Journal of Anatomical Sciences

Email:anatomicaljournal@gmail.com

J Anat Sci 7 (1)

Teratogenic Effect of Maternal Vitamin A Consumption on the Cerebellum, Limbs and Other Morphological Parameters of the Pups of Wistar Rats

[*1]Aguwa US, [2]Olatunde O, [1]Olu SI, and [1]Leko BJ

[1]Department of Human Anatomy, Faculty of Basic Medical Sciences Madonna University Elele.
[2]Department of Human Anatomy, University of Ibadan

Corresponding Author: Aguwa US

E-mail :ugochukwuaguwa@yahoo.com; +234-7030885803

ABSTRACT

Vitamin A is a commonly consumed vitamin in raw foods and in form of supplement. Previous researches showed that single doses of this vitamin when administered at targeted gestation periods produced varying degrees of congenital malformations in different organs in the pups of rats including the brain. This study intends to evaluate the teratogenic potentials of vitamin A. A total of twenty (20) female Wistar rats weighing an average of 150g were randomly separated into four groups of five rats each. The rats were mated and given increasing doses of vitamin A- 400mg/kg, 500mg/kg and 600mg/kg for groups B, C and D respectively. Group A was the control group and received only food and water like the rest of the groups. Administration lasted for 14days, after which the rats were allowed to deliver. Most of the rats in the experimental groups displayed various signs of toxicity, ranging from significant weight loss, well reduced activity, reduced food and water consumption, peeling of hairs, obvious signs of spontaneous abortion as well as death. Physical examination was carried out on the pups using a magnifying hand lens to check for any form of physical malformation on the limbs, face and other morphological parameters. This was done on days 1, 7 and 16 post- partum. The pups were then sacrificed. The histology of the cerebellum showed no significant differences between the experimental groups and the control. However, the results of the oxidative stress markers showed significant levels of oxidative stress in the homogenized brain samples as compared to the control. Oxidative stress is a possible indication of underlying tissue damage. Our results revealed that the damages done to the pup tissues were better appreciated at the molecular levels than were seen in H&E preparations.

Key words: Vitamin A, teratogen, cerebellum, morphological, anti-oxidant.

INTRODUCTION

Vitamin A has been implicated as causing diverse kinds of birth defects in several studies including malformations of the limbs and the nervous system[1]. This is an intriguing discovery considering the fact that there has been a lot of emphasis on Vitamin A consumption in recent years. Most of the common food additives like salt, sugar, vegetable oils etc. are specially fortified with Vitamin A and is prominently displayed on the packs having the diagram of an "eye with a big 'A' " inside it. More so, a lot of the common fruits consumed by the majority of the populace contain lots of vitamin A. Even the common table palm oil used is an important source of vitamin A. If this vitamin is as teratogenic as studies have shown, then somehow many people should have had malformed babies consequent to consumption of this vitamin. So, do we attribute the persistently high level of limb malformations reported by Vargession[2] to ignorance in consumption of high doses of vitamin A? These among others were the questions we set out to answer.

Vitamin A is the name of a group of fat-soluble retinoids, including retinol, retinal, retinoic acid, and retinyl esters[3]. Vitamin A is involved in immune function, vision, reproduction, and cellular communication[4]. Vitamin A also supports cell growth and differentiation, playing a critical role in the normal formation and maintenance of the heart, lungs, kidneys, and other organs[5]. Common sources of vitamin A are food and dietary supplements. Two forms of vitamin A are available in the human diet: preformed vitamin A (retinol and its esterified form, retinyl ester) and provitamin A carotenoids[6,5]. Preformed vitamin A is found in foods from animal sources, including dairy products, fish, and meat (especially liver). By far the most important provitamin A carotenoid is beta-carotene; other provitamin A carotenoids are alpha-carotene and beta-cryptoxanthin. The body converts these plant pigments into vitamin A. It is also available as multivitamins and as a stand-alone supplement, often in the form of retinyl acetate or retinyl palmitate[4].

The cerebellum otherwise called 'little brain' develops from thickenings of dorsal parts of the alar plates. The cerebellum receives spinal (somatosensory, ipsilateral)

input via the inferior cerebellar peduncle (ICP), by way of the dorsal spinocerebellar (lower extremities) and cuneocerebellar (upper extremities) tracts. Other major input from the level of the brainstem originates in the vestibular nuclei and ganglion, the lateral reticular nuclei. The functions of the cerebellum are broadly categorized into two – motor functions and non-motor functions. There is mounting evidence that the cerebellum likely plays a crucial role in various aspects of nonverbal learning, as well as in general cognition, arousal, and emotional and autonomic responses, although its most commonly recognized role is in the control and/or modulation of motor activity[7]. It always takes place against a backdrop of the effects of gravity; the relative position of one's limbs, head, and trunk in space and to each other; and/or some other ongoing activity. Because of its direct interconnections with the vestibular system, the proprioceptive feedback it receives from stretch receptors via the spinocerebellar pathways, and its influence on those same antigravity muscles via the vestibulospinal (and probably the reticulospinal) pathways, the cerebellum can learn to mediate the subconscious, reflex adjustments necessary to maintain one's equilibrium[7].

Antioxidants are substances that are capable of counteracting the damaging, but normal effects of the physiological process of oxidation in animal tissue. Antioxidants are nutrients (vitamins and minerals) as well as enzymes[8]. They are believed to play a role in preventing the development of such chronic diseases as cancer, heart disease, stroke, Alzheimer's disease, rheumatoid arthritis, and cataracts. Oxidative stress occurs when the production of harmful molecules called free radicals is beyond the protective capability of the antioxidant defenses. Free radicals are chemically active atoms or molecular fragments that have a charge due to an excess or deficient number of electrons. Because they have one or more unpaired electrons, free radicals are highly unstable. They scavenge the body to grab or donate electrons, thereby damaging cells, proteins, and DNA (genetic material). Free radicals arise from sources both inside (endogenous) and outside (exogenous) our bodies. Oxidants that develop from processes within our bodies form as a result of normal aerobic respiration, metabolism, and inflammation. Exogenous free radicals form from environmental factors such as pollution, sunlight, strenuous exercise, X-rays, smoking and alcohol. Our antioxidant systems are not perfect, so as we age, cell parts damaged by oxidation accumulate[9]. Antioxidants from our diet appear to be of great importance in controlling damage by free radicals. Each nutrient is unique in terms of its structure and antioxidant function. The antioxidant enzymes superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) serve as your primary line of defense in destroying free radicals[8]. SOD first reduces (adds an electron to) the radical superoxide (O^{2-}) to form hydrogen peroxide. Catalase and GPx then work simultaneously with the protein glutathione to reduce hydrogen peroxide and ultimately produce water (H_2O). The oxidized glutathione is then reduced by another antioxidant enzyme - glutathione reductase[9]. Together, they repair oxidized DNA, degrade oxidized protein, and destroy oxidized lipids.

MATERIALS AND METHODS

20 female rats of 3months old, weighing an average of 150g were randomly separated into 4 groups A,B,C and D. The individual rats were marked for identification and acclimatized for 2weeks. From day 15 the rats were introduced to males for mating. From the next morning (day 16) the rats were tested for pregnancy using the vaginal lavage and microscopic examination for the presence of spermatozoa. The first day of pregnancy was counted as day 1. From day 1 of pregnancy the rats received their corresponding dosages of Vitamin A for 14 days uninterrupted. Group A was the control and took only food and water. Group B received 400mg/kg/day of vitamin A. Group C received 500mg/kg/day of vitamin A. Group D received 600mg/kg/day of vitamin A. Vitamin A was administered orally using a gavage attached to a syringe.

Method of Analysis

Physical observation of the limbs of the pups was carried out immediately after their delivery with the aid of a hand lens. Physical examination was also employed to search for other observable anomaly in the different groups. This was repeated on days 7 and 16, after which the pups were sacrificed. From each group we had more than 10 pups from the mothers. Five pups were sacrificed from each group and their cerebellum harvested for histological studies with H&E. Another set of five (5) pups from each group was also sacrificed and their brain harvested and homogenized for anti-oxidant tests. Phosphate buffer solution (PBS) was prepared and poured into the specimen bottle containing each brain sample (the volume is four times the volume of the weight of the brain specimen). Then the sample was homogenized using a homogenizing machine and cold centrifuged at 12,000 rpm (rounds per minute) and the supernatant was collected. The rest of the assay was done using the supernatants. Antioxidant parameters investigated includes Supraoxide dismutase (SOD), Hydrogen peroxide (H_2O_2), Gluthathione peroxidase (GPx), Gluthathione (GSH) and Lipid Peroxidase (LPO). This was carried out at the laboratory of the Department of Biochemistry, University of Ibadan.The variations between the different groups for the anti-oxidant assay were compared using the Student's T-test.

RESULTS

Results of histological studies

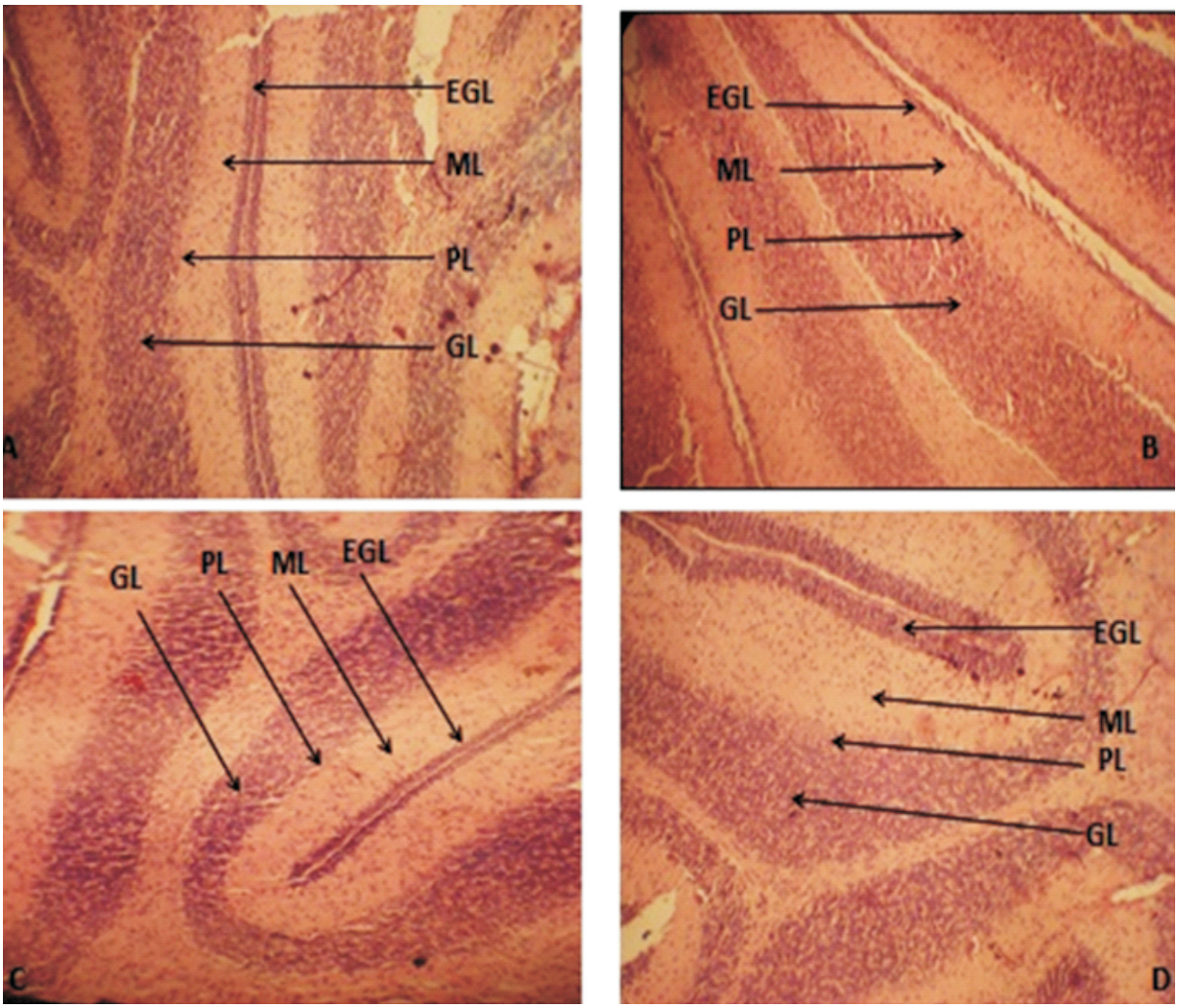

Plate 1: Representative photomicrograph of cerebellum of rats. Group A is the control group that took rat chow and water only. Group B - 400mg/kg of Vitamin A. Group C-500mg/kg of Vitamin A and Group D- 600mg/kg of Vitamin A. The picture micrographs show the four basic histological layers of the fetal cerebellum viz. external granular layer (EGL), molecular layer (ML), purkinje layer (PL) and granular layer (GL). There were no observable differences in the appearance of the layers in the experimental groups when compared to the control. H&E x240.

Anti-Oxidant Results

Table 1: Effects of maternal vitamin A consumption on markers of oxidative stress in Wistar rats.

GROUP	PROTEIN	GSH	GPx	LPO	H_2O_2	SOD
A	7.82±0.31	11.8±1.30	29.97±1.33	1.17±0.34	24.73±1.19	1.2±0.28
B	5.56±1.11*	19.33±0.58*	35.06±2.17*	0.81±0.69	25.88±1.31	1.07±0.19
C	6.62±0.86*	13±0.82	38.78±1.30*	1.98±0.29*	23.25±2.90	1.07±0.33
D	6.32±1.07*	17±3.83*	33.91±2.93*	0.64±0.25*	32.5±4.33*	0.4±0.00*

Values are expressed as mean ± standard deviation of 5 animals in each group.
* Statistical significance at $p < 0.05$.

Results of the protein analysis showed significant decrease in the protein levels of brain of the experimental groups B, C and D compared to those of the control A at $P<0.05$. The decline in SOD activities in groups A and B that received 400mg/kg and 500mg/kg of vitamin A respectively was not significant. However, the table shows a significant reduction in the SOD activity of group D that received 500mg/kg of Vitamin E in addition to 600mg/kg of Vitamin A.

Here also, no statistically significant difference was observed between the control group and groups B and C. However, there was a statistically significant increase in levels of H_2O_2 in group D that received 600mg/kg as well as 500mg/kg of Vitamin E. This increase in H_2O_2 level does not show dose dependence.There were no significant differences in the level of LPO between the control group A and group B that received 400mg/kg of Vitamin A. However, there was significant increase in LPO levels for groups C (500mg/kg) and D (600mg/kg of Vitamin A and 500mg/kg of Vitamin E) compared to the control. This increase does not show a dose-dependent pattern. There were significant increases in the levels of GPx in all experimental groups B, C and D as compared to the control. However, the increase does not show a dose-dependent pattern. There was a significant increase in the GSH level in groups B and D compared to the control but there was no significance between group C and the control. However, there was significant increase in GSH levels in group D compared to the control.

Table 2: Effects of maternal vitamin A consumption on the gross morphological features of the pups of Wistar rats.

	Group A Days			Group B Days			Group C Days			Group D Days		
	1	7	16	1	7	16	1	7	16	1	7	16
Upper Limb Defects												
Polydactyly	-	-	-	-	-	-	-	-	-	-	-	-
- Pre-axial	-	-	-	-	-	-	-	-	-	-	-	-
- Post-axial	-	-	-	-	-	-	-	-	-	-	-	-
Syndactyly	-	-	-	-	-	-	-	-	-	-	-	-
Amelia	-	-	-	-	-	-	-	-	-	-	-	-
Meromelia	-	-	-	-	-	-	-	-	-	-	-	-
Synostosis	-	-	-	-	-	-	-	-	-	-	-	-
Hemimelia	-	-	-	-	-	-	-	-	-	-	-	-
Ecrodactyly	-	-	-	-	-	-	-	-	-	-	-	-
Adactyly	-	-	-	-	-	-	-	-	-	-	-	-
Brachydactyly	-	-	-	-	-	-	-	-	-	-	-	-
Lower Limb Defects												
Polydactyly	-	-	-	-	-	-	-	-	-	-	-	-
- pre-axial	-	-	-	-	-	-	-	-	-	-	-	-
- post-axial	-	-	-	-	-	-	-	-	-	-	-	-
Syndactyly	-	-	-	-	-	-	-	-	-	-	-	-
Amelia	-	-	-	-	-	-	-	-	-	-	-	-
Meromelia	-	-	-	-	-	-	-	-	-	-	-	-
Synostosis	-	-	-	-	-	-	-	-	-	-	-	-
Hemimelia	-	-	-	-	-	-	-	-	-	-	-	-
Ecrodactyly	-	-	-	-	-	-	-	-	-	-	-	-
Adactyly	-	-	-	-	-	-	-	-	-	-	-	-
Brachydactyly	-	-	-	-	-	-	-	-	-	-	-	-
Craniofacial Defects												
Eye defects	-	-	-	-	-	-	-	-	-	-	-	-
Facial defects	-	-	-	-	-	-	-	-	-	-	-	-
Cranial defects	-	-	-	-	-	-	-	-	-	-	-	-
Exencephaly	-	-	-	-	-	-	-	-	-	-	-	-
Spina bifida	-	-	-	-	-	-	-	-	-	-	-	-
Abdominal wall Defects	-	-	-	-	-	-	-	-	-	-	-	-

Note: $\surd$ Indicates present, - Indicates absent

Gross morphological observations of the pups of all groups did not show observable anomalies as viewed by normal vision and hand lenses.

DISCUSSION

This study investigated if maternal vitamin A consumption will affect (and to what degree) the development of the cerebellum, limbs and gross morphological features of the pups. The results of this study showed that overdose of vitamin A can be toxic, leading to threatening health conditions like spontaneous abortion, general body weakness, inactivity, ulcerations and death. These were the observation as the rats experienced these signs in a dose dependent manner (that is, the effects increased as the dose administered increased).This is in agreement with the report of[10]. All the rats in the experimental groups (B, C and D) lost their pregnancies and their level of activity decreased by the day. This was manifested as the rats became smallish as though shrunk, weak and waif. Food as well as water consumption reduced drastically and the rats showed several degrees of ulceration in their mouth as well as body regions. This is in agreement with the report from[11] which showed that retinoid prescribed to treat skin disease used during pregnancy could cause spontaneous abortions and congenital malformations including limb defects. However, the control group remained healthy, and increased in size as gestational age increased. They also delivered their litters between 21 and 23 days of gestation. All the deaths recorded took place between the 12th and 14th day of administration. After stopping the administration, it took the surviving rats between 26 to 30 days to bring forth their litters. Based on this report, calculating from the normal 21 days gestation of rats, it took the rats between 5 to 9 days of discontinuation of the drug to reconceive.

Histological sections of the cerebellum revealed no significant differences in the fetal arrangement of the layers and cells of the cerebellar cortex. However, antioxidant studies confirmed that the pups in the experimental group were under oxidative stress. There was a significant increase in the level of glutathione peroxidase (GPx) and a significant decrease in the level of superoxide dismutase (SOD) when compared to the control. The reduction in SOD activity may be due to the inactivation by interaction with oxygen radicals. Reports have shown that decline in SOD activity could be caused by hydroxyl radicals and H_2O_2 .This is obvious from this report as there was significant increase in the level of hydrogen peroxide (H_2O_2) in groups B and C. The depression in SOD activity may result in cellular injury by superoxide radicals. Therefore when SOD activity reduces, levels of superoxide radicals rises and could result in cellular injury. To further establish the presence of oxidative stress, our result shows an increase in lipid peroxidation (LPO) in the experimental groups compared to the control. Previous works confirm that the higher the level of LPO, the stronger the oxidative stress. Our results show that the reduced glutathione (GSH) levels were significantly higher in experimental groups A and C.GSH in the mitochondria is the major defense available against hydrogen peroxide. Our report shows that the level of GSH was increased in the experimental groups. This is contrary to the findings of[8] which shows depletion in the level of GSH in oxidation stress. A lot of factors could be responsible for this disparity ranging from the stage of the oxidative stress and the fact that this research was carried out in pups and not adult rats. It appears from this report that the alterations seen in these pups were at the molecular level which could not be revealed by light microscopy. This is inferred, as the significant differences seen in the biochemical studies were not obvious under the light microscope.

The result of gross morphological examination done on all the litters of the experimental groups as well as the control to examine limb deformities, craniofacial and any other physically observable anomalies shows that there was neither any limb abnormality nor any observable craniofacial or other gross morphological anomalies seen. This is contrary to the results of Rutledge et al.,[1] that reported limb abnormalities and limb duplications in mice after receiving single doses of Vitamin A ; 68mg/kg, 53mg/kg and 41mg/kg on days 4.5,5 and 5.5 post-fertilization respectively. Our result is also contrary to this same report which shows that mice which received 21.5mg/kg and 15mg/kg on days 6 and 7 post-fertilization respectively had craniofacial abnormalities. The variations in these reports could be due to the fact that these were targeted administration that took effect on particular days of development in which the observed features were supposed to develop. Also, our work was carried out on pups of rats and not mice and the administered doses are different. It is obvious from the report of these studies that the mere fact that a new born does not show any physical anomalies at birth does not mean that all is well. Apart from phenotypic manifestations of abnormalities, there are possibilities of underlying metabolic as well as biochemical anomalies which may still manifest later. To this end, women of child bearing age must be very careful in taking medications and this should only be by the prescription of a qualified medical practitioner.

CONCLUSION

Vitamin A is very important for optimum body and developmental functions. High doses of vitamin A are very toxic and can lead to the manifestation of various kinds of toxicity symptoms, including death. Women of child bearing age who consume high doses of vitamin A are likely to lose the pregnancy and when they don't, the baby may come down with various kinds of abnormalities including limb abnormalities as well as central nervous system defects. However, to remove these effects, the best solution is to first discontinue the administration. Our report has shown that in rats recovery may be attained within 5 to 9 days. This work also shows that the damaging effect of over-dose of vitamin A increases as the days of consumption increases. No rat died from days 1 to 11 of receiving the over-dose of vitamin A (even up to the highest doses),

although other signs of toxicity were already present, including increased inactivity, loss of appetite for food and water and spontaneous abortion. This implies that probably none of the rats would have died if we stopped administration of vitamin A at day 10. Days 13 and 14 were the most critical and at day 14 we thought all the rats will die before the next morning. This further buttresses the fact that withdrawal is an essential first step in terminating the toxic effects of overdose of vitamin A. However, this does not in any way mean that high doses can be consumed for few days during pregnancy without any effect. Death may not occur, but damage may be done to the offspring. Previous works[1] has shown that consumption of doses as low as a single dose of 12.5mg/kg produced birth defects including exencephaly, eye defects as well as facial anomalies. Therefore women of child bearing age must not take vitamin A (or any other drug) in excess of the recommended dose.

RECOMMENDATION

1.) We did not investigate the tissues and different maternal parameters to ascertain the level of damage done to them and the rate of recovery recorded with time. This is a fresh ground for further research.

2.) We observed that biochemical studies revealed better details which were not appreciated with light microscopy. Therefore further studies can be done, utilizing other forms of light and electron microscopy.

REFERENCES

1. Rutledge JC, Shourbaji AG, Hughes LA, Polifka JE, Cruz YP, Bishop B. Limb and lower-body duplications induced by retinoic acid in mice. *Proc. Natl. Acad. Sci. USA*.1994; 91:5436-5440.
2. *Vargession N. Thalidomide-induced limb defects: resolving a 50-year old puzzle. Bioassay.2009; 31:1327-1336.*
3. Institute of Medicine. Food and Nutrition Board. Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc Washington, DC: National Academy Press; 2001.
4. U.S. Department of Agriculture, Agricultural Research Service. USDA National Nutrient Database for Standard Reference, Release 24. Nutrient Data Laboratory Home Page; 2011
5. Tanumihardjo SA. Vitamin A: biomarkers of nutrition for development. *Am. J Clin.Nutr.* 2011; 94:658S-65S.
6. Otten JJ, Hellwig JP, Meyers LD. Dietary Reference Intakes: The Essential Guide to Nutrient Requirements Washington, DC: The National Academies Press; 2006.
7. Mendoza EJ, Foundas AL. Clinical Neuroanatomy. A Neurobehavioral approach. New York. Springer; 2008.
8. Devipriya N, Srinivasan M, Sudheer AR, Menon VP. Effect of ellargic acid, a natural polyphenol on alcohol-induced prooxidant and antioxidant imbalance: a drug dependent study. SMJ.2007; 48 (4):311-318.
9. Institute of Medicine. Food and Nutrition Board.Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids Washington, DC: National Academy Press; 2000.
10. *Holder N, Hill J. Retinoic acid modifies development of the midbrain-hindbrain border and affects cranial ganglion formation in Zebrafish embryos. Development 1991; 113, 1159-1170.*
11. *Ross D. Recommendations for vitamin A supplementation. J. Nutr. Suppl. 2002; 132:2902S-2906S.*