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COMPARATIVE STUDY OF THE EFFECTS OF TRADITIONAL AND CHEMICAL ADDITIVES ON ORGANOGENESIS OF CHICK EMBRYO (*GALLUS DOMESTICUS*).

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Introduction:-

Food additives are used to enrich the value of food products. (Tawfek et al., 2015). Plants used in food enhancement are used dating back to 60,000 years ago (Solecki, R.S. Shanidar).The food additives used in adorning the food which causes health issues.(Amin and Al-Shehri, 2018). Colorants used on large scale in drugs ,cosmetics and food according to guidelines of (FDA) (Pressman et al., 2017).Countries like Egypt has tremendous increase for demand of synthetic color (Abd Elhalem et al., 2016; Ali et al., 2016) El-Borm et al. (2019) studied effect of coloring agents on uriniray system and hepatic sytem of chick embryo.

Science times immortal medicinal plants are apart of our kitchens across civilizations. Even if their biochemistry is still being studied their health benefits have served the mankind for centuries used in home remedies & disease cure.**Hassan BAR(2012)** 80 % of the civilizations use medicinal plant extracts or products to cure diseases even today. **McKay DL, Blumberg JB (2006)**.

Peppermint or mint (*Mentha piperita L.*), is a hybrid (*Mentha aquatic L.*). **Khalil AF, Elkatry HO, El Mehairy, HF (2015).Spirling LI, Daniels IR (2001)** cosmopolitian in distribution due to cultivation but originally from Maditeranian region (**Iscan G et.al (2002)**)The volitile oils is the key component in flavouring industries **Dorman H D et.al (2003)**.

Curcumin is a derived from Turmeric (*curcuma longa*) immensely used in It is widely used as presrvering and colouring agent. It has protective properties and science long used by us Indians in our daily food. (Pulido-Moran et al., 2016).

Stevia rebaudiana has about 150 species is known as sweet herb a natural sweetner. In Progressive countries it is used at industry level. It has health hazardous as a substitute for sugar yet is used in China and Japan on large scale.

Article History

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Material & Methods:-

The natural food additives like *Curcuma longa* (Turmeric powder from farmer) *Mentha piperita L.* (from own garden without pesticides) and *Stevia rebaudiana* (wild variety) were used as natural additives. The artificial (Synthetic /Chemical) additives like aginomoto & tartrazine

Experimental Setup

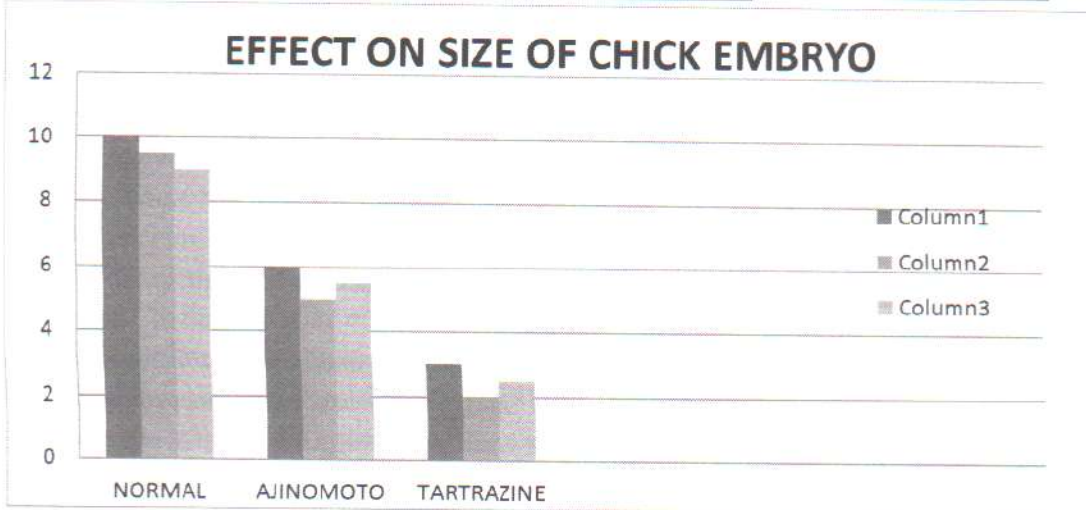
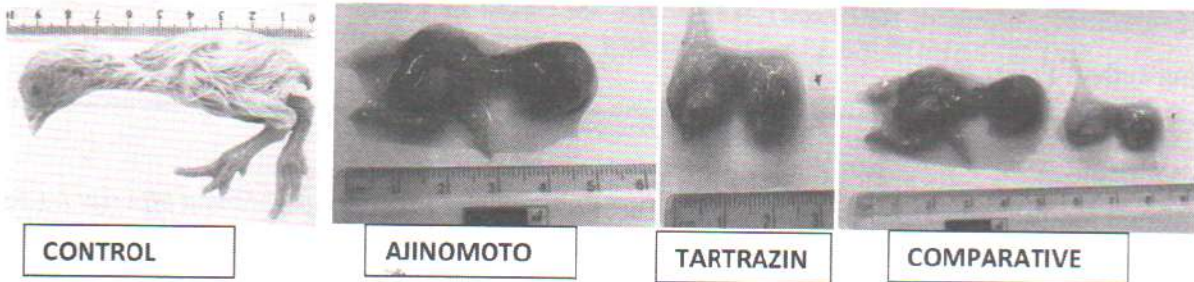
Eggs were incubated at 37.5°C and humidity was maintained by keeping a water bowl in incubator. The eggs were randomly assigned to 3 equal treatment groups, 10 eggs each, as follows: group 1: Control group (medicinal herbs together), embryonated eggs injected with aginomoto, tartrazine 0.5 ml/egg into the yolk sac. On day 4 of incubation, the eggs of group 2 treated with aginomoto group 3 with tartrazine. Embryos received treatment by direct injection into the yolk sac according to the standard techniques. Embryos were reincubated posttreatment and allowed to develop. The viability of the embryos was checked throughout the incubation period by candling.

All embryos were necropsied on day 18 of incubation and examined for macroscopic and microscopic lesions. The treatment protocols and procedures in this study were conducted according to local ethical guidelines. At the end of the experiment, on day 18, embryos were humanely killed by placing on ice and then the eggs were opened at the wider end. After washing in normal saline solution, embryos were observed under stereomicroscope to study any gross abnormalities on the external body surface. The membranes and yolk sac were also inspected. Then, the tissues of embryos were dissected out and fixed in 10% neutral buffered formalin. Following routine preparation of tissues, serial sections of paraffin embedded tissues of 5 µm thicknesses were cut using a microtome and stained with hematoxyline and eosin and studied under light microscope. Statistical analysis was performed using a bar diagram. The graph was used to determine the significant differences in occurrence between experimental groups.

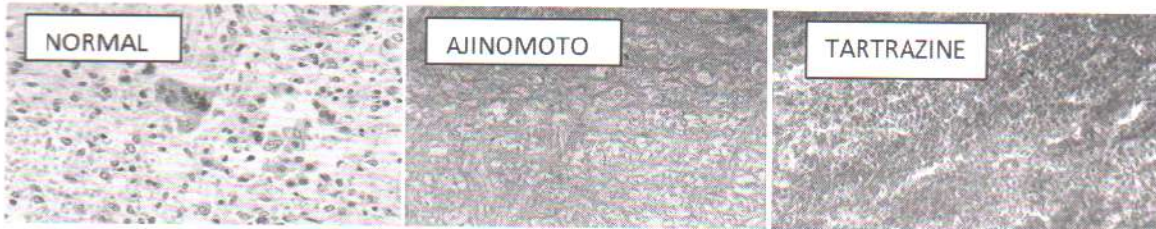
OBSERVATIONS

A] Chart showing legs length in cm.

.No.	Concentration	Length of legs in cm.		
		Control	Ajinomoto	Tartrazine
1	0.5	10	6	3
2	0.5	9.5	5.5	2
3	0.5	9	5	2,5



Brain



Heart



Photomicrograph of the chick embryo control brain was normal. The treated embryos were with deformities. Histology of the control set for brain was normal epidermis, dermis, mesoderm, and amnion were clearly visible and well distinguished with normal cell size shape and organelles. In embryos treated with

ajinomoto the cell size has decreased cells cannot demarked. The cell size & shape was abnormal, tendency of rosette formation. The embryos treated with tartrazine showed cell abnormalities, neoplastic growth. They consist small densely packed cells, indicating mitosis. Rosette formation was seen with cell debris. Photomicrograph of the chicken embryo treated with the ajinomoto & tartrazine solution into the yolk sac are abnormal, the structure of the heart seen in (control) was normal.

In the normal (control) the histology of heart is normal endothelium, pericyte, mesoderm, angioblast were with normal cell size shape and organelles. The embryos treated with ajinomoto are not similar to control. The cell size has decreased they cannot be distinguished there is abnormality in cell size and shape. Cells were highly damaged. The embryos treated with tartrazine detected cell abnormalities, neoplastic growth. They consist small densely packed cells, mitosis occurs in such cells. Mitosis was observed in cells which indicated formation of neoplasia with cell debris.

RESULTS

Macroscopic results indicate that as compared to the control, it was seen that there was normal development up to day 18 in ajinomoto, but tartrazine injected eggs were found with dead tissue and weak embryos. The number of blood vessels were also few and there was clotting seen. The tissues of the embryos were normal in group 1. In ajinomoto injected group, group 2, there was gross abnormality in the tissues and external body surfaces (plate no.3). The obtained tissue samples of these embryos were analyzed for weight and length ratio. **Microscopic findings** Histopathological evaluation has been revealed that all organs were normal in group 1. In embryos which received the ajinomoto & tartrazine solution treatment, all microscopic structures were abnormal.

Histopathological examination of the control shows normal growth it has also been revealed that all organs were abnormal in treated embryos. Therefore, these results suggest that the food additives like ajinomoto and tartrazine have adverse effect on cell histology. Some embryos even didn't reached the 18th day of incubation. Death of embryos was a serious hurdle in our investigation. Nevertheless, further efforts are needed to evaluate in ovo administration of various food additives. In conclusion, based on macroscopic and microscopic findings, it is concluded that ajinomoto and tartrazine have toxic effect on chicken embryo. In addition, from our previous experiment carried out on garlic meristematic tissue it was concluded that tartrazine is more carcinogenic as compared to ajinomoto. The same procedure was followed for avian eggs were the result is the same. It is recommended not to use ajinomoto and tartrazine above the specified limit.

As compared to chick embryos human fetus is larger but both the additives have adverse effect on it also. The previous studies indicate both these additives are carcinogenic and higher dose can cause serious injuries and abnormalities. It has a higher effect on the growth of different organs of the embryo. It is revealed that the administration of Histopathological examination of the control shows normal growth it has also been revealed that all organs were abnormal in treated embryos. Therefore, these results suggest that the food additives like ajinomoto and tartrazine have adverse effect on cell histology. Some embryos even didn't reached the 18th day of incubation. Death of embryos was a serious hurdle in our investigation. Repeated efforts are needed to evaluate in ovo administration of various food additives. In conclusion, based on macroscopic and microscopic findings, it is concluded that ajinomoto and tartrazine have toxic effect on chicken embryo. In addition, from our previous experiment carried out on garlic meristematic tissue it was concluded that tartrazine is more carcinogenic as

compared to ajinomoto. The same procedure was followed for aveian eggs where the result is the same for ajinomoto and tartrazine. There was cell necrosis some of the embryos were found dead.

DISCUSSION

The food adatives has flurished in past decade.The stress causes many health hazardus. There has been extensive research in food industry ehancing colour and taste .The hatching eggs are injected with food adatives to increase weight and laying performance. MSG belongs to the non essential amino acids groups.It has been used successfully for several decades in many Countries such as Canada, Spain, France, Austria, Polish, Denmark, Germany, Turkey, Africa, United States and China.In recent years, its use has increased rapidly in the Iranian poultry industry, but there is little information available about the pathological effects of injecting it into the bird's egg.

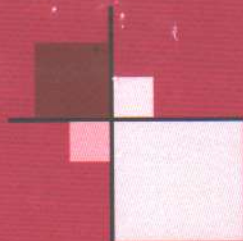
Besides, determining the side effects of drugs on the development of bird embryo is a useful method for studying the biological properties of drugs. The present investigated toxicity of ajinomoto and tartrazine solution for in ovo administration in chicken egg. Lesions & organ injuries following administration were also inspected.The results of these studies displayed that in ovo administration of amino acids may be an effective method to increase chick embryo development and body weight.Dosage and the route of injection can have an influence on the outcome.

Our results obviously showed no gross abnormality in the tissues and external body surfaces of the chicken embryos exposed to control solution by yolk sac route. The study evaluates safety of *Mentha piperita L*, *curcuma longa* & *Stevia rebaudiana* on chick embryo development. The results of this study suggested no abnormalities in the brain, heart and morphology of chick embryo model. Where as studies by Hend T. El-Borm 2020) recommended avoiding the use of tartrazine and ajinomoto during pregnancy causing bone deformity.Our results also coincide with the results which have hazardous effect on brain and heart tissue .

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