The possible ameliorative effect of Grape Seed Extract against of Monosodium Glutamate (MSG) on retina of chick embryo during the incubation period (to Manifest Scientific Miracles in Quran)

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Abstract: Monosodium Glutamate (MSG) is used in the food industry as a flavor enhancer added to thousands of food, This study aims investigate the Effect of Monosodium Glutamate (MSG) on the retina of chick embryo and The possible ameliorative effect of Grape Seed Extract during the incubation period (to Manifest Scientific Miracles in Quran (. In this study, we use 300 eggs were divided into six groups based on injection period and the injected substances, which are the first group control and the second group treated with GSE, the third group treated MSG, the fourth group common, the fifth group protective and the sixth group is therapeutic, We are studying the effect of experimental materials in the fetal development during the following ages (14, 16) day. The results showed that the treatment of embryos with MSG (0.1 ml) caused abnormalities at the level of the gross morphology and tissue abnormalities at the level of the retina. After the treatment of the fetus with the grape seed extract, the ability of the grape seed extract to reduce the effects of MSG. This improvement in the treatment group has been shown more and better than the preventive and joint group. The current study concludes that treating the fetus with grape seed extract and giving a longer period of time to allow the fetal body interact with the active grape seed compounds leads to the risk of MSG damage. We recommend future studies to study the effect of GSE at different doses and different stages of development.


Key words: chick embryo, monosodium glutamate, Retina, Grape seed extract, Scientific Miracles.

1. Introduction:

There is no doubt that the modern food industries facilitated the diversity, availability of food and save them from the damage in different seasons of the year. So that we can find winter's yield in summer season and vice versa. In addition to the export of foods in the international trade, which requires the addition of some external materials to be homogeneous with the food content, whether to preserve or improve the taste. These materials known as food additives.

With continued technological progress in the industrialized food system, the number of approved additives increased because their multiple properties. Recently, the number of chemical additives became very large. Since 1987, a certain percentage of each category of additives has been determined on a global scale, Per day, per person, but that is not enough for many reasons.

Firstly, the dose involved and its relationship to sensitive groups in society, such as children, pregnant women and the elderly remains a neglected point when discussing the impact of chemical additives. Second, food labels do not specify the percentage of chemical additives in the food category and the maximum amount allowed, The amount of consumed dose the most important factors to reduce the risk of use. The consumer consumes many food products manufactured in his daily life, the problem is not in the use of these substances or others, but the problem in the proportion and the frequency of using several times a day, as a result may the dose exceeds the safe limit in the body. So, various types of substances enter the body and this leading to their accumulation in the body. Thus, bypassing food safety limits.

One of the most famous chemicals added to the food is a substance called Monosodium glutamate (MSG), which is one of a number of salt forms of glutamic acid, a non-essential amino acid, with unique flavor-enhancing qualities, that is widely used as a food additive (Quines et al., 2014), has caused widespread debate among consumers, researchers and scientists, they debated about the safety of this substance on human health.

Monosodium Glutamate (MSG) is the sodium salt of amino flashing alglutamic that exists in our bodies and in many foods and materials in additional pain and alglutamic acid involved in configuring mono glotamitwedion levels equivalent to 78% and 22% of sodium and water. (Eweka & Om’Iniabohs, 2007); ( FDA, 2012 ). Many studies highlighted the adverse effects of MSG when consumed. Eye and retina tissue was severely affected when chick embryos were treated with monosodium glutamate (Al-Jahdali and Al-Qudsi 2012).
MSG is considered as an excitotoxin which can alter the normal functions of neurotransmission in animal biological systems (Mahalihana et al., 2016). These effects such as, brain damage potential, Retinal degeneration, and hepatic toxicity (Farombi & Onyema, 2006); El-Sayyad et al., 2016; Foran et al., 2017).

It has been shown to promote a neuroendocrine dysfunction when large quantities are administered to mammals during the neonatal period (Collison et al., 2012). It has been established that the blood–brain barrier of neonates is immature and that glutamate can traverse this barrier and exert excitotoxic effects on adjacent brain regions (McCall et al., 1979; Shah et al., 2015), several studies have shown that MSG administered to pregnant animals can cross the placenta and reach the foetus. (Park & Choi, 2016)

Therefore, it was necessary to search for practical solutions associated with the use of food additives and limit their effects. When we look at the research that is available to us, we found that it is concerned to the prevention of the effect of additives on the tissues of rats and adult mice; (Afteefy et al., 2012;) John et al., 2015 without addressing the protection of the tissues of the fetus during the period of composition.

After understanding and well informed to many researches, we found that grape fruit is one of the most consumed fruits in the world and has different biological functions due to the components of polyphenols, which is mostly in the seeds of grapes by 60-70% and in the crust by 30%, seeds Grapes contain grape prolyl phenolate, which form 5-8% of its weight (Hassan, 2012).

A variety of naturally occurring grape seed extract have been found to have beneficial effects on health (Waggas, 2012). The grape seed extract has been reported to possess a broad spectrum of pharmacological and therapeutic effects such as antioxidative, anti-inflammatory, and antimicrobial activities, as well as having cardioprotective, hepatoprotective, and neuroprotective effects (Hala et al, 2010). In addition, the word of grape repeated around 11 times in the Holly book (AL Quran). For these reasons, grape seeds were chosen as a study site.

And many studies have indicated that the ability of the GSE antioxidant enzyme system may return to discourage responsible for producing free radicals (Hala et al, 2010).

And another study found that grape seeds are a preventive role in the protection of various body parts, tissues (Hasseeb et al., 2011).

And chicken embryos were selected as samples for the experience and to get it, and the short duration of incubation, and chicken embryos are also good laboratory animals to accurately determine the age of the fetus, fetal incubation within the lab and get the freak El Geneina (Al-Ghamdi, 2007).

Retina in chicken embryo age 7 days it's composed of sensory and pigment layer, the layer of pigment cells long sitoblastoma-vertical base kernel contain small pills. Either the sensory layer of outer membrane nerve layer selected (neuroblastic layer) and the internal network layer and nodal cells layer and finally selected membrane bounded fiber layer. It also pointed out that external cells are nerve layer which will be rods and cones and sticks cells and then characterized (amacrine cell) and is an occasional cells followed by (bipolar cell) and supportive cells contain fiber (Muller fibres) (Romanoff, 1960).

And with age stated that the retina at age sixteen days after chicken incubation until hatching of other sensory and pigment layer, became dark pigment layer, either sensory layer is composed of eight layers as follows: 1. External limiting membrane, 2. Rods and Cones layer, 3. Outer reticular layer, 4. Inner nuclear layer, 5. Inner reticular layer, 6. Ganglion cell layer, 7. Nerve fiber layer, 8. Internal limiting membrane. (Romanoff, 1960); (Hamilton, 1965).

And the retina was chosen for study from members of the fetus and to meeting many research on retina affected a MSG as stated by both (Tamás et al., 2004); (Rácz et al., 2006); (Szabadfi et al, 2009); (Ali et al, 2012); (Blanks et al, 1981); (Bellhorn et al, 1981).

Ganglion cell layer in the retina of mice in the groups treated with MSG material less dense cellular of the retina in the control group (Van Rijn et al, 1986).

The treatment of adult rats with a mono sodium glutamic MSG dose (2 mg/g body weight) by mouth and 12 weeks, led to the damaged retina dramatically as most retinal cells with multiple degrees of damage and that was ruptured and pigment layer (PE), and neurosensory nucleus of rods and cones (R & C), also bipolar neurons damaged and lost most of The organelles they need to transfer signals from light receptors (R & C) to the Ganglion cells and cells that appeared disjointed and contains many gaps (Swelim, 2004).

2. Materials and methods:

Materials:

This experiment was using number (300) fertilized egg, where obtained from fagiha for livestock. Monosodium Glutamate (MSG) was purchased as powder from Al-Mizani medical corporation – Saudi Arabia, and that dose 0.1 ml according to method (Al-Jahdali, 2009). And use Grape seed extract was purchased from GNC, And use a dose 0.3 ml according (El-Awdan et al, 2013).
Experimental design:
We use Fertile chicken eggs (n = 300) (average weight 60 gm) were divided into six groups based on injection period and the injected substances. The first group is control, the second group is treated with GSE, the third group is treated MSG, the fourth group is mix (MSG and GSE), the fifth group is preventive (GSE then MSG) and the sixth group is therapeutic (MSG then GSE). 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. We are studying the effect of experimental materials in the fetal development during the following ages (14, 16 days)

Results:
1- Morphological studies:
Congenital malformations
In 14-day proportion distortions in the group treated with MSG and were not to be eye and congenital fetal form and complete his hernia and deformity of the bones of the neck and limbs, and in the Group (G-M) noted the small size of the fetus and delayed growth. And at the end of the age of experience and is a 16 day observed in MSG group not to weld the abdominal wall as a result of the internal organs have emerged through a sprained neck of fetus, besides having congestion in the body of the fetus and its small size (Figure 1; A and B).

2) Morphometric study:
The weight body:
At the age of 14 and 16 days, there was a significant decrease in the weight of the fetus in the treatment group (MSG) and the protective group (G - M) where the mean weight difference was greater and showed a significant difference from the mean body
weight in the control sample. (Table1-A) (Histogram1-A) and (Figure 2; A and B)

**Length body:**

At the age of 14 days there was a significant decrease in the length of the fetus in the protective group (G-M). At 16 days there was a significant decrease in the length of the fetus in the treated group (MSG) and the protective group (G-M). (Table1-B) (Histogram1-B) and (Figure 2; A and B)

**Eye diameter:**

At age 14, there was a significant decrease in eye diameter in the MIX and G-M groups, where the statistical significance of p = .000

At the age of 16 days, there was a significant decrease in eye diameter in only two groups, first group is MSG and second group (M-G). (Table1-C) (Histogram1-C).

**Beak Length:**

At the age of 14 days there is a significant decrease in beak length in the treatment group (G-M) and at the age of 16 years of incubation, a significant increase was observed in the joint group (MIX). (Table1-D) (Histogram1-D)

**Neck length:**

At the age of 14 days, there was a significant decrease in the length of the fetal neck in the group treated with MSG. There is also a significant increase in the length of the fetal neck in the joint group (MIX) and at the age of 16 days there is a significant lack of neck length in the common group (MIX) and the therapeutic group (M-G). (Table 1-E) (histogram 1-E).
**Table 1**

showing the difference mean between experimental groups in Morphometric study (A) whole body weight, (B) whole body length, (C) diameter eye, (D) beak length, (E) neck length.

* The mean difference is significant at the .05 level.
3) Histological studies:

Effects on Retina development

There was no significant difference in the thickness of the retina in the treated group using GSE, while a significant decrease in the thickness of the retina was observed in all groups treated with MSG in (14) day.

At the age of 16 days, a significant increase in thickness of the retina was observed in all groups treated with MSG except for the therapeutic group (G-M) a significant decrease was observed. (Table 2-histogram 2).
Accidental sectors scans of chicken embryos retina in control group for 14-day shows that the retina is composed of two pigment epithelium (PE) and neural retina layer (NR) which in turn consists of 8 layers. And in (GSE group) shows the convergence of total thickness retina and histological structure with control group In the (MSG group) note that the layer sticks and cones R & C (2) less cellular density and separated, and stretched the inner nuclear layer effects to I.N.L (4) and that appeared disjointed and spaced cells and internal plexiform layer I.P.L (5) which appeared thinner and contain many gaps, and as for effects in nodal class G.C.L (6) contains fewer cells with nuclear atrophy occurs for nucleus, for fiber layer Neuron N.F.L (7) following streptococcal class have appeared torn and thinner than in the control group. In (Mix group) Note in this group that the total thickness of the retina than the sample control, inner nuclear layer (I.N.L) and nerve fibers layer (N.F.L) surfaced thinner than in control sample. While in the (G-M) group noted in this group to total fish retina compared with control group where nodal class notes (G.C.L) contains fewer cells, and also a lack of nerve fiber layer (N.F.L). as well as in the (M-G) group It was noted in this group relative affinity in total thickness and structure retina with control group. 

**Table 2**

<table>
<thead>
<tr>
<th>DAY</th>
<th>J groups</th>
<th>J groups</th>
<th>Mean</th>
<th>Difference</th>
<th>Std. Error</th>
<th>Sig.</th>
<th>95% Confidence Interval</th>
<th>Lower Bound</th>
<th>Upper Bound</th>
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<td>0.05</td>
<td>0.05</td>
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<td>0.055</td>
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<tr>
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<td>ctrl</td>
<td>M.S</td>
<td>0.422</td>
<td>0.05</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>14 day</td>
<td>ctrl</td>
<td>M.G</td>
<td>0.573</td>
<td>0.05</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>16 day</td>
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<td>S.E</td>
<td>0.004</td>
<td>0.055</td>
<td>0.942</td>
<td>1.056</td>
<td>0.010</td>
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<tr>
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<td>M.S</td>
<td>0.536</td>
<td>0.05</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>16 day</td>
<td>ctrl</td>
<td>M.G</td>
<td>0.536</td>
<td>0.05</td>
<td>0.00</td>
<td>0.00</td>
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* The mean difference is significant at the .05 level.

**Figure (3 A;B;C- 4 A;B;C)**

**In 16-day**

Histological examination shows the (R) of the eye for 16-day chicken embryos sample as a former age in structure and number of layers. In the (GSE group); the retina also featured as the control group thicker and structure. in the (MSG group); It was noted that a layer rods and cones R & C separated and inner nuclear layer appeared (I.N.L) are ruptured and crushed, and the internal nuclear layer contains I.P.L)) many gaps, while Ganglion cell layer (G.C.L) smaller and consist of many cells and pyknotic also noted that the rate of fiber in nerve fibers layer (N.F.L) less than
in the control group. And in (MIX group) there was a thermonuclear atrophy in the inner nuclear layer (I.N.L), and increased thickness of internal nuclear (I.P.L) and Ganglion cell layer appear (G.C.L) thinner and contain cells have atrophied. In the (G-M group) there was a thermonuclear atrophy in the inner nuclear layer (I.N.L), and increased thickness of internal nuclear (I.P.L) with a nuclear layer cells atrophy (G.C.L) and nerve fibers layer appears (N.F.L) thinner compared to the control group, while in (M-G group) this group appeared similar in thickness and shape layers with the control group. Figure (5 A:B:C - 6 A:B:C).

We noted that the Group were treated with grape seed extract GSE nearer to control group followed by preventive and therapeutic group; Group treatment by MSG alone hardest of all three treatment groups together.
4. Discussion:

Treatment of embryos textured (MSG) led to many of the distortions and it has been disappeared (the disappearance of one or both eyes, the small size of the eye, swelling in the head area, the small size of the fetus, deformation of the neck and limbs). The apparent examination of embryos revealed clear congenital abnormalities in a number of embryos of experimental groups during the following days: (14, 16).

Many studies showed that MSG caused different effects such as elongated heart, cardiac sac edema, yolk-sac edema and spinal kyphosis. (Abdelkader et al., 2012)

Growth retardation and subcutaneous bleeding, abdominal hernia and other congenital malformations such as brain and beak deformation. (Al-Qudsi & Al-Jahdali, 2012)

The cause of malformations in groups treated with MSG is that fetuses have a high level of O2 consumption, making them vulnerable to oxidative damage, resulting in poor fetal growth. (Park et al., 2014); (Tan et al., 2015).

And perhaps because of distortions in MSG treatment groups due to the toxic effect of MSG on the baby's body, since that article MSG disable endocrine function responsible for regulating many body processes including metabolism, and this might play a role in causing deformities including skeletal deformities (Elefteriou et al, 2003). Excessive use of MSG can cause metabolic disorders and biological abnormalities in fetal development, and added that MSG has the ability to cause damage to the endocrine function and may be a cause of this occurrence results regarding teratogenic and disrupt body processes and development of Genetic. (Abdelkader et al., 2012).

That because of the high antioxidant in the body due to excessive production of free radicals or flaw in antioxidants in the body, and that adverse effects on the mother and the fetus (Lappas et al., 2011).

And on the other hand, it was noted that no abnormalities or deaths in Group b and group therapeutic GSE treatment and this agrees with (El-Ashmawy & Bayad, 2016) Telling that grape seed extract and folic acid were protective factors against fetal abnormalities resulting from the use of property azathiobrin.

Perhaps the reasons for the non-occurrence of deformities in the group returns to the GSE treatment captured the first is used for security and not named after a living organism and affirming that the results recorded research done in Japan to 344 rats to study safe use of grape seed body and found that grape seed extract sharp poisoning does not occur and that a daily dose of 2-4 g/kg of body weight orally doesn't make any toxicity in animals and It has no known side effects (Yamakoshi et al, 2002); (El-Ashmawy & Bayad, 2016)

The second reason may be due to their high antioxidant capacity as research studies reported that grapes contain Proanthocyanidin alwrwanthosianidin the phenol compound that has stronger antioxidant effectiveness of vitamin E and 20 times C (Bagchi et al., 1997). As it also has the ability to mitigate damage, oxidative stress by inhibiting enzymes responsible for generating free radicals or detoxification of some carcinogens or mutagens caused by free radicals (Abeer, 2012).

As demonstrated by the above surveys and conducted for morphological characteristics (weight – length – Qatar eye-length beak – the length of the neck) to the group that was treated with MSG solo latest damage to fetus, while the group that was treated with grape seed extract It did not adversely affect members of fetus. And agree with us too.; The embryos of treatment b MSG showed less weight than the control group. (Miškowiak et al, 1992) (Al-Qudsi & Al-Jahdali, 2012).

The length of zebra fish embryos treated with MSG appeared shorter than in control sample (Abdelkader et al., 2012) (Mahaliyana et al, 2016).

The results of the current study also showed a lack of moral dimetereye and beak length and neck length and agree with us (Al-Qudsi & Al-Jahdali, 2012).

And may have interpreted small embryos injected with a substance MSG unable to obtain adequate nutrition from intimated leading to the inability of cells to grow and divide the required speed and efficiency, and returned to see a lot of blood when opening eggs injected with MSG and that what has been observed in the current study, said that it might be blood nath about bleeding not forbid carrying vessels delivering alluded to embryonic cells.

To increase the toxic effects of glutamate on hypothalamus area which led to reduced growth hormone secretion in the body so poignant and after processing b GSPE managed to increase body weight. (Selima et al., 2017) the GSE prevent oxidative stress and prevent adverse effects associated with high-fat food...
that causes heart failure and deposition of abdominal fat.

And either because of improvements in treatment group reported improvement in gross weights to collapse due to stimulate mitosis to body cells and protein biosynthesis by active compounds in the extract of albrobols, which is the antioxidant (Akaberi & Hosseinizadeh, 2016).

In the results of the histological studies, the treatment of chicken embryos with MSG with a dose of 0.1 milliliters led to tissue damage in the retina of the chicken's eye. Retinal thickness statistics indicated that the thickness of the retina treated with MSG was lower than that of the control sample.

Many studies showed that MSG caused changes in all layers, the pigment epithelium showed vaculation of the cytoplasm and numerous dense granules at the periphery of the cell, many nuclei of the outer nuclear layer appeared diffused with lack of chromatin details. Inner nuclear and inner plexiform layer were less in the thickness.

Some cells of the inner nuclear layer were pyknotic and appeared very dense; the ganglion cells were swollen (Ali et al., 2012).

mother rats revealed massive degeneration of ganglion cells, spongy- appearance of inner plexiform layer, thinning of inner nuclear layer and striking missing of outer nuclear and photoreceptor layer in 8 of 20 mother retina. There was a comparative decrease in the number of pigment epithelium. (El-Sayyad et al., 2016).

As that Microscopical analysis revealed a substantial inner retinal degeneration in MSG-treated animals: the retinal thickness was less than 25% of the normal retinas (Szabadfi et al., 2009).

Treatment of retina by MSG led to dissolution in the layers of the retina, where the thickness is less to half the thickness of the retina, the layer appears natural internal nuclear (I.N.L) Ganglion cells layer (G.C.L) welded, and un-differentiation of the (I.P.L) (Tamás et al., 2004).

Treatment by MSG leads to separation of pigment layer (PE) and distort optical receptors and erratic, and necrosis of many nucleus of Outer nuclear layer (O.N.L) and the inner nuclear layer cell and Ganglion cells layer are degeneration, and the expansion and congestion in some blood vessels, and internal limiting membrane (I.L.M) outage, are detected as statistically significant decrease in the thickness of the retina (Moustafa & Okasha, 2016).

Reason for retinal function and composition influenced by MSG to lglotamat receptors exist in most layers of the retina, and destroying the barrier works high glutamate bloody website Organizer to enter material into the retina and thus lead to prevent polarization by stimulating cell death (Swelim, 2004)

That a diet containing high proportions of MSG for years may increase the concentration of glutamate in vitreous body which destroy the cells in the retina. (Ohguro et al., 2002)

The embryos were treated with GSE and then the study of the individual GSE effect on the retina to determine whether there was any harm caused by the individual treatment of GSE and its safe use on the body. We have observed through experiment and histological examination the safety of single use of grape seed extract on the retina There was no tissue damage to the retina and the effect of GSE on the histological damage caused by MSG was also studied.

The histopathological examination of these groups showed that the GSE treated group was similar to the control sample, and that the tissue structure in MIX, GM, MG was observed in some ages. The tissue began to regenerate its tissue, where the degeneration of the layer (INL) and the disappearance of gaps in the internal plexus (IPL), and the cells regained relative form in the nodal layer (GCL) at age (14). While at age 16We also noticed that the improvement in the treatment group (M-G) was reported the best than two groups (MIX / G-M).

(Patel et al, 2016) mentioned that a diet containing grapes saves the retina and its function of oxidative stress that destroys the retina.

Yu et al. (2012) added that taking antioxidants prevents loss of vision due to oxidative stress, as well as promotes the visual functions of the eye, and continue to take antioxidants delay the weakness of vision related to age.

Several studies have suggested that extracts obtained from grape seeds have the potential to inhibit enzymes responsible for free-radical production (Li et al., 2000) (Maier et al., 2009). Recently, research has reported that GSE inhibits Oxidation by altering the expression of enzymatic systems (Puiggros et al., 2005).

The Proanthocyanidin Able to protect cells of G.C.L of death caused by hydrogen peroxide H2O2, by restricting the operations of death and coded inner track control of cell death coder (Wang et al., 2013).

GSE has a high therapeutic value in mitigating the retinal damage to the retina, that may be due to oxidative stress and toxic effects of glutamate.

This improvement effect may be due to the inclusion of GSE on active compounds with vital effects, It contains high concentrations of phenolic compounds known as polyphenols. Antioxidants have the ability to influence the different pathogenesis process by free radicals through the ability to reach the cellular components (mitochondria - nuclei) of the chemical activity in the cell, The active compound is Proanthocyanidin. (Gabetta et al., 2000); (Al-Sowayan & Mahmoud, 2014)
Many studies have indicated that extracts obtained from grape seeds have the capacity to inhibit the enzymes responsible for the production of free radicals (Li et al., 2000) (Maier et al., 2009).

And lately, there is research stating that GSE prevent oxidation by modulating enzymatic systems expression (Puiggros et al., 2005).

And through the results of this study, it was clear to us the improvement of therapeutic and impact by GSE against harmful effects of MSG and add this therapeutic effect to many of the benefits of grape fruit and grape fruit has singled out miracles on 11 themes from the book of Allah Karim (Quran) was mentioned once solo and gathers ten times, and is the number that did not reach any fruits, like this striking.

5. Conclusion:

The treatment for MSG caused much damage in fetus body’s and treatment with GSE was reduced this damage, We recommend to do a further research to study the effect of GSE at different doses and different period, as well as recommend eating grape seed and not to throw it, and so to get health usefulness.

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