

## Effect of saffron on mouse embryo development

Fatma Al-Qudsi and Amal Ayedh

Biology Department, Science Faculty, King AbdulAziz University, Jeddah, Saudi Arabia

P.O. Box 42650, Jeddah 21551, Saudi Arabia

[faqudsi@kau.edu.sa](mailto:faqudsi@kau.edu.sa)

**Abstract:** Saffron is widely used as a food additive, as an important ingredient of Arabic coffee and as an herbal medicine. The aim of this study was to evaluate the effect of high and low doses of aqueous saffron extract on mice embryos development. Pregnant mice were divided into three groups of fifteen animals each. Group 1 received 10ml/kg body weight double distilled water as control, group 2 was treated with 100 mg saffron / kg body weight and group 3 was treated with 2.5 mg saffron / kg body weight. Doses were administered for 5 days during the first and second weeks of gestation and for four days during the third week of gestation. Embryos were extracted on day 14, 18 of gestation and day 1 neonates. Whole body weight, whole body length, tail length, half head circumference and eye dimensions of the embryos and neonates were recorded. Congenital malformations of all groups were studied. Both treatments caused embryonic growth parameters to be significantly less than the controls. Congenital malformations were seen in treated embryos and neonates such as subcutaneous bleeding and head malformations. It was concluded that oral administration of both doses of saffron might cause intrauterine growth retardation and congenital malformations to mouse embryos.

[Fatma Al-Qudsi and Amal Ayedh. **Effect of saffron on mouse embryo development.** *J Am Sci* 2012;8(12):1554-1568]. (ISSN: 1545-1003). <http://www.jofamericanscience.org>. 207

**Key words:** mouse embryo, saffron, head malformation, intrauterine growth retardation, placental blood flow.

### 1. Introduction

Commercial saffron is the dried tripartite stigma of the *Crocus sativus* L. flowers. It is cultivated in many countries such as Azerbaijan, France, Greece, India, Iran, Italy, Spain, Turkey, Egypt, and Mexico (Abe and Saito, 2000; Akhondzadeh *et al.*, 2004; Lage and Cantrell, 2009). Saffron is used largely in folklore medicine and modern pharmacy because it is useful in the treatment of many human diseases such as antispasmodic, hypolipemic, cancer chemopreventive drug and to improve learning and memory activity. Saffron was widely used as a food additive and coloring, flavoring agent (Abdullaev, 1993; Abdullaev *et al.*, 2000; Hosseinzadeh and Younesi, 2002).

Large numbers of patients are resorting to the use of medicinal plants and herbs for the purpose of improving their health (Chermahini *et al.*, 2010). In Saudi Arabia studies have shown that 42% families are using complementary and alternative therapies without medical advice (Jan *et al.*, 2009). In the United States it was found that one person out of every three people uses alternative medicine (Abdullaev and Frenkel, 1999; Chermahini *et al.*, 2010). Human beings have used carotenoids as food flavors and colors for centuries. Saffron is one of the most used pigments. It consists of mixtures of pigments and other unidentified substances. Carotenoids have very important biological activities and their use as food is common today and recommended largely due to their content of vitamin A and antioxidant activities (Delgado-Vargas and Paredes-López, 2003). Biological antioxidants

(carotenoids components of saffron) play important roles in human health via the protection of cells and tissues from damaging effects of free radicals (Edgeet *et al.*, 1997; Tavana *et al.* 2012).

Saffron's ability to protect cells from oxidative stress by scavenging free radicals comes from the two main chemical components of saffron crocin and crocetin (Borset *et al.*, 1982; Tavana *et al.*, 2012).

Most of the research done on the effect of saffron used high but not lethal doses. However no research used the doses that are actually used in everyday food preparation. Many human females consume saffron daily in water, tea or coffee. About 50% of the congenital abnormalities seen in newborns are unknown. There is a need to perform research on the effect of saffron on embryonic development to direct the pharmacological use of saffron later on. Therefore came the importance of doing a research using normal everyday used doses of saffron. Mice were used in this research as a mammal model, as their reaction to substances is likely to be like human reactions. Their main biological body systems work is covered by many previous studies (Theiler 1989; Nagy *et al.*, 2003). In this study the term embryo was used to express all ages during gestation and the term neonate was used for after birth stages. Mice duration of gestation is very short therefore the distinction between embryo and fetuses are not considered important, but the developmental age post -conception is important (Kaufman and Bard, 1999; Crawford *et al.*, 2010).

As no studies have been done on the effect of saffron consumed on a daily basis on embryonic development the aim of the present study was to study the effect of saffron aqueous extract using doses such as those used in food preparation and other doses from previous studies on the development of mice embryos.

## 2. Materials and methods

All experimental procedure was approved by biology department at King Abdulaziz University. Ninety SWR (Swiss White Rodeless) mice were used in this study (45 mature female and 45 mature male mice) from King Saud University Animal house, their weight was between  $\pm 25$ -30 grams. They were maintained at  $22 \pm 2^\circ\text{C}$  on 12 hours light: 12 hours dark daily in plastic cages, and fed on corn cob pellets with water bottles.

Plant material Saffron, the dried stigmas of *Crocus sativus* flower were purchased from Al-alawy market in Jeddah. It was identified by our Botany taxonomist, in the Biology Department at the Faculty of Science, King Abdulaziz University. **Determination of estrus stage** Estrus stage in female mice was figured by performing the vaginal smear according to the methods of (Walmer *et al.*, 1992; Caligioni, 2009). For mating purposes, a male was placed with a female (in estrus stage) in one cage and left overnight. The next day, female vagina was examined for the presence of vaginal plug, as fertilization evidence. This was considered the first day of pregnancy and sexes were separated. On the other hand, in case of vaginal plug absence both sexes were left another 24 hours.

**Dose determination:** In this experiment two doses of saffron solution were used. One dose was taken from a previous study (Premkumar *et al.*, 2003) (100 mg/kg body weight) called in this study (TP for treated paper). While the other dose was calculated according to a questionnaire used in this study (2.5 mg/kg body weight) called in this study (TQ for treated questionnaire). A questionnaire (116 copy) (Figure1) was distributed to various age groups of women to determine the amount of daily consumption of Arabic coffee with saffron on a normal basis. It was observed that the highest rate of drinking Arabic coffee with saffron was eight cups/ day (small Arabic coffee cups = 50 ml). The dose calculated from the questionnaire is explained in figure 2.

### Preparation of the aqueous extract of saffron

The aqueous extract of saffron was prepared according to the method described by (Premkumar *et al.*, 2003). The required amount of dried stigmas of *Crocus sativus* L. was weighed and then soaked in double distilled water for one hour. Then it was homogenized using a homogenizer model Ultra-Turrax

T25. The mixture was then centrifuged at 2000 rpm for 10 minutes to remove the particles. The supernatant was used for the experiment.

Pregnant mice were divided into three groups each one consisting of 15 pregnant mice. Control group (C) given (10 ml / kg body weight) of double distilled water, treated group (TP) given (10 ml / kg body weight from the saffron aqueous extract concentration 100 mg / kg body weight) (Premkumar

*et al.*, 2003) and treated group (TQ) given (10 ml / kg body weight from the saffron aqueous extract concentration (2.5 mg / kg body weight) which was calculated from the questionnaire. Saffron solution or distilled water doses were orally administrated to pregnant mice using oral gavage that was cleaned with distilled water after administration of each experimental group. Doses were administered for 5 days during each of the first and second weeks and for four days during the third week of gestation (Table1). A pilot study was done to see the effect of giving double distilled water to pregnant females 10 ml / kg and 7.3 ml / kg as no significant difference was seen with the outcome. It was decided to keep one control group with 10 ml / kg double distilled water dose.

**Sample collection:** All mothers were weighed on day 1 of experiment then weighed before dissection. Embryos were collected from all groups on day 14, 18 of gestation and day 1 neonates. Pregnant mice were weighted, anesthetized, and then dissected. The complete process of sample collection was photographed (Sony camera model DSC-T900) for each dissected mother including the photos of each embryo the camera had a fixed zoom and fixed distance from the specimen this was done to record all data during sample collection. Collected embryos of all ages and experimental groups were washed in saline solution (0.99 grams of sodium chloride in 100 ml of distilled water) they were patted dry then weighed. After that they were examined and photographed by a dissecting microscope model (Olympus SZX10) connected with a camera model (Olympus DP25) at the central laboratory, Faculty of science, King Abdulaziz University, girl's section. Morphometric measurements were performed using DP2-BSW software. In embryos morphometric measurements studied were embryos whole body length, tail length, half head circumference, eye dimensions (diameter and area of the eye) (figure 2).

**Statistical analysis:** Data was analyzed using SPSS 16. The test used with normal distribution was Anova, Student-Neuman Keul test. In case of abnormal distribution Man-Whitney U test was used from the non-parametric test. Significance (\* was at  $p < 0.05$ ).

### Questionnaire

There are many questions about the effects caused by consuming saffron during pregnancy on embryo and if it has a role in the incidence of abortion.

The objective of the questionnaire: to estimate the amount of daily saffron consumption by adult females.

Name: Amal Saeed Ayedh

Please fill in the questionnaire with credibility and clarity, grateful to you for your cooperation.

1- Where is the place of your stay?

A- Jeddah B- Makkah AL-Mokaramah C- Al-Taief D- Abhaa E- AL-Riadh F- Others → to know the city

2- What is the origin?

A- Northern region B- Southern region C- Eastern region D- Western region E- Central region → To study the distribution

3- what is your weight in ?

40-50      50-60      60-70      70-80      80-90      90-100 → To estimate the weight of consumer

If you do not mind please write the exact weight to its importance in the research \_\_\_\_\_

4- Do you add saffron when cooking certain foods?

Yes      No

→ To find out whether saffron is used when cooking

5- Do you put saffron in the drinking water?

Yes      No

→ To see if they add the saffron with water or not

6- Do you drink Arabic coffee?

Yes      No

→ To find out if they drink Arabic coffee

7- Do you put the saffron in the coffee?

Yes      No

→ To estimate the proportion of females who add saffron to the coffee

8- How many times do you drink Arabic coffee with saffron on a day?

Once      Twice      Three times      More than Three times

9- How many cup of coffee you drink every time?

One      Two      Three      More than three cups

→ To see how many times they drink coffee in one day

→ To know the number of cups consumed each time

Figure 1 showing the questionnaire used to determine the amount of daily consumption of Arabic coffee with saffron on a normal basis

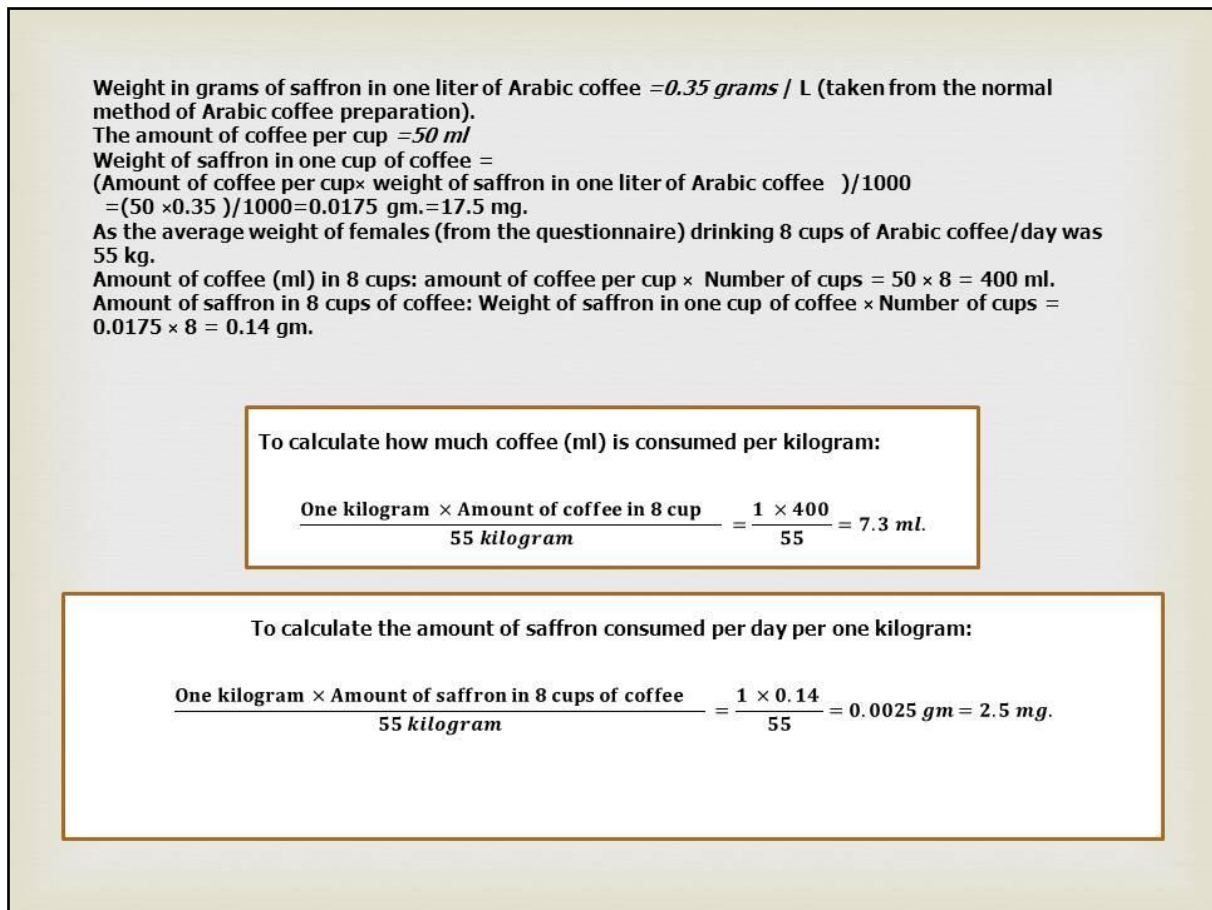
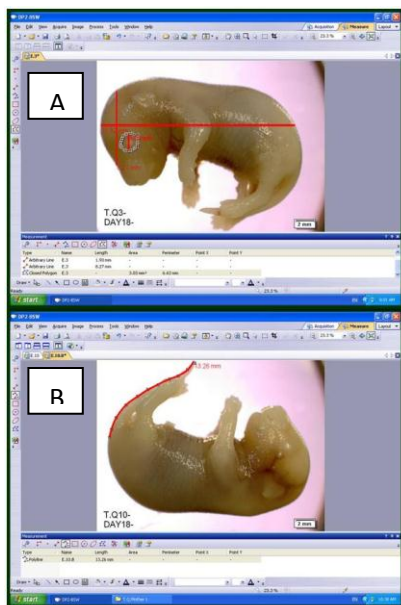


Figure 2 explains the dose calculated from the questionnaire



**Table 1 shows the dose administration time and type**

Groups Days of gestation	Dose type	Doses amount	Days of administration during pregnancy (according to gestational age)	Number of doses	The number of pregnant female
C day 14	double distilled water	10 ml / kg body weight	On days 1, 2, 3, 4, 7, 8, 9, 10, 11 and 14	10	5
TP day 14	100 mg saffron / kg body weight	10 ml / kg body weight	On days 1, 2, 3, 4, 7, 8, 9, 10, 11 and 14	10	5
TQ day 14	2.5 mg saffron / kg body weight	7.3 ml / kg body weight	On days 1, 2, 3, 4, 7, 8, 9, 10, 11 and 14	10	5
C day 18	double distilled water	10 ml / kg body weight	On days 1, 4, 5, 6, 7, 8, 11, 12, 13, 14, 15 and 18	12	5
TP day 18	100 mg saffron / kg body weight	10 ml / kg body weight	On days 1, 4, 5, 6, 7, 8, 11, 12, 13, 14, 15 and 18	12	5
TQ day 18	2.5 mg saffron / kg body weight	7.3 ml / kg body weight	On days 1, 4, 5, 6, 7, 8, 11, 12, 13, 14, 15 and 18	12	5
C day 1 neonate	double distilled water	10 ml / kg body weight	On days 1, 2, 3, 6, 7, 8, 9, 10, 13, 14, 15, 16, 17 and 20	14	5
TP day 1 neonate	100 mg saffron / kg body weight	10 ml / kg body weight	On days 1, 2, 3, 6, 7, 8, 9, 10, 13, 14, 15, 16, 17 and 20	14	5
TQ day 1 neonate	2.5 mg saffron / kg body weight	7.3 ml / kg body weight	On days 1, 2, 3, 6, 7, 8, 9, 10, 13, 14, 15, 16, 17 and 20	14	5

### 3.Results:

#### Effect of saffron on pregnant mother's weight

A decrease in treated TP and TQ mother's weight was seen on day 14 of gestation compared to the controls. However this decrease was only significant in treated TP mother's  $p=0.008$ . A non significant increase in treated TP and TQ mother's weight was seen on day 18 of gestation compared to the controls. However on day 1 after birth TP mother's weight decreased, whereas TQ mother's weight increased non significantly compared to the controls (Figure 4).

#### Effect of saffron on uterus morphology during pregnancy

TP saffron treatment seemed to cause unclear smaller embryonic lobules within the uterus of pregnant mother; this was seen on day 14 and 18 of gestation. Also embryonic placentas within the uterus seemed to have lighter colors compared to the controls. On the other hand TQ treatment seemed to produce darker placentas compared to the controls (Figure 5).

#### Effect of saffron on the vitality of embryos and neonates

The number of live 14 day embryos extracted from TP and TQ treated mothers decreased compared

to the controls, however it was only significant in TP  $p=0.016$ . The number of live 18 day embryos extracted from TP and TQ treated mothers increased compared to the controls, however it was only significant in TQ  $p=0.032$ . Mortality was seen in 18 day old TP embryos only where two embryos were dead. A non-significant decrease in the number of neonates of TP was seen on day1 neonates compared to the controls; however in the TQ group numbers of neonates were equal with the control group (Table 2).

#### Effect of saffron on congenital malformations

Congenital malformations were observed in some embryos, such as the presence of subcutaneous bleeding in several TP and TQ embryos of age 14 days of gestation. Swelling of the hindbrain, and configuration in the front of the head were seen in TP embryos compared to controls (Figure 6). There was also subcutaneous bleeding in several places in the TP and TQ embryos at age 18 days of gestation (Figure 7). At the age of day 1 neonates a little subcutaneous bleeding was seen however no congenital malformations were observed (Figure 8).

#### Effect of saffron on whole body weight

A non-significant decrease in the whole body weight was detected in TP and TQ embryos of age 14 days of gestation compared to the controls. A significant

decrease in the whole body weight was detected in TP embryos of age 18 of gestation compared to the controls  $p= 0.001$ . While a non-significant decrease in the whole body weight was seen in TQ embryos of age 18 days of gestation compared to the controls. A slight decrease in the whole body weight of day 1 neonates TP and TQ was seen compared to control. However this decrease was not significant (Figure 9).

#### Effect of saffron on whole body length

A significant decrease in the whole body length of TP and TQ embryos was seen on day 14 of gestation compared to the controls  $p=0.038$  for TP,  $p= 0.008$  for TQ. Also a significant decrease of whole body length of TP and TQ embryos on day 18 of gestation was seen compared to the controls,  $p= 0.006$  for TP and  $p= 0.004$  for TQ. A non-significant decrease in the whole body length of treated day1 neonates TP and TQ was seen compared to the controls (Figure 9).

#### Effect of saffron on half head circumference

A non-significant decrease in the half head circumference of treated embryos TP and TQ was seen on day 14 compared to the control. While a slight non-significant increase in the half head circumference of TP and TQ embryos was seen on day 18 and day 1 neonates respectively compared to the controls.

#### Effect of saffron on eye dimensions

A significant decrease occurred in the eye diameter of TP embryos on day 14  $p= 0.007$  compared to the controls and on day 1 TQ neonates compared to controls  $p= 0.040$ . This was also seen in TP embryos and TQ embryos on day 18 and day 1 neonates TP, however it was not significant. A significant increase in the eye diameter was detected in TQ embryos of day 14 compared to the controls  $p= 0.025$  (Figure 9). Eye area was also non significantly smaller in 14, 18 days TP embryos compared to controls, respectively. This was also seen day 1 neonates TP and TQ compared to control group. On the other hand there was a slight non-significant increase in the eye area TQ embryos day 18 compared to the controls.

#### Effect of saffron on tail length

A slight non-significant decrease in the tail length of TP and TQ embryos was seen on day 14 compared to the controls. A significant decrease in the tail length of treated TP and TQ embryos was seen on day18 compared the controls  $p=0.007$  for TP and  $p=0.017$  for TQ. A non significant increase in the tail length was seen in TP day1 neonates compared the controls. On the other hand a significant decrease in tail length was seen on day 1 TQ neonate compared the controls  $p= 0.009$  (Figure 9).

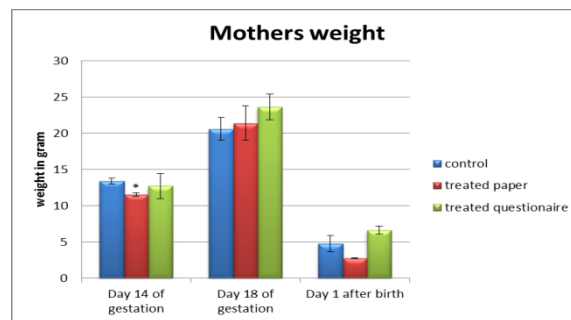


Figure 4 Graph showing the effect of saffron on mother's weight. Values are mean  $\pm$  SE taken from 5 samples for each age treatment (\*)  $P < 0.05$ .

Table 2. The number of live and dead, and cases of subcutaneous bleeding and the percentage of bleeding in embryos and neonates in the control and treated groups. Note that the highest percentage of bleeding in the treated group was on 18 days of gestation

Groups	The number of female pregnant	The number of embryos (alive)	The number of embryos (dead)	No of embryos with subcutaneous bleeding	Percentage of embryos and neonates having bleeding
C 14 days of gestation	5	63	-	2	3.17
TP 14 days of gestation	5	50	-	13	26.00
TQ 14 days of gestation	5	62	-	20	32.26
C 18 days of gestation	5	43	-	3	6.98
TP 18 days of gestation	5	51	2	29	56.86
TQ 18 days of gestation	5	57	-	26	45.61
C day 1 post natal (at birth)	5	50	-	5	10.00
TP day 1 post natal	5	47	-	20	42.55
TQ day 1 post natal	5	50	-	16	32.00

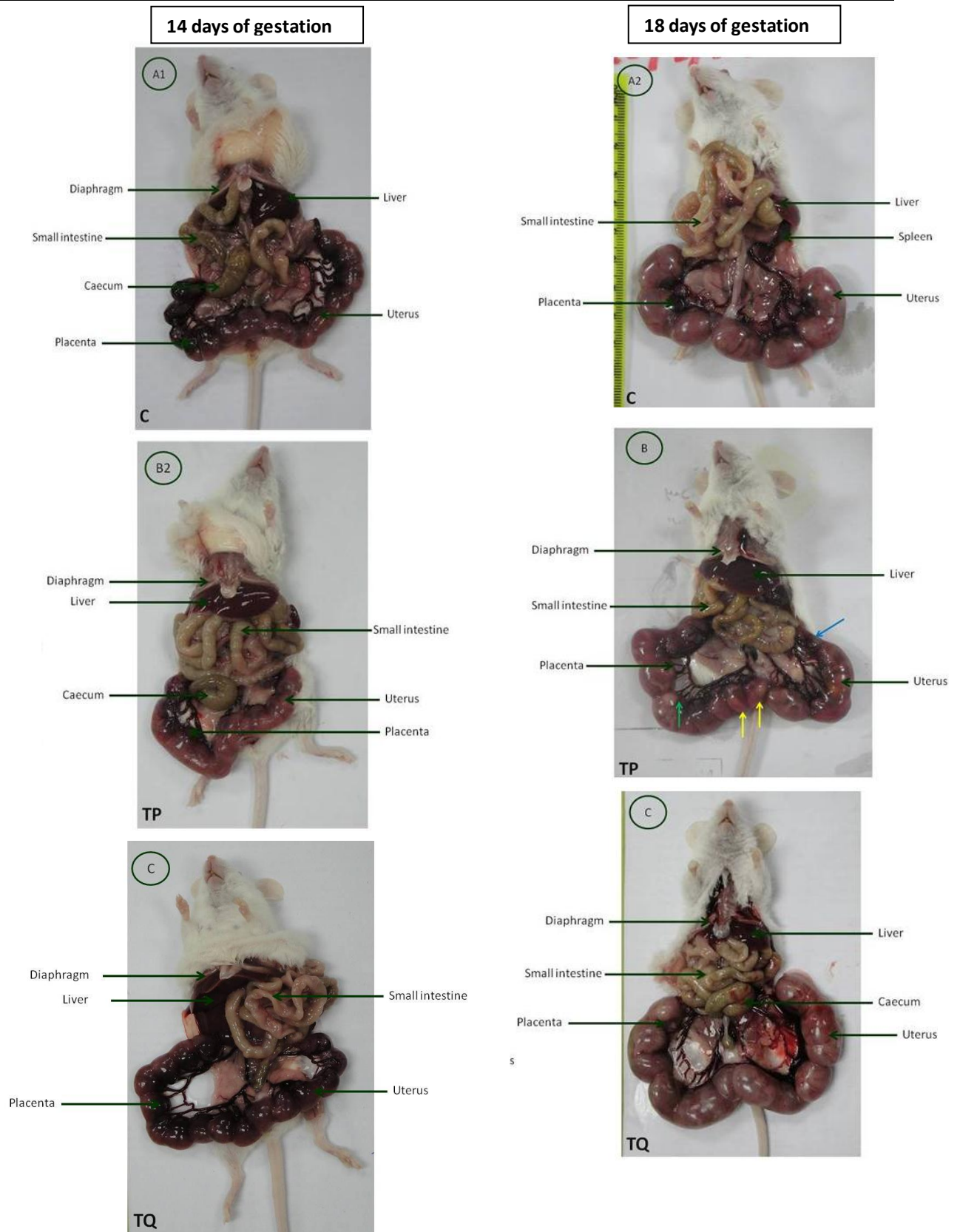
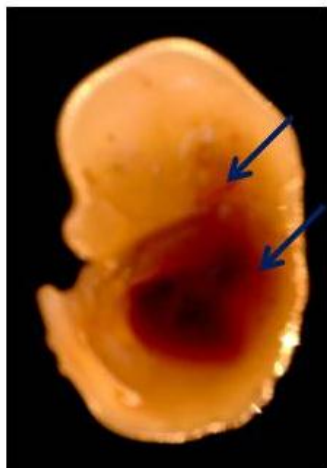


Figure 5 shows the effect of saffron on uterus in pregnant female mice at 14 (left) and 18 (right) days of gestation. At 14 days of gestation (left) Note that the placenta in TP seems to have a lighter color compared to the controls. The embryos are not very clear in the uterus. The embryonic lobules in the uterus are not very clear as in control (A). In 18 days of gestation (right) Note in (B) the decrease in embryo size compared to the controls. Note the absorbed embryo (blue arrow), very small size embryos (yellow arrows) and light colored placenta (green arrow). (C): placenta of TQ embryos of 14 and 18 days seems to be darker compared to the controls.



C. Day 14

Figure 6 showing the sites of subcutaneous bleeding in mouse 14 days of gestation TP and TQ embryos (blue arrows) compared to control (upper left). Note the anterior head swelling (red arrow) in TQ embryo , the posterior head swelling of TP embryo (red arrow) and the malformation on the front of the head in Tp mouse embryo (green arrow) (lower right).



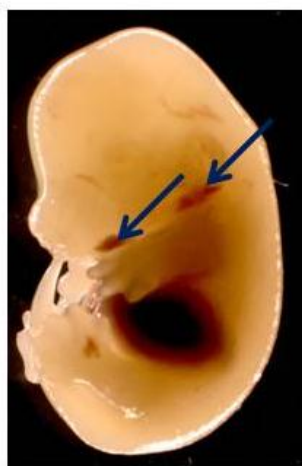
TQ. Day 14



T.Q Day 14



T.Q Day 14



TP. Day.14



TP. Day 14



TP. Day 14



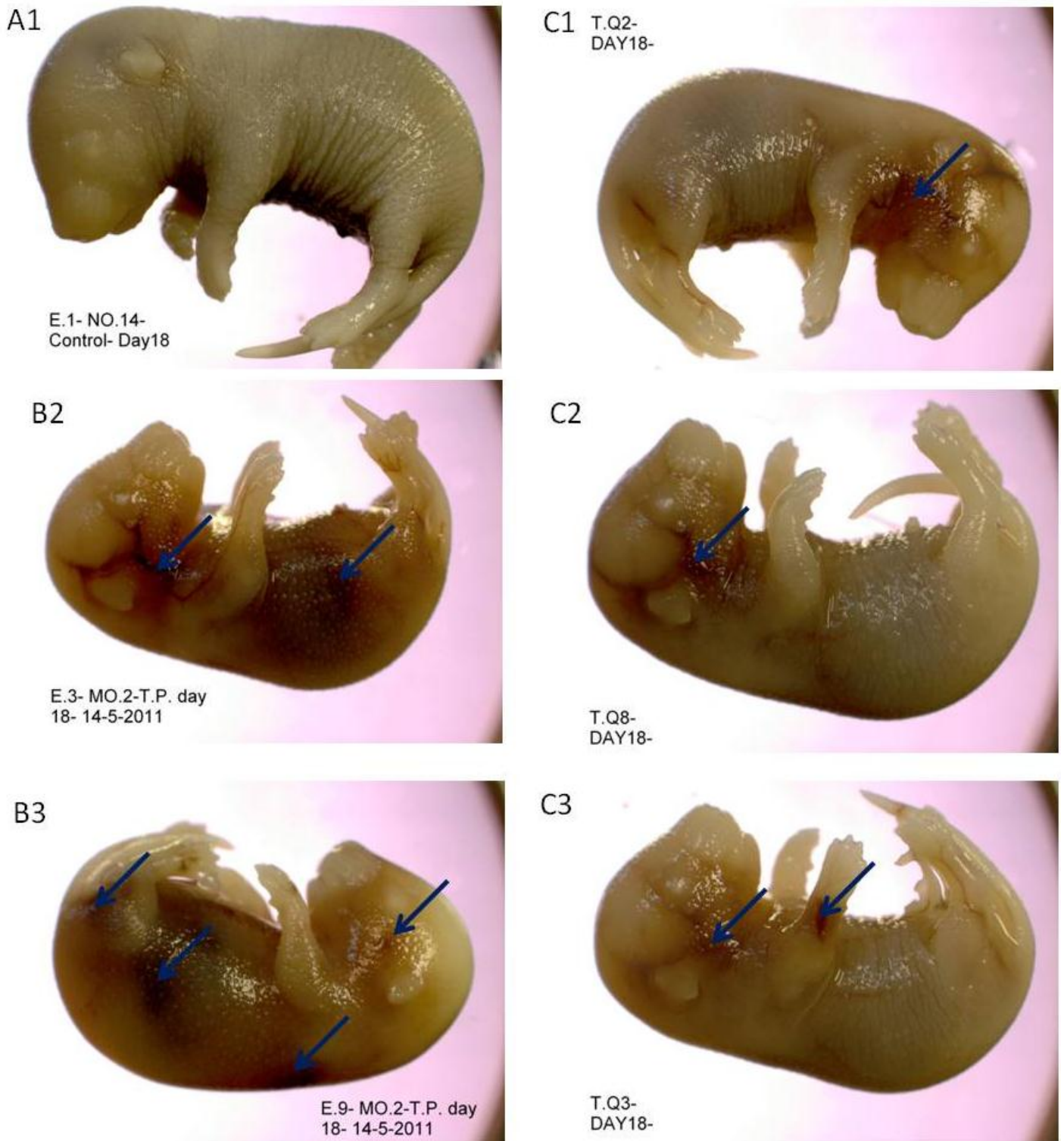


Figure 7 showing the sites of subcutaneous bleeding (blue arrow) in mouse TP and TQ embryos day 18 of gestation compared to the control. A1 = Controls, B2 & B3= TP and C1, C2 & C3= TQ.



Figure 8 Showing the sites of subcutaneous bleeding (blue arrows) in mouse TP and TQ day 1 neonates  
A1 = Controls,  
B2= TP  
C1 = TQ.

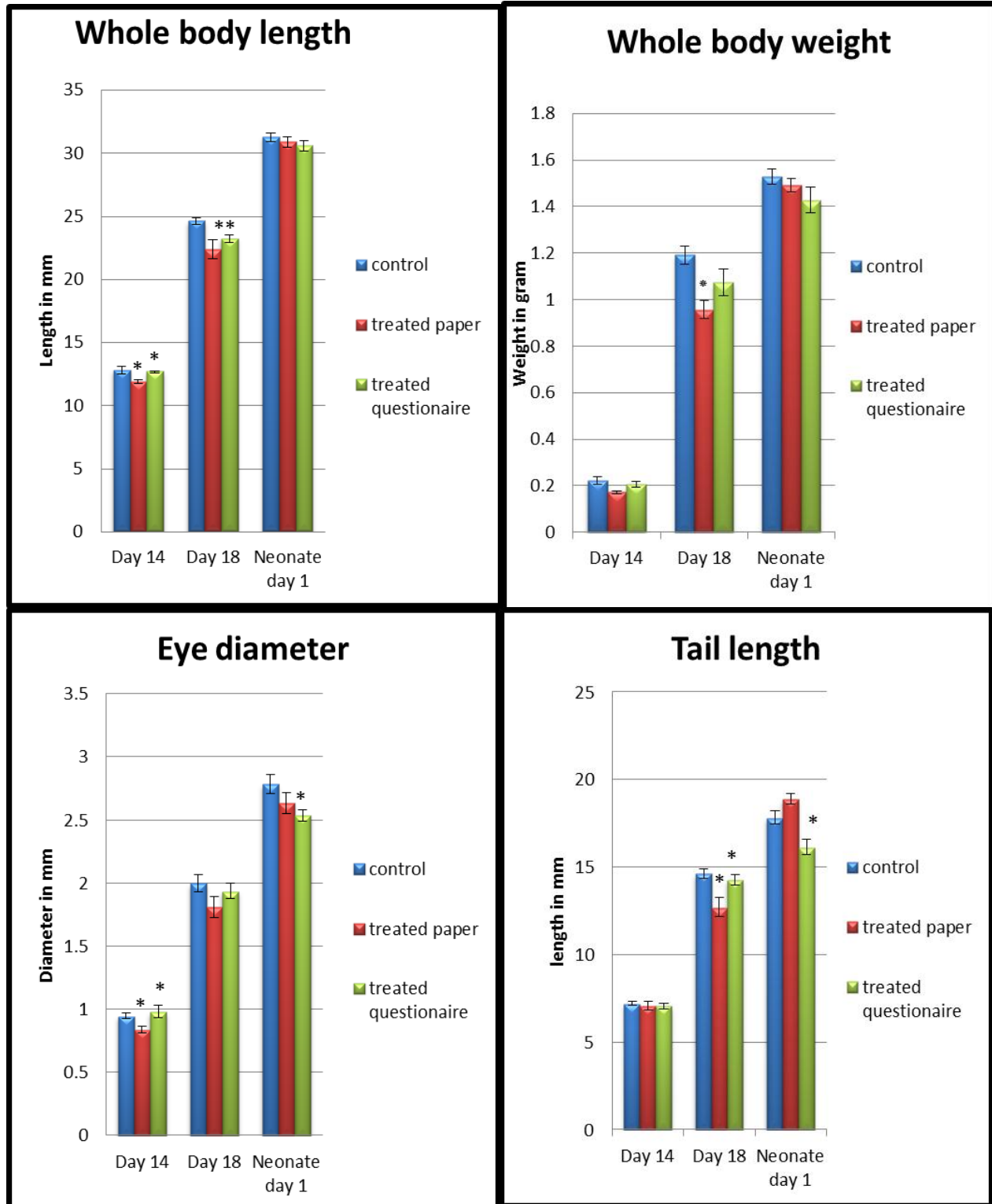


Figure 9 Graphs showing the effect of saffron on embryos and neonates whole body length, whole body weight, eye diameter and tail length. Values are mean ± SE taken from 15 samples for each age treatment (\*) P<0.05.

#### 4. Discussion

Saffron is widely used as a food additive and coloring, flavoring agent and in folk medicine as an antispasmodic, sedative, antidepressant, respiratory decongestant, eupeptic, carminative, diaphoretic, gingival sedative (Rios *et al.*, 1996; Abdullaev and Espinosa-Aguirre, 2004; Hosseinzadeh *et al.*, 2008). Pharmacological studies of saffron proved that it has antitumor properties (Abdullaev, 2002; Abdullaev and Espinosa-Aguirre, 2004), hypolipidemic effects, anticonvulsant (Hosseinzadeh and Khosravan, 2002), antidepressant (Akhondzadeh *et al.*, 2004; Hosseinzadeh *et al.*, 2004), anti-inflammatory (Hosseinzadeh and Younesi 2002) and gastric ailments (Al-Mofleh *et al.*, 2006) as well as learning and memory improving properties (Abe and Saito, 2000; Pitsikas *et al.*, 2007).

This study showed that aqueous saffron extract 100 mg / kg body weight (TP) caused a significant decrease in treated mother's weight on day 14 of gestation and a non significant decrease on day 1 after birth; however an increase in TP treated mother's weight on day 18 of gestation was seen. On the other hand the lower dose given in this study 2.5 mg / kg body weight (TQ) caused a decrease in treated mother's weight on day 14 of gestation then an increase in treated mother's weight on day 18 of gestation and on day 1 after birth. Many studies recorded the effect of saffron in reducing weight. Mohajeri *et al.* (2007) reported that ethanolic extract of (0.35, 0.70 and 1.05 g/ kg of saffron stigma produced a significant decrease in body weight after two weeks and reduced appetite in male rats. Zeinali *et al.* (2009) studied the effect of saffron on intra abdominal fat deposition in pregnant mice. They concluded that saffron might prevent deposition of fat and this may be due to its action on food intake and its antioxidant effect (Zeinali *et al.*, 2009). These symptoms were seen in other studies. Asdaq and Inamdar (2010) studied the potential effect of saffron and its active constituent, crocin, in hyperlipidemic adult rats. Their results of administration of saffron (100, 50, and 25 mg/kg body weight) for five consecutive days showed that saffron and crocin have an overall protective effect against hyperlipidemia in rats. The reason for maternal weight decrease in 14 day pregnant mothers TP and TQ might be due to the reduction of intra-abdominal fat deposition in mice bodies. Another study noted that there was no significant difference in mother's weight between treated pregnant female mice given 0.5% saffron decoction and control mice (Dashti-Rahmatabadi *et al.*, 2012). In a study carried out by both Asdaq and Inamdar (2010) it was noted that high doses of

saffron (100 mg/kg body weight) led to significant increase in body weight with reduction in daily diet intake in treated rats group (Asdaq and Inamdar, 2010). The increase in weight seen in this study on day 18 might be a result of the increase in the number of embryos observed in this study at the age of 18 days of gestation compared to the controls. In the present study uterus blood flow of the TP treated mothers seemed to be less compared to the controls as the placenta of TP mothers had a lighter color compared to the controls and embryonic lobules in the uterus of TP mothers were not very clear as in the controls. As a result embryo's size of 18 day TP treated mothers decreased compared to the controls and had a light colored placenta. In contrast mothers treated with 2.5 mg saffron / kg body weight (TQ) had an increased blood flow in placenta as seen from the color of uterus and placenta. However this did not produce bigger embryos.

This study showed that the aqueous extract of saffron caused a decrease in the number of live embryos at day 14 of gestation compared to the controls in both doses used in the study as well as on day 1 neonates. Also the number of embryos at the age of 18 days of gestation increased compared to the controls, however mortality was seen. These symptoms were seen in another study where aqueous saffron extract (0.8, 0.2, and 0.4%) was given during the first or second trimester throughout the gestational period, produced resorbed and dead embryos were seen. Mice treated with 0.8% saffron solution on first trimester had a greater percentage of resorbed fetuses while mice treated on second trimester had a greater percentage of dead embryos. This has been interpreted that embryonic implantation occurs in the first trimester of gestation; it might be that saffron affected embryonic implantation in this period and thus led to abortion (Hosseini *et al.*, 2009).

The results of this study showed that saffron aqueous solution given to pregnant mice affected embryo and neonate growth as growth parameters (whole body weight, whole body length, tail length, half head circumference and eye parameters) showed a decrease in the treated compared to the controls.

Many factors are able to contribute to intrauterine growth retardation (IUGR) such as inadequate blood flow through placenta which was demonstrated with the light color of the placenta of TP treated mothers and the fact that organogenesis might have been disturbed by the presence of saffron. Some studies have showed the same results. A study reported that organogenesis takes place in the second trimester of mouse gestation and giving mothers 0.8% saffron solution in this period may affect the development and lead to abnormalities such as a

decrease in weight and diameter of the placenta, fetal weight, tail length and biparietal diameter (Hosseini *et al.*, 2009). Other studies reported that decrease of embryos weight in treated groups with saffron may be due to genotoxic effect of saffron, which led to blocking cell growth. Saffron or crocin might cause reabsorbing of extra cellular liquid in the fetus. The extra cellular liquid forms the main part of weight and volume in the fetuses which leads to decreasing weight of embryos in treated experimental groups (García -Olmo *et al.*, 1999; Golalipour *et al.*, 2006; 2008; 2011). Eye parameters showed a decrease compared to the controls which might affect the visual system. Similar symptoms were mentioned in other studies. Dashti-Rahmatabadi *et al.* (2012) reported that saffron affected mice visual system. Contact between the embryo or fetus and the agent that may cause a teratogenic effect during the development process may cause a kind of alteration. These alterations may be morphological or functional abnormalities of the embryo or fetus, delay in intrauterine growth and development, fetal death. This all depends on the nature of the agent, the age of the fetus when the mother has taken these materials and the dose that was taken (Garcia-Pelaez *et al.*, 2010). In a study it was noted that 50mg of saffron given twice a day cause a decrease susceptibility of lipoprotein oxidation in healthy individuals and this is evidence of the possibility of saffron on the hypolipidemic by increasing antioxidant (Verma and Bordia, 1998; Asdaq and Inamdar, 2010).

In this study aqueous saffron extract caused different congenital malformation such as subcutaneous bleeding that was seen in all ages of all test groups. Swelling of the hindbrain and configuration in the front of the head was also seen. Previous studies showed a significant increase in embryo absorption and abnormality in mice saffron treated group (Tafazoliet *et al.*, 2004). Dashti-Rahmatabadi *et al.* (2012) reported that saffron might cause preterm labor and had a teratogenic effect. In a study by Martin and others it was proved that crocetin isolated from saffron is effective in treating many types of cancer; however it was considered as a teratogen (Martinet *et al.*, 2002).

Fetal development depends on the ability of the fetus to take nutrients from maternal diet and circulation (Aldoretta and Hay, 1995). The *Crocus sativus* might be transported through placenta to fetuses. Placental volume and the rate of placental growth may affect the size of fetuses. Blocker effect on cell growth also can cause weight decreased fetuses (Golalipour *et al.*, 2011). In addition many studies have shown that *Crocus sativus* extracts has antidepressant properties, where it inhibits reuptake of dopamine, norepinephrine and serotonin (Karimiet

*al.*, 2001; Akhondzadeh *et al.*, 2004 ). The use of antidepressants (Selective serotonin reuptake inhibitors) during pregnancy may be associated with an increased risk for specific types of malformations (neural tube defects, ventricular septal defects, craniosynostosis, omphalocele, or right ventricular outflow tract defects) (Alwan *et al.*, 2007; Malm *et al.*, 2011).

Regarding the cytotoxic effect of saffron on different types of cancer cell lines in different studies, a question arises, does saffron cause toxicity to embryonic cells, taking into account that embryonic cells and cancer cells are very similar in many aspects. Would saffron block the growth of embryonic cells under special doses, during special timing of embryonic development? All these remarks should be taken into consideration.

It should be noted here that the low dose used in this study (based on the daily use of saffron by females in Jeddah- Saudi Arabia) affected mouse embryos by causing growth retardation and subcutaneous bleeding; therefore women should be more careful when consuming saffron during pregnancy

#### **Conclusion:**

The results of this study showed that oral administration of high and low doses of aqueous saffron extract to mice during pregnancy caused a fluctuation in mother's weight. The light color of the uterus of TP treated mothers suggested poor blood flow to the placenta resulting in IUGR of the embryos which was indicated by significant decrease in embryonic growth parameters.

Congenital malformations were seen in treated embryos and neonates (subcutaneous bleeding and head malformation) compared to the controls. The results also showed that saffron caused a decrease in eye dimension compared to the controls.

#### **Acknowledgement**

The authors would like to thank King Abdulaziz City for science and technology for funding this research, and King Abdulaziz University for providing labs and apparatus. Special thanks to Dr. Najia Alzanbagi for providing the animal house in the girl's campus at KAU.

**Corresponding author:** Fatma Al-Qudsi, Biology Department, Science Faculty, King Abdulaziz University, Jeddah, Saudi Arabia P.O. Box 42650, Jeddah 21551, Saudi Arabia  
[falqudsi@kau.edu.sa](mailto:falqudsi@kau.edu.sa)

#### **References**

1. Abdullaev F. I. and Frenkel G. D. (1999) *Saffron in biological and medical research*, In: Negbi, M. Ed. *Saffron Crocus sativus L.*, 103–113, Amsterdam: Harwood Acad.

2. Abdullaev F. I., Rivera, L. R., Roitenburd, B. V. and Espinosa, A. J. (2000) Pattern of childhood cancer mortality in Mexico, *Archives of Medical Research*, vol. 31(5): 526–531.
3. Abdullaev, F. I. (1993) Biological effects of saffron, *Biofactors*, vol. 4(2):83–86.
4. Abdullaev, F. I. (2002) Cancer chemopreventive and tumoricidal properties of saffron (*Crocus sativus* L.), *Experimental Biology and Medicine*, vol. 227(1):20-25.
5. Abdullaev, F. I., and Espinosa-Aguirre, J.J. (2004) Biomedical properties of saffron and its potential use in cancer therapy and chemoprevention trials, *Cancer Detection Prevention*, vol. 28(6): 426–432.
6. Abe, K. and Saito, H. (2000) Effects of saffron extract and its constituent crocin on learning behaviour and long-term potentiation, *Phytother. Res.*, vol. 14:149-152.
7. Akhondzadeh, S., Fallah-Pour, H., Afkham, K., Jamshidi, A. H. and Khalighi- Cigaroudi, F. (2004) Comparison of *Crocus sativus* L. and imipramine in the treatment of mild to moderate depression: a pilot double-blind randomized trial, *BMC Complementary and Alternative Medicine*, vol. 4(12): 1–5.
8. Aldoretta, P. W., Hay, W. W. (1995) Metabolic substrates for fetal energy metabolism and growth, *Clinics of Perinatology*, vol. 22(1): 15–37.
9. Al-Mofleh, L. A., Alhaider, A. A., Mossa, J. S., Al- Sohaibani, M. O., Qureshi, S. and Rafatullah, S. (2006) Antigastric ulcer studies on 'saffron' *Crocus sativus* L. in rat, *Pakistan Journal of biological sciences*, vol. 9 (6):1009-1013.
10. Alwan, S., Reefhuis, J. , Rasmussen, S. A. , Olney, R. S. and Friedman, J. M. (2007) Use of selective serotonin-reuptake inhibitors in pregnancy and the risk of birth defects, *The New England Journal of Medicine*, vol. 356(26):2684–2692.
11. Asdaq, S.M. B. & Inamdar, M. N. (2010) Potential of *Crocus sativus* (saffron) and its Constituent, Crocin, as Hypolipidemic and Antioxidant in Rats, *Appl Biochem Biotechnol*, vol. 162:358–372.
12. Bors W, Saran, M. and Michel, C. (1982) Radical intermediates involved in the bleaching of the carotenoid crocin. Hydroxyl radicals, superoxide anions and hydrated electrons, *International Journal of Radiation Biology & Related Studies in Physics Chemistry & Medicine*, vol. 41(5): 493-501.
13. Caligioni, C., (2009) Assessing reproductive status/stages in mice, *Current Protocols in Neuroscienc* A. 4I, 1–8.
14. Chermahini, S. H., Abd. Majid, F. A., Sarmidi, M. R., Taghizadeh, E. and Salehnezhad, E. (2010) Impact of saffron as an anti-cancer and anti-tumor herb, *African Journal of Pharmacy and Pharmacology*, vol. 4(11), pp. 834-840.
15. Crawford, L. W., Foley, J. F. and Elmore, S. A. (2010) Histology atlas of the developing mouse hepatobiliary system with emphasis on embryonic days 9.5-18.5, *Toxicologic Pathology*, vol. 38: 872-906.
16. Dashti-Rahmatabadi, M. H., Nahangi, H., Oveisi, M. and Anvari, M. (2012) The effect of saffron decoction consumption on pregnant mice and their offspring, *Journal of Shahid Sadoughi University of Medical Sciences*, vol. 19(6): 831-37.
17. elgado-Vargas, F. and Paredes-López, O. (2003) *Natural colorants for food and nutraceutical uses*, 143-148, Washington: CRC Press.
18. Edge, R., McGarvey, D. J. and Truscott, T. G. (1997) The carotenoids as anti-oxidants—a review, *Journal of Photochemistry and Photobiology B: Biology*, vol. 41(3): 189-200.
19. García-Olmo, D. C., Riese, H. H., Escribano, J., Ontañón, J., Fernandez, J. A., Atiénzar, M. and García-Olmo, D. (1999) Effects of long-term treatment of colon adenocarcinoma with crocin, a carotenoid from saffron (*Crocus sativus*): an experimental study in the rat, *Nutrition Cancer*, 35(2):120-6.
20. Garcia-Pelaez, I., Aguirre-Luna, O. M., Saavedra-Ontiveros, D. and Arteaga-Martinez, M. (2010) Teratogenic effect of ethylene glycol-methyl cellosolve mixture in rats. II. Craniofacial and limb abnormalities, *International Journal of Morphology*, 28(4):1173-1180
21. Gotalipoor, M. J., Khori, V. and Afshar, M. (2006) Teratogenic effect of saffron on mice, *Reproductive Toxicology*, 22: 272.
22. Gotalipoor, M. J., Ghafari, S., Maleki, A., Kiani, M., Asadi, E. and Farsi, M. (2011) Study of embryotoxicity of *Mentha piperita* L. during organogenesis in Balb/c mice, *International Journal of Morphology*, 29(3):862-867.
23. Gotalipoor, M. J., Gharravi, A. M., Ghafari, S., Afshar, M. and Khori, V. (2008) Effects of *crocus sativus* on the fetal development of NMRI mice, *Saudi Medical Journal*, 29(2): 309- 10.
24. Hosseini, S. M., Dashti, M. H., Anvari, M., Zeinali, F. and Miresmaeili S. M. (2009)

- Studying teratogenic and abortifacient effects of different doses of saffron (*Crocus Sativus*) decoction in 1st or 2nd trimesters in mice, *Iranian Journal of Reproductive Medicine*, vol. 7: 35.
25. Hosseinzadeh, H. and Khosravan, V. (2002) Anticonvulsant effects aqueous and ethanolic extracts of *Crocus sativus* L. stigmas in mice, *Arch. Irr. Med.*, vol. 5: 44- 47.
  26. Hosseinzadeh, H., Abootorabi, A. and Sadeghnia, H. R. (2008) Protective Effect of *Crocus sativus* Stigma Extract and Crocin (trans-crocin 4) on Methyl Methanesulfonate Induced DNA Damage in Mice Organs, *DNA AND CELL BIOLOGY*, vol. 27(00): 1–8.
  27. Hosseinzadeh, H., and Younesi, H. (2002) Antinociceptive and anti- inflammatory effects of *Crocus sativus* L. stigma and petal extracts in mice, *BioMed Central Pharmacology*, vol. 2: 7.
  28. Hosseinzadeh, H., Karimi, G. and Niapoor, M. (2004) Antidepressant effects of *Crocus sativus* stigma extracts and its constituents, crocin and safranal, in mice, *Acta Horticulturae (ISHS)*, vol. 650: 435–445.
  29. Jan, M. M., Basamh, M. S., Bahassan, O. M. and Jamal-Allail, A. A. (2009) The use of complementary and alternative therapies in Western Saudi Arabia, *Saudi Medical Journal*, vol. 30(5): 682-686.
  30. Karimi, G., Hosseinzadeh, H. and Khaleghpanah, P. (2001) Study of antidepressant effect of aqueous and ethanolic of *Crocus sativus* in mice, *Iranian Journal of Basic Medical Sciences*, vol. 4:11-15.
  31. Kaufman, M. H., and Bard, J. B. L. B. (1999) *The Anatomical Basis of mouse development*, San Diego: Academic Press.
  32. Lage, M. and Cantrell, C. L. (2009) Quantification of saffron (*Crocus sativus* L.) metabolites crocins, picrocrocin and safranal for quality determination of the spice grown under different environmental Moroccan conditions, *Scientia Horticulturae*, vol. 121: 366–373
  33. Malm, H., Artama, M., Gissler, M. and Ritvanen, A. (2011) Selective serotonin reuptake inhibitors and risk for major congenital anomalies, *Obstetrics and Gynecology*, vol. 118(1):111–120.
  34. Martin, G., E. Goh and A.W. Neff, (2002) Evaluation of the developmental toxicity of crocetin on *Xenopus*, *Food and Chemical Toxicology*, vol. 40(7): 959-964.
  35. Mohajeri, D. , Mousavi, G., Mesgari, M., Doustar, Y. and Nouri, M. H. K. (2007) Subacute toxicity of *Crocus sativus* L. (saffron) stigma ethanolic extract in rat, *American Journal Of Pharmacology and Toxicology*, vol. 2 (4):189-193.
  36. Nagy, A., Gertsenstein, M., Vintersten, K. and Behringer, R. (2003) *Manipulating the mouse embryo, A laboratory manual*, third edition, New York: Cold Spring Harbor Laboratory Press.
  37. Pitsikas, N., Zisopoulou, S., Tarantilis, P. A., Kanakis, C. D., Polissiou, M. G. and Sakellaridis, N. (2007) Effects of the active constituents of *Crocus sativus* L., crocins on recognition and spatial rats' memory, *Behavioural Brain Research*, vol.183(2): 141–146.
  38. Premkumar, K. , Abraham, S. K. , Santhiya, S. T. and Ramesh, A. (2003) Inhibitory effects of aqueous crude extract of saffron (*Crocus sativus* L.) on chemical-induced genotoxicity in mice, *Asia Pacific Journal of Clinical Nutrition*, 12 (4):474-476.
  39. Rios J., Recio M., Giner R. and Mañez S. (1996) An update review of saffron and its active compounds, *Phytother Res.*, vol. 10(3):189–193.
  40. Tafazoli, M. , Kermani, T. and Sadat Jou, S. A. R. (2004) Effect of saffron on abnormal and its side effect on mice balb/c, *Ofogh-E-Danesh Fall*, vol. 10(3):53-56.
  41. Tavana, S., Eimani, H., Azarnia, M., Shahverdi, A. and Eftekhari Yazdi, P. (2012) Effects of Saffron (*Crocus sativus* L.) aqueous extract on *in vitro* maturation, fertilization and embryo development of mouse oocytes, *Cell Journal*, vol. 13(4): 259-264.
  42. Theiler, K. (1989) *Thehouse mouse atlas of embryonic development*, New York: Springer-verlag.
  43. Verma, S. K. and Bordia, A. (1998) Antioxidant property of saffron in man, *Indian Journal of Medical Sciences*, vol. 52(5):205-207.
  44. Walmer, D. K., Wrona, M. A., Hughes, C. L., Nelson, K. G. (1992) Lactoferrin expression in the mouse reproductive tract during the natural estrous cycle: correlation with circulating estradiol and progesterone, *Endocrinology*, 131:1458–1466.
  45. Zeinali, F., Anvari, M., Dashti, R. M. H. and Hosseini, S. M. (2009) Saffron (*Crocus sativus*) decoction induces intra abdominal fat deposition in pregnant mice, *Planta Medica*, vol. 75: 125.

12/12/2012