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Study of the effect of grape seed extract on the harmful effects of Monosodium Glutamate on chicken embryo Development.

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ABSTRACT

Monosodium Glutamate (MSG) is used in the food industry as a flavor enhancer added to thousands of food.Several studies have proven the damage caused by MSG on laboratory animals. Therefore, this study aims to study the effectiveness of grape seed extract(GSE) in mitigating MSG damage on chicken embryos, as part of the solutions provided in the event of the long term use of MSG and its accumulation in the body Human.

In this study, we use 300 eggs were divided into six groups which are the control, MSG Group, GSE group, MSG+GSE , GSE than MSG and MSG than GSE. We are studying the effect of experimental materials in the fetal development during the following ages (7, 10, 12, 14, 16) day.

The results showed that the treatment of embryos with MSG dose (0.1 ml) caused of the affect growth retardation, congenital malformations and Significant decrease in the weight and height of the fetuses and after the treatment of the fetus GSE was noted to reduce the damage. Grape seed extract has a curative effect in reducing the damages of mono-glutamate sodium on the development of chicken embryos.

INTRODUCTION

There is no doubt that the modern food industries facilitated the diversity of food and availability and conservation of damage in different seasons of the year, we find that winter crops are available in the summer and spring crops are in winter, in addition the export of food in international trade requires addition of some external materials to be homogenized with the food content, whether to preserve or improve its taste. These materials are known as food additives, and with the continued technological progress in the field of food manufacturing, the number of approved additives has increased because of the multiple properties, so chemical additives for food has become used in a very large scale.

Since 1987, a certain percentage has been determined for each category of additives on a global scale, allowing consumption per person per day, but this is not enough for several reasons:

First , the dose and its relationship to sensitive groups in society, such as children, pregnant women and the elderly, remain a neglected point when discussing the effects of chemical additives.

Second, some food labels do not specify the percentage of chemical additives in the food category and the maximum allowable intake of these chemical additives.

Third, the amount of the dose consumed is one of the most important factors to reduce the risk of use.

The consumer consumes many processed food products in his daily life, And not the problem in

the use of these substances or others, but the problem in the focus used and repeated use several times a day, which may make the dose beyond the safe limit of health enters the body a large amount of these substances which leads to accumulation in the body beyond the safe limit , in addition, the harmful effect of some additives may appear only in the long run.

One of the most common chemical substance used as food additives is called Monosodium Glutamate (MSG), which caused widespread debate among consumers, researchers and scientists about how safe it is for human health, So it was necessary to search for practical solutions that accompany the use of food additives and limit their effects, and when we look at the research available to us, we found that it is limited to the prevention of the impact of MSG on the tissues of rats and adult mice without addressing the protection of tissues of the fetus during the period of composition despite Of the MSG has the ability to cross the placenta and reach the fetus. (Park , 2016) ; (Afeefy *et al* , 2012);(John *et al* , 2015) .Hence the idea of this research is to find alternative solutions have health benefits in the protection of fetal organs and at the same time be accessible to all categories of consumers, through the development of therapeutic mechanisms to mitigate the effects of harmful MSG on the organs of the fetus.

After searching and looking at many researches, we found that the grape fruit is one of the most consumed fruits in the world and has different biological functions due to the components of polyphenols, which are mostly in the seeds of grapes by 60-70% and in the crust by 30%, Grape seed contains 5% to 8% of its weight (Hassan, 2012).

In addition, grapes were mentioned about 11 times in the Holy Book. For these reasons, grape seeds were selected as a study site.

MATERIAL AND METHODS

Materials :

In this study was using Fertilized chicken eggs (n=300) were obtained from Fakieh Poultry Farms , Monosodium Glutamate (MSG) was purchased as powder from Al-Mizani medical corporation – Saudi Arabia, with a 0.1-millimeter dose into air chamber of the egg (Al-Qudsi and Al-Jahdali , 2012) and Grape Seed Extract, obtained from GNC - Makkah Al Mukarramah - Saudi Arabia and used 0.3 ml according (El-Awdan *et al,* 2013).

Experimental design:

Fertilized chicken eggs were divided into 6 groups:

1- Control Group:It is used to study the normal structure and growth of the retina in fertilized chicken embryos, This group consisted of (50) of fertilized eggs and was injected with a dose (0.1 ml) of distilled water into air chamber of the egg during the first week of incubation and that in the following days (0, 1).

2- The group treated with GSE : It consists of 50 fertilized eggs, this group is injected with GSE at a dose (0.3 ml) in the following days (0, 1)

3- Group treated with MSG: it is a number (50) of fertilized eggs are used, and they are injected with MSG at a dose (0.1 ml) in the following days (0, 1(.

3- The group treated with MSG and GSE together. (Mix):In it, 50 fertilized eggs are used, and they are injected by MSG and GSE together in following days (0, 1)

5- The group treated with GSE then MSG (G-M) :This is to study the protective effect of grape seeds, in which a number (50) of fertilized eggs are used and injected with GSE in the following days (0, 1) and then they are injected with MSG in the following days (3, 4).

6 - The group treated with MSG then GSE(M-G) :that is to study the therapeutic effect of grape seeds, in which a number (50) of fertilized eggs are used and injected with MSG in the day (0, 1) and then they are injected with GSE in the following days (3, 4) (called the treatment group).

And I've been injecting embryos in age (0) day nursery before and to study the effect of GSE and MSG on organs configuration process and been administered by (1) a day and that it is the beginning of the embryonic brain as members constitute the head and eye, and they've been injecting fetus on (3, 4) to study the effect of injection of MSG, GSE during complete fetal configuration for many members of the organs.

After bringing the fertilized eggs from Fakih Poultry Farms to the factory, the following was done:

Set the air chamber in the wide part of the egg and divide the eggs into work groups and write data to the groups.The wide segment was sterilized and two holes were made in the wide part of the egg shell using a sterile and sterile pin (Allam *et al*., 1976) , inject the groups with extract or distilled water and then close the holes using small pieces of paraffin wax, incubate the eggs in incubation at a temperature of 37.5 m with a special placement of eggs so that the wide part of the egg containing the air space to the top and the part of the point down, and follow the growth process through the optical examination and extracted embryos from the egg at the age of 14 days And fixation of samples in formalin solution.(figure 1)

RESULTS AND DISCUSSION

3-1: Congenital malformations:

The fetal examination reveals that there are clear congenital abnormalities in a number of embryos in the MSG treated group, which is a lack of eye, complete congenital malformation of the fetus, a hernia in the viscera, deformation of the bones of the neck and limbs, small size of the fetus and delayed development. (Figure 2).

(Al-Qudsi and Al-Jahdali , 2012) reported that the treatment of chicken embryos with MSG caused many congenital malformations such as delayed development and bleeding under the skin at the age of 7, 10 and 14 years of incubation and abdominal hernias On day 7 and 10, most congenital malformations of the 10th age of incubation, such as brain deformity, were seen as only one sample and deformation of the fetus's beak.

In a study conducted by (Mahaliyana *et al* ,2016) to investigate the effect of (MSG) on embryonic development in zebrafish, the acute toxicity of MSG was performed for 4 days and zebrafish eggs were injected with ten different concentrations of MSG. The results of the study indicated that at low concentrations Of MSG such as 10,30, 50 mg no mutations in embryonic development were observed, and with increased MSG various abnormalities occurred in zebrafish embryo and at high concentrations such as 100, 150, 200, 250, 300, 400, 500 mg, and These abnormalities were delayed growth, shrinkage of the placenta, edema of the sacral sacrum, absence of pigmentation in the fetus's body, The tail.

The cause of malformations in MSG groups is due to the toxic effect of MSG on the fetus's body.(Elefteriou *et al*,2003) reported that MSG treatment disrupts the endocrine function responsible for regulating many biologic processes including metabolism, This disruption may play a role in causing deformities, including skeletal deformities, as supported by (Abdelkader *et al*, 2012) where excessive use of MSG can cause metabolic disorders and biological deformities in embryonic development. The ability to cause damage to the endocrine function may be a cause This occurrence of teratogenicity results and disrupt vital processes in the body.

The adverse effects of toxicity caused by high levels of glutamate have shown a blockage of the central artery, and the disappearance of one or both eyes may be due to the central artery occlusion of the eye and therefore not being . (Al-Qudsi and Al-Jahdali, 2012)

And may be due to MSG's ability to induce oxidative stress (Park *et al*, 2014).

(Tan *et al* ,2015) reported that embryos have a high level of O2 consumption, making them susceptible to oxidative damage.

(Guerin *et al*, 2001) added that oxidation is a cause of congenital malformations and that the free radicals created by this oxidation may originate from fetal metabolism or surrounding areas.

Correspondingly, there were no abnormalities in the treatment group(MSG-GSE) This is consistent with (El-Ashmawy & Bayad 2016) those who noticed that, the extract of grape seeds and folic acid is a protective factor against fetal abnormalities resulting from the use of azathioprine.

The researchers added (Siman & Eriksson, 1997a); (Siman & Eriksson, 1997b) that congenital malformations caused by experimental diabetes can be prevented by antioxidants such as vitamin C and E.

3-2Body growth parameters:

The weight : There was a significant decrease in the weight of the fetus in the group treated with MSG where the mean difference of body weight was greater and showed a significant difference from the mean body weight in the control sample where P = .002 and the average difference between them is 4.230, while no significant differences In the weight of other groups. (Table 1), Figure 3

The Length : There was a significant decrease in the weight of the fetus in the group treated with MSGG- M , (Table 2), Figure 4

In general, it is noticed through the previous results that weight and height were not affected by MSG significantly during the young ages (7, 10) days, and with the advancement of the fetus's age, specifically on days (12, 14, 16) the significant effects were limited to certain groups that are shown by table (1, 2) Figure (3,4).

As we notice a significant decrease in the weight and length of the embryos treated with MSG, and it is coincide with (Miśkowiak et al , 1992) that the administration of MSG (4 mg / g body weight) under the skin in newborn rats on day 2, 4, 6, 8, 10 Led to a decrease in the weight of treated rats. (Al-Qudsi and Al-Jahdali, 2012) reported that MSG-treated embryos showed less weight than the control group.

(Alalwani ,2014) reported that treating rats with MSG daily oral doses of 30 and 60 g / kg bw for two months caused a significant increase in the weight of the mouse body.

This finding explains that, deficiency of fetal weight is that the embryos treated with MSG become small in size, this is indicated by (Cohen ,1967), where he was reported that the treatment of embryos with MSG leads to small embryos, as pointed out also (Al-Qudsi and Al-Jahdali , 2012) explained the embryos treated with MSG to be unable to obtain adequate food from the Which led to the inability of cells to grow and divide efficiently and speed required, and returned to watch a lot of blood when opening eggs injected with MSG and this is what was observed in the current study, and added that this blood may be caused by bleeding in the vascular led To the absence of delivery of cells to the fetus, In addition, (Hermanussen et al. 2006) and (Mahaliyana et al , 2016) reported that increased glutamate had a toxic effect on the hypothalamus, resulting in decreased growth hormone in the body, thus affecting fetal growth and reproduction of its cells.

As for the therapeutic effect GSE and reduction of MSG damage, agree with (Sun et al., 2016) who reported that, rats with diabetes developed with STZ resulted in a decrease in body weight and after treatment with GSPE was able to increase body weight and Restore it.

(Akaberi & Hosseinzadeh, 2016) also noted that the biologically active ingredients in grapes inhibit a wide range of chronic disorders associated with metabolic syndrome, (Selima et al ,2017) added that GSE prevents oxidative stress.

(Fahd *et al* ,2013) reported that the improvement in the body weights of the chick was due to the activation of the mitosis of the cells of the body and the synthesis of the protein protein in the protein biosynthesis by the active compounds in the extract of Propolis, which is considered an antioxidant.

Several studies have also indicated that melatonin, an antioxidant, plays an important role in regulating the growth hormone secretion in the body through its effect on the area of the thalamus in the brain and inhibition of the hormone peptide Somatostatin (Valcavi et al, 1993) , (Kasuya et al, 2006).

Conclusion:

The treatment for MSG caused many damage in fetus body's and treatment with GSE was reduced this damage, We recommend to do a future research to study the effect of GSE at different doses and different period.

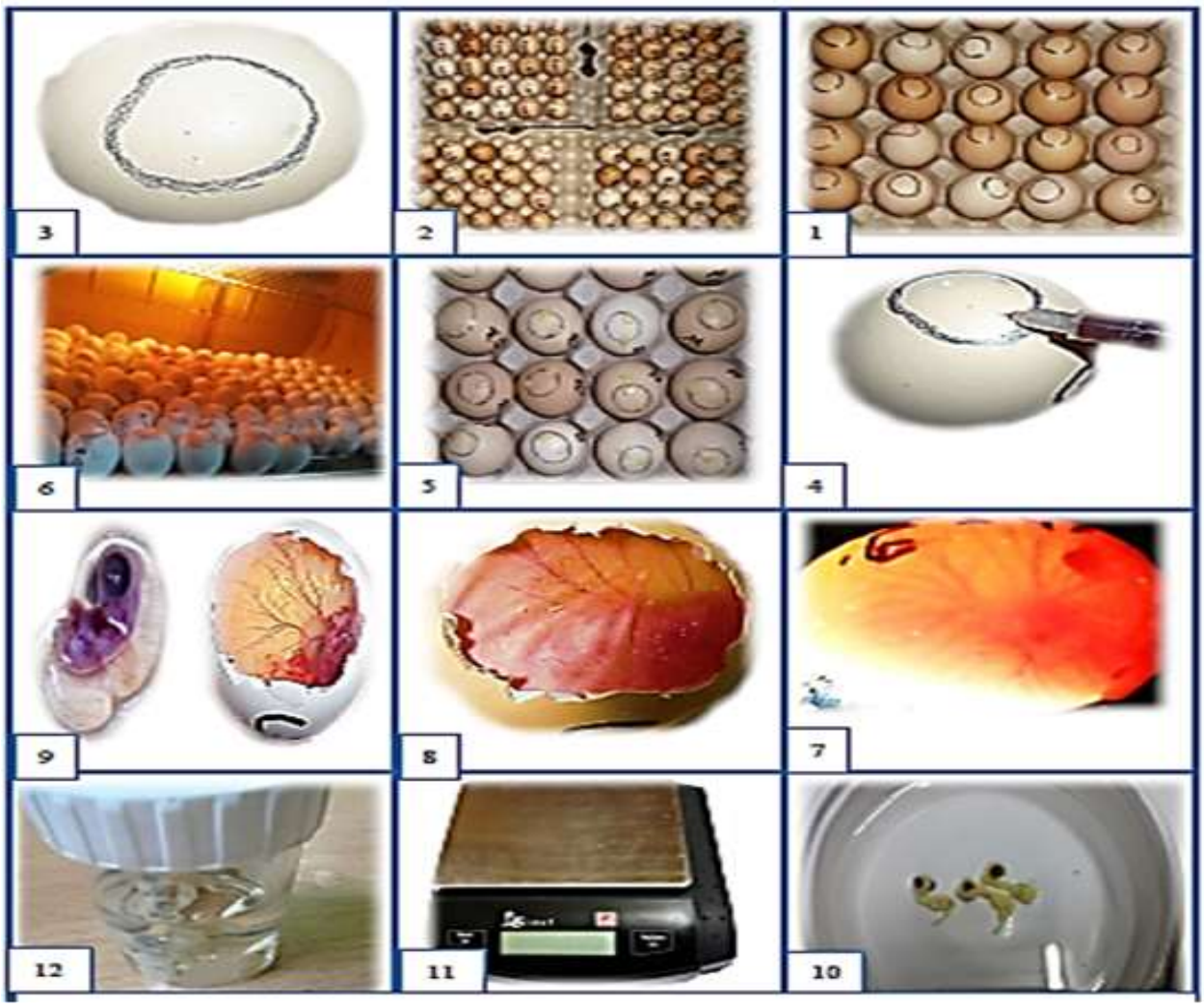

Figure 1 : Showing the steps of the research experimen

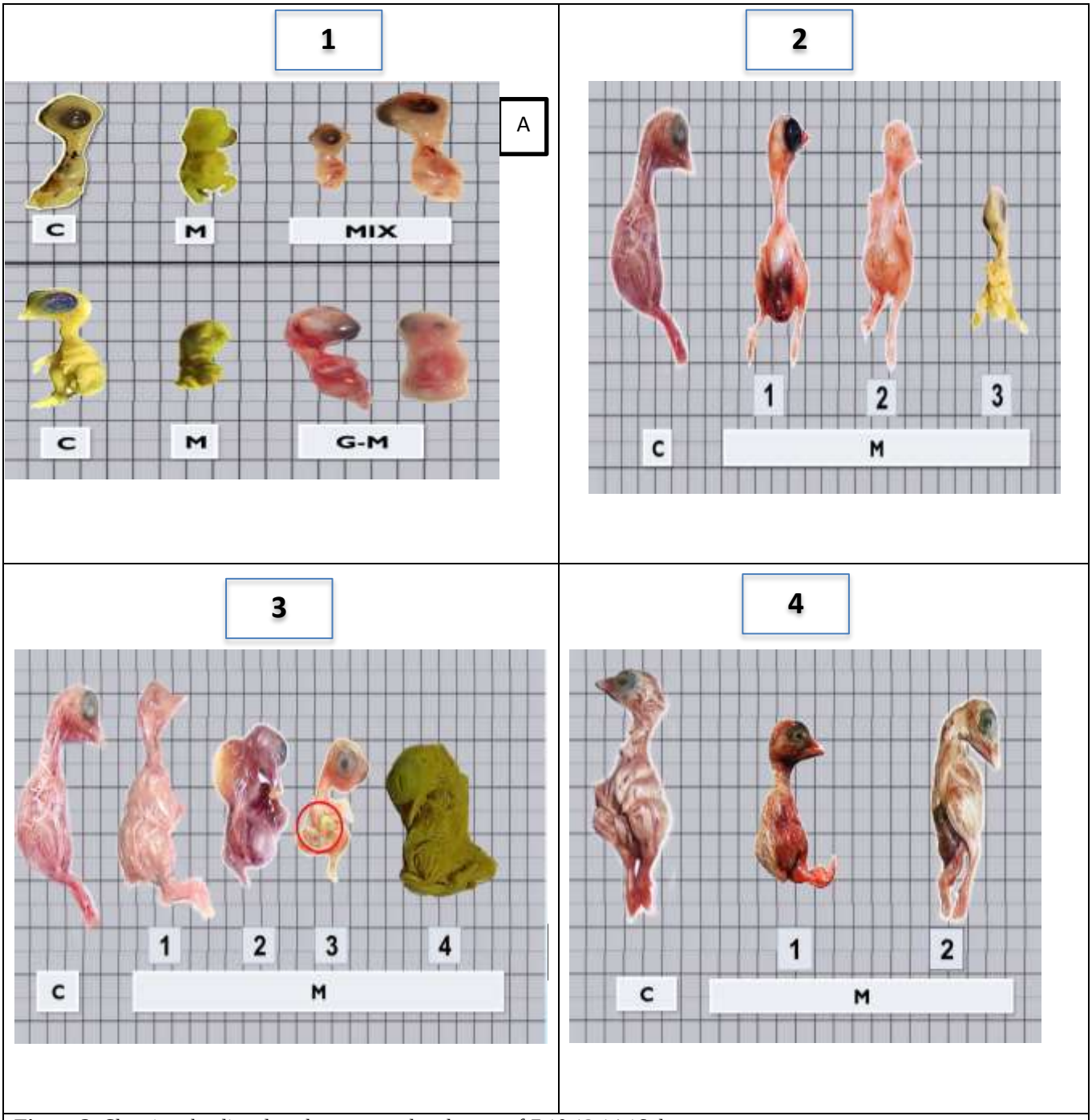

Figure 2: Showing the disorders that appeared at the age of 7,10,12,14,16 days
1-A : Abnormalities that appeared at 7 days of age is the right eye is not formed
Incision in the eye + small size of the fetus.
1-B : Abnormalities that appeared at 10 days of age is Complete deformation of the fetus.
Swelling in the head area , small eyes of the fetus and its size.
2-Abnormalities that appeared at 12 days old : Lower area enlargement , disappearance of the eye and delayed growth of the fetus in MSG group.
3-Malformations that appeared at 14 days of age: The disappearance of the eyes , a complete deformation of the fetus , a hernia in the abdomen and a deformity of the limbs and neck in MSG group.
4-Abnormalities that appeared at 16 days old ; small size of the fetus , the presence of blood congestion in the body of the fetus, Abdominal hernia and neck twisting.

DAY	(I) groups	(J) groups	Mean Differene (I-J)	Std. Error	Sig.[a]	95% Confidence Interval for Difference[a]	
						Lower Bound	Upper Bound
7 day	ctrl	GSE	-.030-	1.338	.982	-2.665-	2.605
		MSG	-.020-	1.338	.970	-2.585-	2.685
		MIX	-.030-	1.338	.982	-2.665-	2.605
		G - M	.210	1.338	.875	-2.425-	2.845
		M - G	-.020-	1.338	.988	-2.655-	2.615
10 day	ctrl	GSE	.400	1.338	.765	-2.235-	3.035
		MSG	.410	1.338	.760	-2.225-	3.045
		MIX	.670	1.338	.617	-1.965-	3.305
		G - M	1.190	1.338	.375	-1.445-	3.825
		M - G	.880	1.338	.511	-1.755-	3.515
12 day	ctrl	GSE	.010	1.338	.994	-2.625-	2.645
		MSG	.410	1.338	.760	-2.225-	3.045
		MIX	.300	1.338	.823	-2.335-	2.935
		G - M	3.360*	1.338	.013	.725	5.995
		M - G	-.420-	1.338	.754	-3.055-	2.215
14 day	ctrl	GSE	2.040	1.338	.129	-.595-	4.675
		MSG	4.230*	1.338	.002	1.595	6.865
		MIX	1.050	1.338	.433	-1.585-	3.685
		G - M	4.110*	1.338	.002	1.475	6.745
		M - G	.880	1.338	.511	-1.755-	3.515
16 day	ctrl	GSE	-2.230-	1.338	.097	-4.865-	.405
		MSG	2.800*	1.338	.037	.165	5.435
		MIX	2.470	1.338	.066	-.165-	5.105
		G - M	6.020*	1.338	.000	3.385	8.655
		M - G	.210	1.338	.875	-2.425-	2.845

Table(1) ; showing the statistical analysis of the average difference between groups in weight of the fetuses in grams .

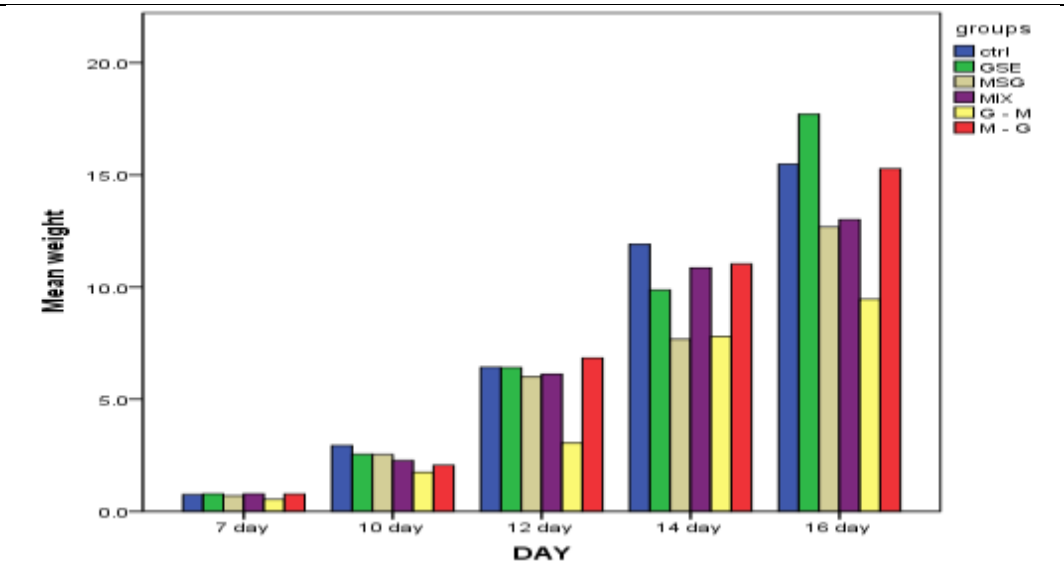

Figure (3) graphs showing the difference in the total weight of the fetuses between experimental groups.

DAY	(I) groups	(J) groups	Mean Difference (I-J)	Std. Error	Sig.[a]	95% Confidence Interval for Difference[a] Lower Bound	Upper Bound
7 day	ctrl	GSE	-.270-	.335	.421	-.930-	.390
		MSG	.120	.335	.721	-.540-	.780
		MIX	-.190-	.335	.571	-.850-	.470
		G - M	-.220-	.335	.512	-.880-	.440
		M - G	-.150-	.335	.655	-.810-	.510
10 day	ctrl	GSE	-.014-	.335	.967	-.674-	.646
		MSG	.396	.335	.238	-.264-	1.056
		MIX	-.100-	.335	.766	-.760-	.560
		G - M	.208	.335	.535	-.452-	.868
		M - G	-.286-	.335	.394	-.946-	.374
12 day	ctrl	GSE	.342	.335	.308	-.318-	1.002
		MSG	.262	.335	.435	-.398-	.922
		MIX	.550	.335	.102	-.110-	1.210
		G - M	1.418*	.335	.000	.758	2.078
		M - G	-.120-	.335	.721	-.780-	.540
14 day	ctrl	GSE	-.402-	.335	.231	-1.062-	.258
		MSG	.120	.335	.721	-.540-	.780
		MIX	.138	.335	.681	-.522-	.798
		G - M	2.486*	.335	.000	1.826	3.146
		M - G	-.282-	.335	.401	-.942-	.378
16 day	ctrl	GSE	.020	.335	.952	-.640-	.680
		MSG	.820*	.335	.015	.160	1.480
		MIX	.630	.335	.061	-.030-	1.290
		G - M	.800*	.335	.018	.140	1.460
		M - G	.370	.335	.271	-.290-	1.030

Table(2) showing the statistical analysis of the average difference between groups in the length of embryos in centimeters

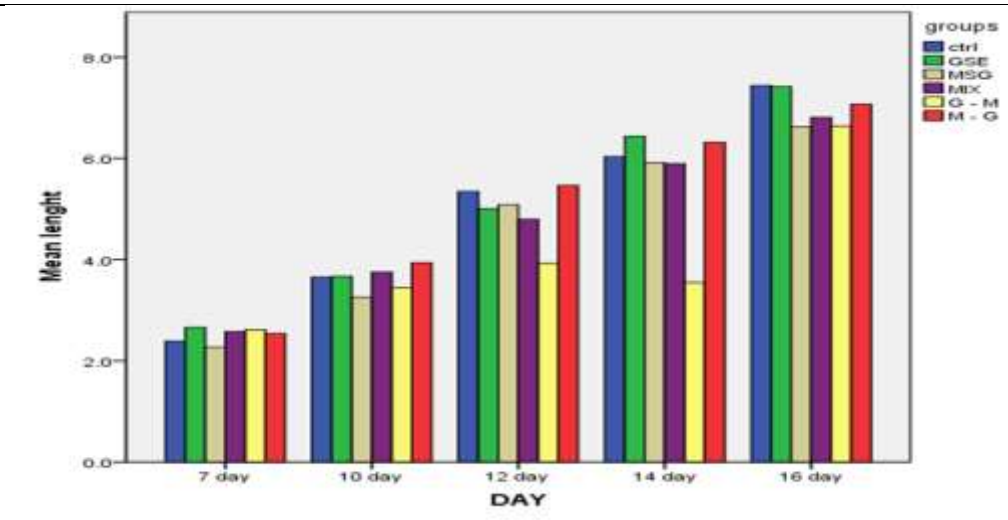

Figure (4) graphs showing the difference in the total length of embryos in centimeters betw experimental groups

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