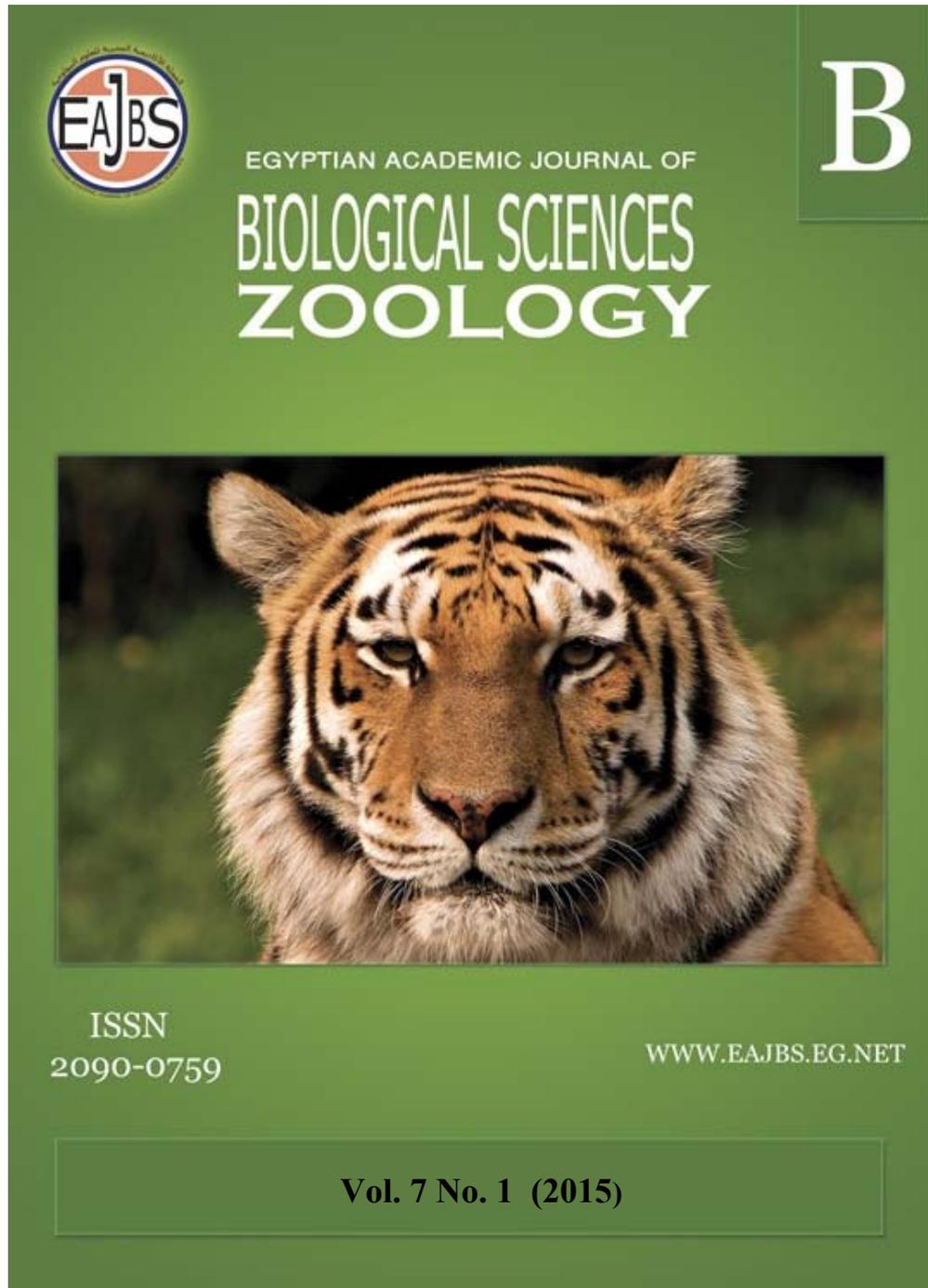


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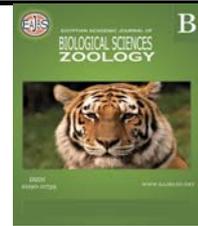
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**Citation** :*Egypt.Acad.J.Biolog.Sci. ( B.Zoology ) Vol.7(1)pp1-12 (2015)*



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

Bashir Ahmad; Sheetla Chouhan and Vinoy K. Shrivastava

Laboratory of Endocrinology, Bioscience Department, Barkatullah University, Bhopal.

E-mail:-[vinoyks2001@yahoo.com](mailto:vinoyks2001@yahoo.com)

### 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 intraperitoneally (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 beneficial.

### 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 (17 $\beta$ -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 17 $\beta$ -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 indigenous 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  $25 \pm 5$ g. 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  $(25 \pm 2^{\circ}\text{C})$  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 intraperitoneally (IP) to female mice for 30, 60 and 90 days. Powder form of ZEA was dissolved in dimethyl sulphoxide (1% DMSO) and stored in  $4^{\circ}\text{C}$  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 sacrificed 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 architecture of vagina, teats, exterior structure of ovary and uterus. However, ZEA treated animals for 30, 60 and 90 days revealed 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 interesting. 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 capacity 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→ Duration↓	Control		ZEA		ZEA+Saffron		Saffron	
	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 <sup>a</sup>	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 <sup>a</sup>	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 <sup>ab</sup>	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  $P < 0.01$ .

<sup>a</sup>Significantly variant from control ( $P < 0.01$ ). <sup>b</sup>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±0.014 <sup>a</sup>	0.320±0.010 <sup>ab</sup>	0.327±0.126 <sup>ab</sup>
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  $p < 0.01$ .

<sup>a</sup> Significantly variant from control ( $P < 0.01$ ) <sup>b</sup>Significantly variant from saffron ( $P < 0.01$ ).

However, animals administered with ZEA+saffron exhibited 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 associated 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 ER $\alpha$  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  $\alpha$  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  $\alpha$  estrogen receptor. Another theory by Kahn (2005) related to vaginal prolapse may explain the mechanism behind it. The female genital organs are attached with ligamentum latum 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 may disturb the binding and saturating mechanism between ZEA and estrogenic receptor. Our study 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 topoisomerase 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-  $\beta$  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.

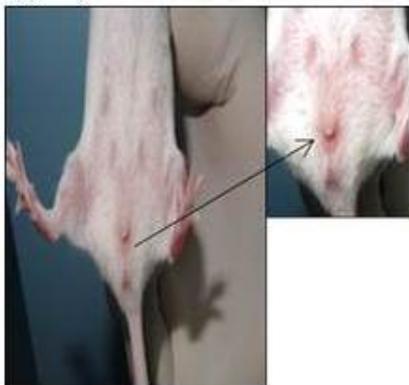
### Morphology of Vagina



**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

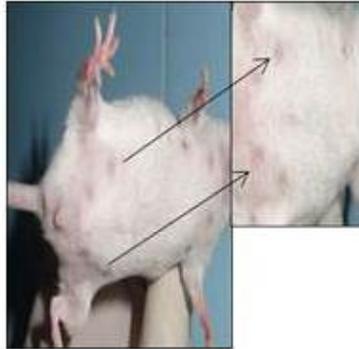


Fig. 2-A Control (Normal Teats)



Fig. 2-B ZEA Treated (Effected Teats)

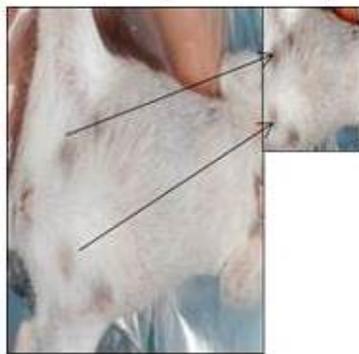


Fig. 2-C ZEA + Saffron Treated (Normal Teats)

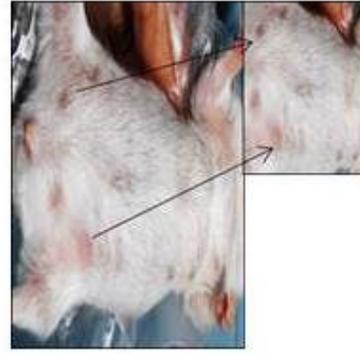


Fig. 2-D Saffron Treated (Normal Teats)

### Morphology of uterus and ovary



Fig. 3-A Control



Fig. 3-B ZEA Only



Fig. 3-C ZEA + Saffron



Fig. 3-D Saffron Only

## REFERENCES

- Abbes S, Salah-Abbes JB, Ouanes Z, Houas Z, Othman O and Bacha H (2006a). Preventive role of phyllosilicate clay on the Immunological and Biochemical toxicity of zearalenone in Balb/c mice. *International Immunopharmacology* 6: 1251-1258.
- Abdullaev FI, Rivern NL, Caballero OH, Manuel HJ, Prez LI, Pereda MR, Espinosa AJJ. (2003). Use of in vitro assays to assess the potential antigenotoxic and cytotoxic effects of saffron (*Crocus sativus* L.). *Toxicol In Vitro*. 17: 731–736.
- Abe K. and Saito H. (2000). Effects of saffron extract and its constituent crocin on learning behaviour and long-term potentiation. *Phytother Res*. 14: 49-52.
- Ahmad AS, Ansari MA, Ahmad M, Saleem S, Yousuf S, Hoda MN. (2005). Neuroprotection by crocetin in a hemi- parkinsonian rat model. *Pharmacol Biochem Behav*. 81: 805-813.
- Alireza TH, Seyed AM, Mahmoud M. and Hosseinzadeh H. (2011). The effect of crocin and safranin, constituents of saffron, against subacute effect of diazinon on hematological and genotoxicity indices in rats. *Phytomedicine J of Phytother&Phytopharmaco*. 48: 28-38.
- Anmar SH, Karkaz MT and Batol ID. (2014). Effects of interaction between Aflatoxins (AFs) and functional materials FM in the hematological, biochemical parameters and enzyme activity in Rats. *Egypt. Acad. J. Biolog. Sci.,(B-Zoology )* 6 (2): 17- 22
- Asai A, Nakano T, Takahashi M, Nagao A. (2005). Orally administered crocetin and crocin are absorbed into blood plasma as crocetin and its glucuronide conjugates in mice. *J Agric Food Chem*. 53: 7302-7306.
- Bilal AW, Amina KRH, Mohiddin FA. (2011). Saffron a repository of medicinal properties. *J of Medi Plan Res*. 5: 2131-2135.
- Bisset NG. (1994). *Herbal drugs and phytopharm*. Boca Raton, FL, CRC Press
- Carcinogenicity bioassay of Zearalenone in F344/N rats and F6c3f1 mice. National toxicological Program, Technical Report series, USA. 1982; 235.
- Croubels S, De Backer P. (2009). *Diergeneeskundige Toxicologie* Course faculty of veterinary medicine, Ghent, 185.
- Doboszynska T, Jarczyk A. and Postek M. (2004). Influence of zearalenone on the structure of uterine horns in young gilts. *Medycyna Wet*. 60: 274-277.
- Drewett RF. (1973). Oestrous and dioestrous components of the ovarian inhibition on hunger in the rat. *Anim. Behav*. 21: 772–780.
- Ennen S, Kloss S, Scheiner BG, Failing K. and Wehrend A. (2011). Histological, hormonal and bio-molecular analysis of the pathogenesis of ovine Prolapsusvaginae ante partum. *Theriogenology*. 75: 212-219.
- Escribano J, Alonso GL, Coca PM, Fernandez JA. (1996). Crocin, safranin and picrocrocin from saffron (*Crocus sativus* L.) inhibit the growth of human cancer cells in vitro. *Cancer Lett*. 100: 23–30.
- Farag IM, Abdel AKB, Nada SA, Tawfek NS, Farouk T. and Darwish HR. (2010). Modulation of ochratoxin-induced oxidative stress, genotoxicity and spermatotoxic alterations by *Lactobacillus rhamnosus* GG in male Albino mice. *J of Am. Sci*. 6: 575-587
- Fatehi M, Rashidabady T. and Fatehi HZ. (2003). Effects of *Crocus sativus* petals' extract on rat blood pressure. *J Ethnopharmacol.*, 84: 199-203.

- Fernandez JA. (2006). Anti-cancer properties of saffron, *Crocus sativus* Linn. In Kahn, MTH, Ather, A. Editors, *Lead Molecules from Natural Products*. Elsevier B.V., 313–330.
- Ford JJ, Wise TH. and Christenson RK. (2004). Lack of an association between plasma follicle-stimulating hormone concentrations and ovarian weight in prepubertal gilts. *J Anim Sci.*, 82: 472–478.
- Gutheil WG, Reed G, Ray A, Anant S. and Dhar A. (2012). Crocetin: an agent derived from saffron for prevention and therapy for cancer. *Curre Pharma Biotech.*, 13: 173-179.
- Heba MA, Abdel R, Hend M T, Hekmat MT. and Hanan T. (2013). Soy isoflavones reduce adiposity via increasing estrogen receptor beta expression in ovariectomized female rats. *Egypt. Acad. J. Biolog. Sci.,(B-Zoology)* Vol., 5(1): 59 -71
- Hosseinzadeh H, Vahidehsadat M. and Farzin H. (2007). Antidepressant effect of kaempferol, a constituent of Saffron (*Crocus sativus*) petal in mice and rats. *Pharmacol.*, 2: 367-370
- Hosseinzadeh H. and Younesi HM. (2002). Antinociceptive and anti-inflammatory effects of *Crocus sativus* L. stigma and petal extracts in mice. *BMC Pharmacol.*, 2: 1-8
- Hou YJ, Zhao YY, Xiong B, Cui XS and Kim NH (2013). Mycotoxin-Containing Diet Causes Oxidative Stress in the Mouse. *PLOS ONE* 8 6037- 6044.
- Jaspreet S, Vineeta T, Mahaveer G, Khalid MS, Tapas CN, Ruma R, Santosh K. and Dharamvir SA. (2012). *Crocus sativus* L (saffron) attenuates isoproterenol-induced myocardial injury via preserving cardiac functions and strengthening antioxidant defense system. *Exp and Toxicol Pathol.*, 64: 557– 564
- Jiang SZ, Yang ZB, Yang WR, Gao J, Liu F X, Broomhead J. and Chi F. (2011). Effects of purified Zearalenone on growth performance, organ size, serum metabolites, and oxidative stress in post weaning gilts. *J Anim Sci.*, 89: 3008-3015
- Kahn C. (2005). Ed. *Merck veterinary manual*. 9<sup>th</sup> ed. Rahway, NJ: Merck,
- Kanakis CD, Tarantilis PA, Pappas C, Bariyanga J, Tajmir RHA. and Polissiou MG. (2009). An overview of structural features of DNA and RNA complexes with saffron compounds models and antioxidant activity. *J Photochem Photobiol B Biol.*, 95: 204–212.
- Karimi G, Hosseinzadeh H. and Khaleghpanah P. (2001). Study of antidepressant effect of aqueous and ethanolic of *crocus sativus* in mice. *Iranian J Basic Med Sci.*, 4: 11-15
- Katariya DC, Nilakshi N, Vijay GR. and Abhyankar MM. (2011). Pharmacological action of *Crocus sativa*. *Int J. Pharma and Bio Sci.*, 2: 530.
- Kim IH, Son HY, Cho SW, Ha CS. and Kang BH. (2003). Zearalenone induces male germ cell apoptosis in rats. *Toxicol. Lett.*, 138: 185–192.
- Klopman G. and Chakaravarti SK. (2003). Structure-activity relationship study of a diverse set of estrogen receptor ligands (I) using Multicase expert system. *Chemosphere*. 51: 445–459.
- Knapczyk K, Duda M, Durliej M, Galas J, Kozirowski M. and Slomeczyn SM. (2008). Expression of estrogen receptor (ER) and estrogen receptor (ER) in the ovarian follicles and corpora lutea of pregnant swine. *Domes Anim Endocrin.*, 35: 170–179.

- Krivohlavkova L, Hoskova K, Krejcarikova A. and Rajmon R. (2013). Activity of Soy Phytoestrogens and Zearalenone on mammalian reproduction: *Scie. Agri. bohemia.*, 44: 119–126.
- Leticia C, Teixeira, Fabiano MF, Rosangela LD, Elizabeth S. and Geraldo CA. (2011). Effects of zearalenone in prepubertal gilts. *Pesq. Vet. Bras.*, 31: 656-662.
- Lioi MB, Santoro A, Barbieri R, Salzano S. and Ursini MV. (2004). Ochratoxin A and zearalenone: a comparative study on genotoxic effects and cell death induced in bovine lymphocytes. *Mutat. Res.*, 557: 19-27
- Margaux K. and Aart De Kruif. (2010-2011). The occurrence of Vaginal Prolapse in Sheep and Cattle. Ghent University Faculty of Veterinary Medicine Academic year.
- Mauvais-Jarvis, F.; Clegg, D.J. and Hevener, A.L. (2013). The role of estrogens in control of energy balance and glucose homeostasis. *Endocr. Rev.*, 34: 309–338
- Mayr U, Butsch A. and Schneider S. (1992). Validation of two in vitro test systems for estrogenic activities with zearalenone, phytoestrogens and cereal extracts. *Toxicology.*, 74:135–149.
- Mokhtar M, Esfandiar S. and Adel D. (2010). Effects of Hydro-alcoholic Extract of Red Dried Stigmas of *Crocus sativus* L. Flowers (saffron) on the Levels of Pituitary-ovary Hormones and Folliculogenesis in Rats. *Int J of Fertil and Ster.*, 3: 185-190.
- Morrison AG, Callanan JJ, Evans NP, Aldridge, TC. and Sweeney T. (2003). Effects of endocrine disrupting compounds on the pathology and oestrogen receptor and distribution in the uterus and cervix of ewe lambs. *Domest Anim Endocrinol.*, 25: 329–343.
- Mueller S, Simon S, Chae K, Metzler M. and Korach KS. (2004). Phytoestrogens and their human metabolites show distinct agonistic and antagonistic properties on estrogen receptor  $\alpha$  (ER $\alpha$ ) and ER in human cells. *Toxicol Sci.*, 80: 14 –25.
- Nair SC, Kurumboor SK. and Hasegawa JH. (1995). Saffron chemoprevention in biology and medicine: a review. *Cancer Biother.*, 10: 257-264.
- Nair SC, Salomi MJ, Pannikar B. and Pannikar KR. (1991). Modulatory effects of the extracts of saffron and *Nigella sativa* against cisplatin induced toxicity in mice. *J Ethno pharmacol.*, 31:75-83.
- Nandan KJ. (2005). *Pytopharm, Crocus sativus* Feature article., 3-12.
- Pestka JJ. and Bondy GS. (1994). Immunotoxic effects of mycotoxins. In: Miller JD and Trenholm HL (Eds.), *Mycotoxins in grain. Compounds other than aflatoxins.* Eagan Press, Pt. Paul (MN) USA, 339-358.
- Premkumar K, Thirunavukkarasu C, Abraham SK, Santhiya ST, Ramesh A. (2006). Protective effect of saffron (*Crocus sativus* L.) aqueous extract against genetic damage induced by anti-tumor agents in mice. *Hum Exp Toxicol.*, 25: 79-84.
- Ranzenigo, G., Caloni, F., Cremonesi, F., Aad, P.Y. and Spicer, L.J. (2008). Effects of *Fusarium* mycotoxins on steroid production by porcine granulosa cells. *Anim. Reprod. Sci.*, 107: 115–130.
- Roesch DM. (2006). Effects of selective estrogen receptor agonists on food intake and body weight gain in rats. *Physiol. Behav.*, 87: 39–44.
- Ruddick JA, Scott PM. and Harwig J. (1976). Teratological evaluation of zearalenone administered orally to the rat. *Bull Environ. Contam. Toxic.* 15: 678-681.
- Russo J, Rivera R. and Russo IH. (1992). Influence of age and parity on the development of the human breast. *Breast Cancer Res Treat.*, 23: 211-215.

- Salomi MJ, Nair SC. and Panikkar PR. (1991). Inhibitory effects of *Nigella sativa* and saffron (*Crocus sativus*) on chemical carcinogenesis in mice and its non-mutagenic activity. *Proc Ker Sci Congr.*, 3: 125–126.
- Sambuu R, Takagi M, Namula Z, Nii M, Taniguchi M, Uno S, Kokushi E, Tshering C, Do Santos RR, Fink-Gremmels J. and Otoi T. (2013). Effects of long-term in vitro exposure of ejaculated boar sperm to zearalenone and  $\alpha$ -zearalenol in sperm liquid storage medium. *Anim. Sci. J.*, 84: 28-34.
- Silvana PB, Carlos BF, Lucian Del’F, Luiz F, Freire R, Cristiano RJ, Mauro SO. And Ana FF. (2012). Possible role for glutathione-S-transferase in the oligozoospermia elicited by acute zearalenone administration in Swiss albino mice. *Toxicol.*, 60: 358–366.
- Stob M, Baldwin RS, Tuite J, Andrews FN. and Gillette KG. (1962). Isolation of an anabolic uterotrophic compound from corn infected with Gibberellae. *Nature.*, 196: 1318
- Szafranska B, Ziecik A. and Okrasa S. (2002). Primary antisera against selected steroids or proteins and secondary antisera against globulins an available tool for studies of reproductive processes. *Repro Bio.*, 2:187-204.
- Urry WH, Weirmeister HL, Hodge EB. and Hidy PH. (1966). The structure of zearalenone *Tetrahedron. Lett.*, 27:3109-3114
- Verma SK. and Bordia A. (1998). Antioxidant property of saffron in man. *Indian J. Med. Sci.*, 52: 205-207.
- Visconti A. (2001). Problems associated with Fusariummycotoxins in cereals. *Bulletin, of the Institute for Comprehensive Agricultural Sciences, Kinki University.*, 9: 39-55
- World Health Organization (2000). Evaluation of Certain Food Additives and Contaminants. WHO technical report series, 896
- Xuan B. (1999). Effects of crocin analogs on ocular flow and retinal function. *J Ocul Pharmacol Ther.*, 15: 143-52.
- Zinedine A, Soriano JM, Molto JC. and Manes J. (2007). Review on the toxicity, occurrence, metabolism, detoxification, regulations and intake of zearalenone an oestrogenicmycotoxin. *Food and Chemi. Toxicol.*, 45: 1-18.