis of uterine estrogen-induced protein (Ford *et al.* 2004). Our results are also supported by Leticia *et al.* (Leticia *et al.* 2011) that showed increased reproductive tract weight, vulvar area and epithelial cell hypertrophy and hyperplasia of the uterine and vaginal mucosa as well as endometrial glands. The increased weight gain of reproductive system clearly showed hyperestrogenic effect of ZEA which directly affected body weight gain of mice in our experimental study. Through various experimental studies, it has been observed that hyperestrogenism process acts as inhibitory effect on body weight gain in animal models (Drewett 1973; Roesch 2006 and Heba *et al*., 2013). Thus, the reduced body weight gain in our study indicates estrogenic inhibitory effect of ZEA on treated animals. We also assumed the arcuate nucleus of hypothalamus is the area of the central nervous system (CNS) in which food intake is controlled, it also includes energy expenditure and body weight homeostasis is also controlled by ventromedial hypothalamus and it is also the fact that ERα is abundantly expressed in these areas of the CNS (Mauvais *et al.* 2013). So the reduced food intake clearly explained the reason of reduced body weight gain in present study. The wonder of vaginal prolapse may be genetical, nutritional or hormonal; all are linked. Unfortunately there is lot that still remains unknown about the causes of vaginal prolapse. Certain researchers have suggested that an increased expression of estrogen receptor α in the genital tract may facilitate an increased estrogenic effect, resulting in vaginal prolapse (Margaux and Arat 2010) while as, Ennen *et al.* (2011) reported that the animals having vaginal prolapse showed lower rate of α estrogen receptor. Another theory by Kahn (2005) related to vaginal prolapse may explain the mechanism behind it. The female genital organs are attached with ligamentiumlatum uteri also known as broad ligaments of uterus. Due to hormonal change, the soft tissues associated with the vagina undergo a varying degree of relaxation. The conjoining tissue relaxation with an increased abdominal pressure brought about by the increased weight of uterus may be risk for vaginal prolapse. So, these are contradicting results which have to be explored in future research work. The contact of animals to ZEA intoxication may encourage the explosion in estrogen-

dependent cells that may lead to enlargement of mammary glands or may lead to neoplasia and hyperplasia in the uterus, ovaries or mammary gland. Some researchers attribute that an endogenous hormones often inhibits unwanted proliferation but excite differentiation and endorse apoptosis (Doboszynska *et al.* 2004; Ranzenigo *et al.* 2008). Prominent and deteriorated teats in current study follows the previous literature that estrogens stimulate the proliferation of the endothelium in ducts and the retention of sodium and water, that leads to edema of stroma and finally to the excessive collagen production. Physiologically, all these functions are balanced by progesterone. It is rational since dysplastic lesions are usually the result of disturbances in estrogen-progesterone balance and the relative deficiency of progesterone (Russo *et al.* 1999). On the other hand a group of mice simultaneously treated with saffron in the current study revealed normal body weight, nearly normal genital organs and preserved architecture of teats as well as uninterrupted vagina. The main components of saffron such as crocetin and saffranal (Asai *et al.* 2005) have a wide range of various beneficial biological activities, often with no toxic side effects. In various studies saffron extract enhance female genital function and showed properties against DNA and RNA damaging (Fernandez 2006; Abdullaev *et al.* 2003; Kanakis *et al.* 2009). It affects the growth of cancer cells by inhibiting nucleic acid synthesis, enhancing anti-oxidative system, inducing apoptosis and hindering growth factor signaling pathways. (Gutheil *et al.* 2012). However, the mechanisms whereby these effects are induced have not been yet fully understood. In connection to this, we assumed that the proliferation of estrogenic-response cells might be inhibited by saffron, which leads significant inhibition of nucleic acid synthesis or it maydisturbthebinding and saturating mechanism between ZEA and estrogenic receptor.ourstudy was also supported by (Nair *et al.* 1995) reported a significant inhibition of nucleic acid synthesis, it appears that saffron (dimethyl- crocetin) disrupts the DNA- protein interaction and is believed that crocetin from crocin interferes with the enzyme to poisomerase II which is important for cellular DNA synthesis. It may be summarized here that saffron can be used for the better functioning of reproductive system and it also protects from ZEA induced reproductive toxicities.

Finally, we conclude that the ZEA may have resemblance with endogenous estrogen 17- β estradiol, because its administration in our experiment evidently showed deleterious effect on reproductive system *i.e.* uterus size and ovarian functions and mammary developments. Despite of ZEA‘s toxic effect, the simultaneous administration of saffron resulted in restoration of the morphological reproductive architecture of mice induced by Zearalenone and can be considered as an efficient protector against ZEA induced reproductive toxicity. These ameliorative effects may be on direct or indirect on gland or it may modulate the hypothalamo-hypophysial gonadal axis.

ACKNOWLEDGEMENT

Authors are thankful to Head of the Department of Bioscience, Barkatullah University, Bhopal for his ardent and technical support and also very thankful to Prof. Hossein Hosseinzadeh. Pharmaceutical Research Center, Department of Pharmacodynamics and Toxicology, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad Iran, for his guidance during the tenure of my research work.

Intrest of Confilict: Authors have no confilict with the work.

Morphology of Vagina

Fig. 1-A Control (Normal Vagina)

Fig. 1-B ZEA Treated (Prolapsed Vagina)

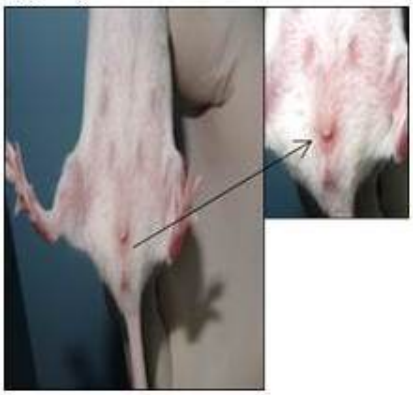

Fig. 1-C ZEA + Saffron (Normal Vagina)

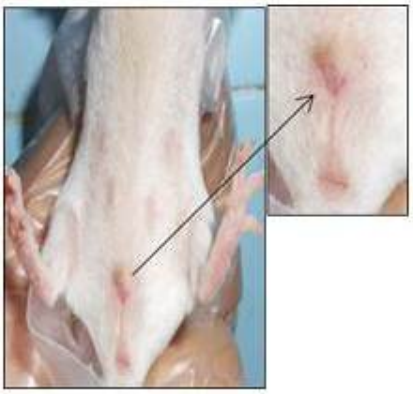

Fig. 1-D Saffron Treated (Normal Vagina)

Morphology of Teats

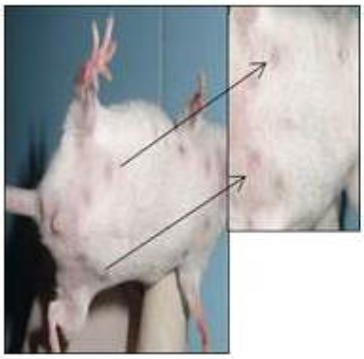

Fig. 2-A Control (Normal Teats)

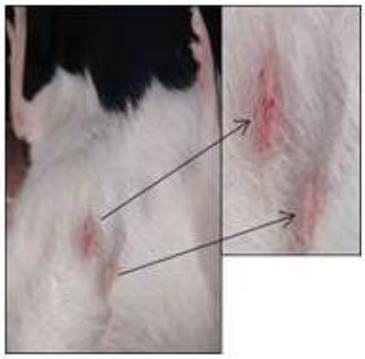

Fig. 2-B ZEA Treated (Effected Teats)

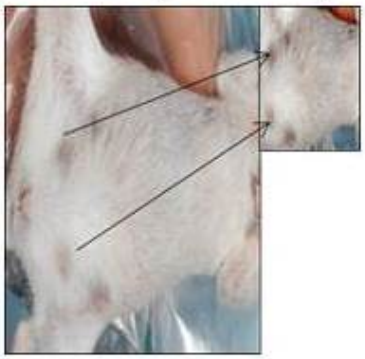

Fig. 2-C ZEA + Saffron Treated (Normal Teats)

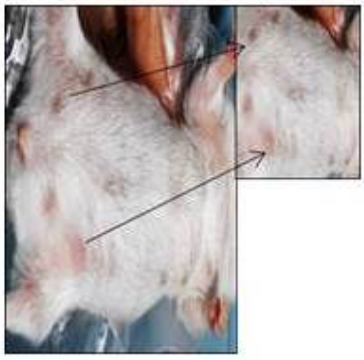

Fig. 2-D Saffron Treated (Normal Teats)

Morphology of uterus and ovary

Fig. 3-A Control

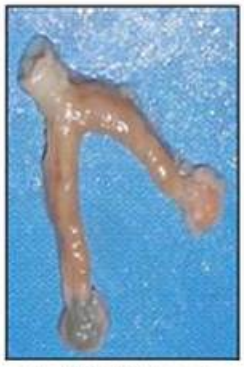

Fig. 3-B ZEA Only

Fig. 3-C ZEA + Saffron

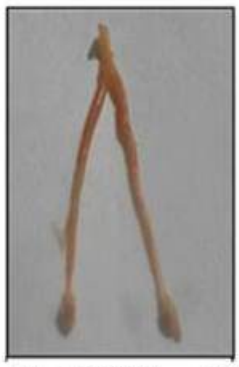

Fig. 3-D Saffron Only

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