

Study the Effect of *Ginkgo biloba* Leaf Extract on Induce Experimental Brain Poisoning in Rabbits

Zinah I. Khaleel

Department of Histology, College of Applied Sciences, University of Samarra, Iraq

Mustafa H. Ali

Ihab A. Salih

College of Applied Sciences, University of Samarra, Iraq

zena28230@gmail.com

Abstract

The current study was designed to investigate the therapeutic role of the water extract of leaves of ginkgo biloba plant against the acute poisoning of the nervous system caused by exposure to mercury. Experimental animals were divided into four groups. The first group was treated as a control group treated with physiological saline solution. The second group was given mercury chloride at 0.12 mg / kg . bw for seven days. The third group was given mercury chloride orally at 0.12 mg / Kg for five days and then injected under the peritoneal membrane with water extract for leaves of ginkgo plant for 25 days at a concentration of 250 mg / kg / day, while The fourth group gave mercury chloride for five days and then injected with the water extract of the leaves of the ginkgo plant at a concentration of 500 mg / kg / day under the peritoneal membrane. All groups were treated daily according to prescribed doses and 24 hours after the last given dose, the animals were explained and the study criteria were met. Mercury treatment caused obvious tissue changes in brain tissue. The treatment with water extract of leaves of the ginkgo plant led to improvement in brain cells and tissues.

Key word: ginkgo biloba, nervous system, mercury chloride, brain cells .

الخلاصة

الدراسة الحالية صُمِّمَت للتحري عن الدور العلاجي للمستخلص المائي لاوراق نبات الجنكة بايلوبا *Ginkgo biloba* ضد التسمم الحاد للجهاز العصبي المتسبب عن التعرض للزئبق . قسمت حيوانات التجربة الى اربعة مجاميع ، المجموعة الاولى عدت كمجموعة سيطرة عوملت بالمحلول الملحي الفسيولوجي ، والمجموعة الثانية اعطيت كلوريد الزئبق فمويًا بجرعة 0,12 ملغم/ كغم من وزن الجسم لمدة سبعة ايام ، والمجموعه الثالثة اعطيت كلوريد الزئبق فمويًا عند الجرعة 0,12 ملغم/ كغم لمدة خمسة ايام ثم حقنت تحت الغشا البريتوني بالمستخلص المائي لاوراق نبات الجنكة ولمدة 25 يوم بتركيز 250 ملغم/كغم / يوم ، فيما اعطيت المجموعة الرابعه كلوريد الزئبق لمدة خمسة ايام ثم حقنت بالمستخلص المائي لاوراق نبات الجنكة عند التركيز 500 ملغم /كغم /يوم تحت الغشا البريتوني . عوملت جميع المجاميع يومياً حسب الجرع المقررة وبعد 24 ساعة على اخر جرعة معطاة شرحت الحيوانات واجريت عليها معايير الدراسة. سببت المعاملة بالزئبق حدوث تغيرات نسجية واضحة في نسيج الدماغ . وأدت المعاملة بالمستخلص المائي لاوراق نبات الجنكة الى التحسن في خلايا ونسيج الدماغ.

الكلمات المفتاحية: الجنكة بيلوبا, الجهاز العصبي, كلوريد الزئبق, خلايا الدماغ .

Introduction

Ginkgo biloba is a type of plant that belongs to the Genco genus. It is the only remaining species of the genus .

The plants attributed to this species are spread throughout the Middle Ages, so this is a type of living fossils .

Ginkgo is also known as the coriander tree of the well, a unique type of tree, the only surviving species of the genocovita, a tree of seedless nests, although it is still present in two small areas in east China's Zhejiang Province.

The Ginkgo biloba tree has several characteristics. Although it is a local tree of Korea, China and Japan, it can be found in gardens and along the sidewalks of cities around the world. It may reach 40 meters high and can live for 1,000 years .

Ginkgo has spread in Europe, and the National Institute of Aging in the United States is funding a clinical trial to assess the effectiveness of ginkgo in treating the symptoms of Alzheimer's disease (Paul *et al*, 2002) .

Uses

Is a herb whose leaves are usually used for ginkgo apoptosis, and have been used in many diseases especially memory disorders including Alzheimer's disease (Brookmeyer *et al*,1998).

It is also used in patients with cerebral hemorrhage, especially in the elderly, such as impaired memory, headache, tinnitus, dizziness, difficulty of concentration, mood disorders and hearing problems. It is also used in patients with peripheral hemorrhage, such as leg pain during walking (intermittent claudication) Raynaud's syndrome, and eye problems associated with diabetes (Paul *et al*, 2002) .

Effects of ginkgo in the brain

In circulation circulatory

- 1- Induces expansion of blood vessels, increasing blood flow to the brain and lowering blood pressure (and may reduce the risk of stroke),(Pappu *et al*, 2008) .
- 2-Reduces cholesterol levels in the blood (excessive cholesterol is associated with increased risk of Alzheimer's disease)
- 3-inhibits platelet aggregation and clot formation, which in turn can reduce the risk of obstructive stroke (caused by clot blocking a blood vessel in the brain), but increases the chance of hemorrhagic stroke (caused by hemorrhagic brain hemorrhage), (Giangrande, 2003) .

Antioxidant

- 1-inhibiting free radicals, which are highly reactive oxygen molecules that may damage neurons and cause brain changes associated with aging .
- 2-The effects of cerebral ischemia reduce the production of toxic free radicals following ischemia (Lewis, 2008) .

In the consumption of glucose

The uptake of glucose (the main fuel of the body) increases in the cortex (front, frontal and cerebral cortex), the two important brain spaces for processing sensory information and for planning complex actions .

glucose absorption in the nucleus accumbens, and in the cerebellum (the two brain regions concerned with the feeling of pleasure for the recombinant nucleus and the movement of the cerebellum) are increased (Wenzel *et al*. 2015)

In neurotransmitter systems

- 1- Neurons in the front brain help absorb choline from the blood. Choline is a component of acetylcholine, which in turn is a brain chemical that transmits signals between certain neurons (Zeisel, Steven, 2012) .
- 2-Slow down the depletion of nerve receptors that direct serotonin response, which is a neurotransmitter that reduces stress and anxiety (Fuller, 1990) .
- 3-The release of gamma-amino butyric acid (GABA), another neurotransmitter that can relieve anxiety, has been enhanced (Watanabe *et al*, 2002) .
- 4-The production of norepinephrine, which is also a neurotransmitter, has increased and the activation of norepinephrine by certain antidepressants has been shown to reduce the symptoms of depression (Aronson, 2000) .

Materials and methods of work

The study was conducted on the Swiss white rabbit and included 16 rabbits obtained from the Medical Center for Control and Scientific Research / Baghdad, and the rabbits were healthy and transferred to the workplace in a room with laboratory conditions uniform in terms of ventilation and temperature, which was up to (25) m and session (6-10) months of average weight (1200-1800 g). They were healthy and placed in laboratory cages dedicated to raising plastic rabbits with metal lattices, and sprinkled with sawdust with care. Clean the cages and disinfect them twice a week .

During the experiment, the animals were placed in uniform laboratory conditions in terms of ventilation and temperature. The special feeding of rabbits in addition to water was provided continuously throughout the experiment period in special containers for not being contaminated with sawdust. The first group, the control group consists of 4 rabbits treated with physiological saline solution Until the dissection .

Group II Infected group: consists of 4 rabbits injected with toxic mercury chloride at a concentration of 0.12 mg/ kg for seven days .

Group III Therapeutic group A: This group consists of 4 rabbits, This group was restricted for 30 days by injecting it with a 250 mg ginkgo extract and they were dissected immediately after the end of the dosage period .

Group IV Therapeutic group B: This group includes 4 rabbits. This group was also removed for 30 days by injecting it with a 500mg ginkgo extract and explained immediately after the end of the dosage period .

The substance that causes poisoning, mercury chloride

The mercury chloride material was obtained from the Applied Chemistry Store with a molecular weight of 472.09 and was dissolved in the solution to prepare a natural solution and was injected orally for a period of seven days with a concentration of 0.12 mg / kg .

Ginkgo Extraction

The leaves of the ginkgo plant were obtained from the herb center in Samarra, 500g of which was then extracted by ethanol alcohol at a concentration of 70% through the use of the rotary evaporator. The extract of the ginkgo extract was obtained completely. The ginkgo biloba extract was used for two groups of rabbits (group3: 250mg and group4: 500mg) in the form of intraperitoneal injection for one month (1 ml) for each rabbit (Abd-Allh, 2014) .

Preparation of tissue sections

The tissue was studied by using (Humason,1978) methods. The samples were examined to determine the effect of the doses on the rabbit's brain .

After dissecting the animals and placing brain in the Formalin 10% solution, the samples to be studied were converted to ethyl alcohol at 70% concentration. The following steps were taken :

a- Dehydration b- Clearing c- Infiltraton d- Embedding e- Sectioning f- Staining g-Mounting

Results

Histological changes in the effect of mercury chloride

In the brain

The results of the study showed that there were many disease groups, namely: vasculitis, vasoconstriction, lymphocytic infiltration, monoprosis, macrophage cells, mitral regions and necrotic regions, image (2) .

In the cerebellum

Through the results of the histological examination, the sections showed many pathological lesions that cause deformities of the nervous system, such as the exploding abnormalities of the neurons and glia, and the small size of the glial cells, as well as the increase of the glial cells, image (3) .

Histological changes by the effect of ginkgo extract

In the brain

The study showed the existence of tissue lesions in the brain. Where the brain showed microscopic edema and slight congestion. Histological examination showed shrinking neurons with focal areas of hemorrhage, in addition to congestion of blood vessels in the brain and meninges with glial cells, images (4,6) .

In the cerebellum

The cerebellum showed edema with degenerative changes and partial dissolution of the bergenic cells, images (5,7) . In addition to spasticity compared with sections exposed to exposure to mercury chloride .

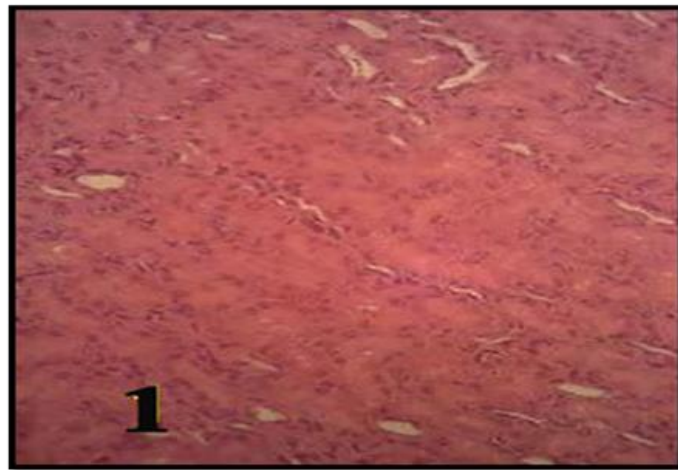


Figure (1) section of the brain of rabbit control group , H & E, 400 X.

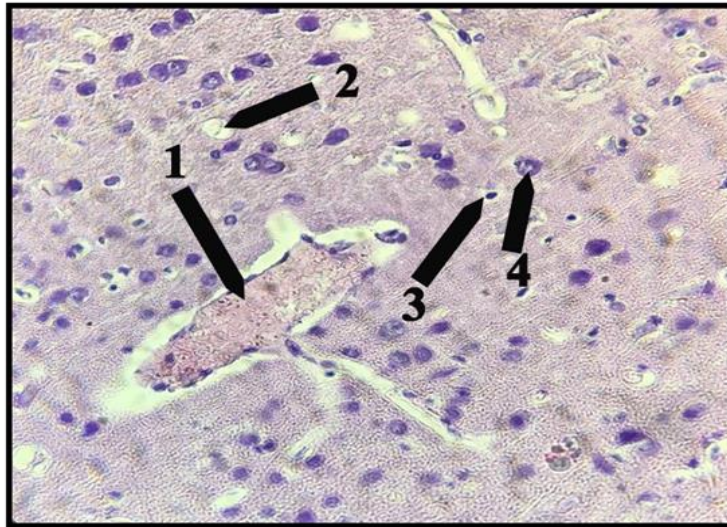


Figure (2) the brains of rabbits at a dose of mercury chloride (1) congestion Con, (2) overgrown nerve cells, (3) microglial cells decomposed GC, (4) different shapes and sizes NC nerve cells, H & E, 400 X.

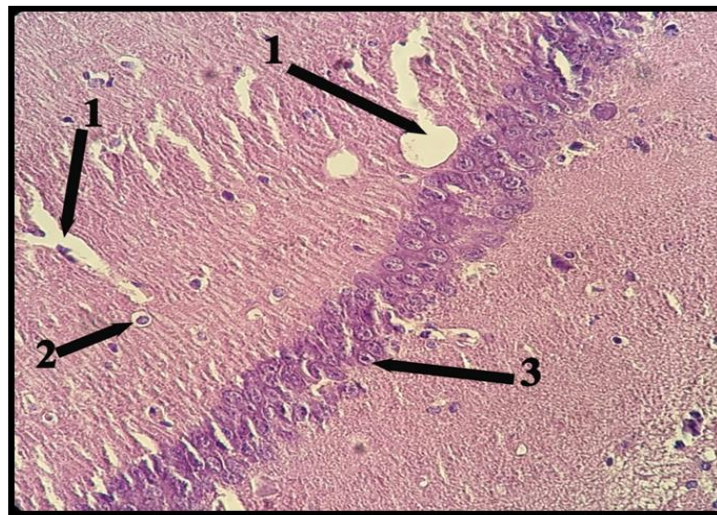


Figure (3) cerebellum dose of mercury chloride rabbit (1) large gaps (2) cells Silaya decomposed (3) decomposing nerve cells, H & E, 400x .

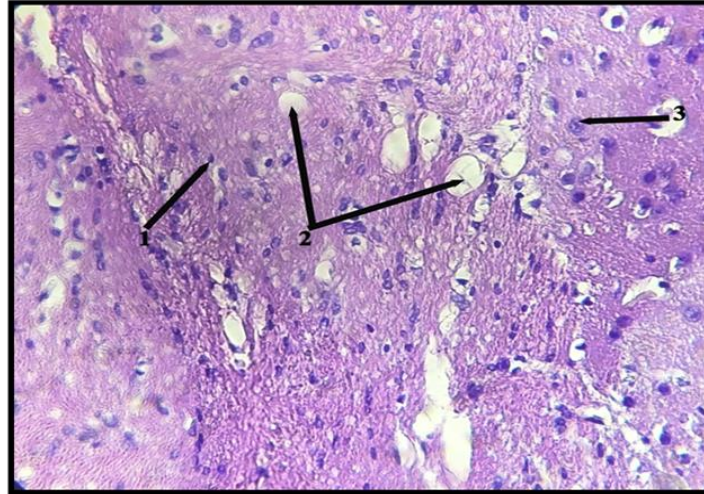


Figure (4) ginkgo extract the brains of rabbits at a dose of extract of Ginkgo 250 mg / kg (1) microglial cells, (2) gaps, (3) normal nerve cells BC, H & E, 400 X .

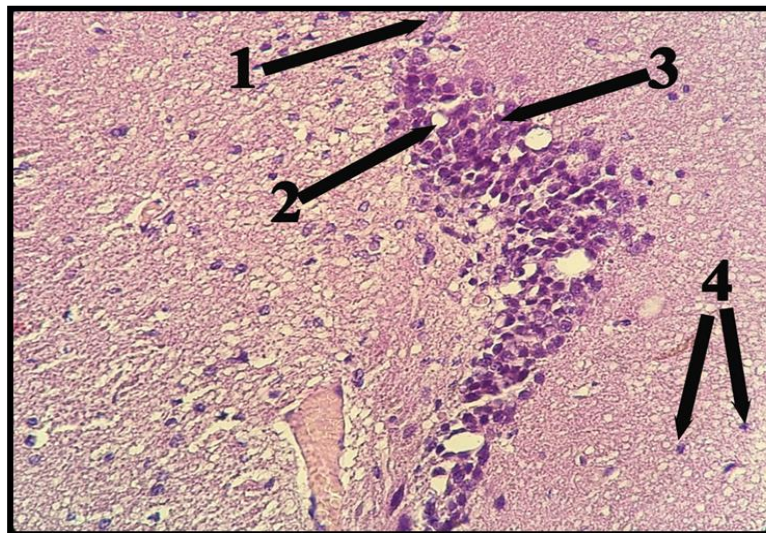


Figure (5) ginkgo extract at a dose of 250 rabbit cerebellum mg / kg (1) clear nodular cells NN, (2) gaps V, (3) Brkenji cells, (4) natural Silaya cells, H & E, 400 X .

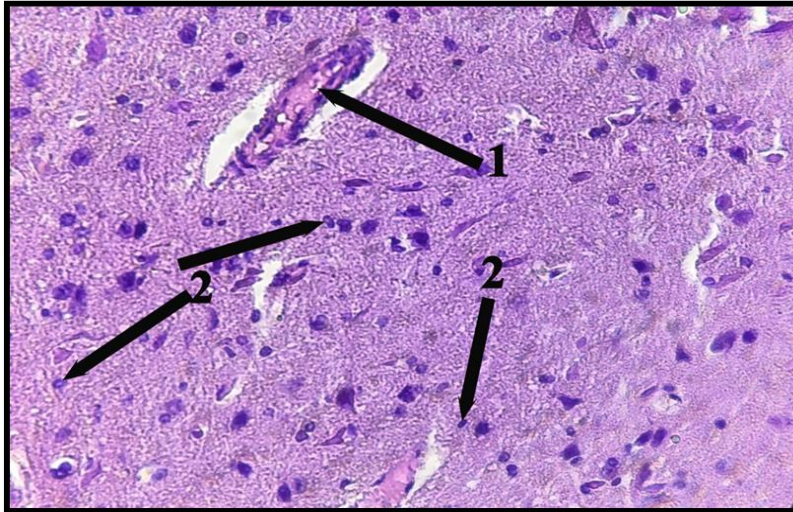


Figure (6) ginkgo extract at a dose of 500 brains of rabbits mg / kg (1) congestion, (2) normal nerve cells, H & E, 400 X .

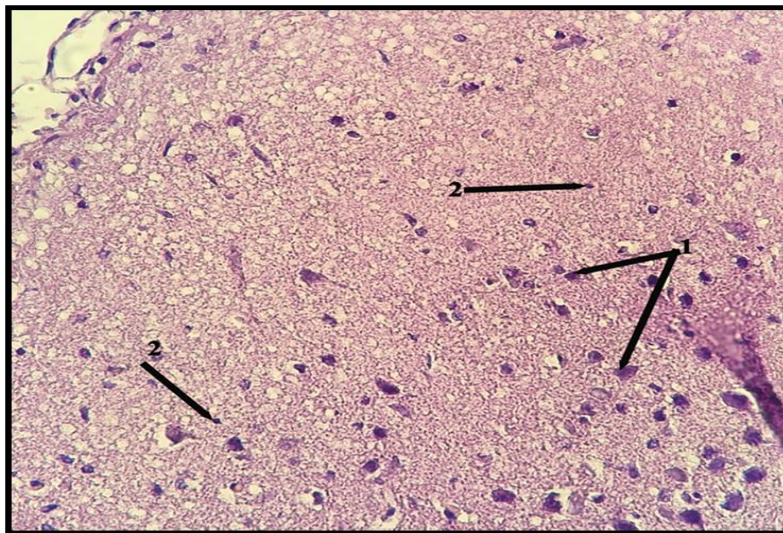


Figure (7) ginkgo extract at a dose of 500 rabbit cerebellum mg / kg (1) normal nerve cells, (2) Silaya clear cells, H & E, 400 X .

Discussion

The effect of mercury chloride on the histological structure of the nervous system of rabbits

Effect on the brain

Spread of the cleft in the brain tissue in different sizes and shapes. This is due to the pooling of water inside the cells. This pool of water was explained by the researchers (Robbins & Angell. 1976) who indicated that the hippocampal eruption is one of the primary signs of response in the water .

The blood congestion was observed in the blood congestion spread in the brain tissue of all concentrations and this is due to blockage in the blood vessels equipped for the part that has been congested has been observed damage to blood vessels is one of the most common damage in the current study and this is consistent with what he said (McEvoy, 2002) that the main effect of the chloride of mercury is associated with blood poisoning .

The results of the microscopic examination showed a change in the shape and size of nerve cells, as well as focal distillation and degeneration of the nuclei caused by mercury chloride. This was confirmed by the researcher (Wallace, 1996) while studying the rats where he observed the effect of mercury causing degeneration and necrosis in different regions of the nervous tissue in the brain Contrary to what is in the control group in terms of clarity and regularity of the cells and differentiation. As a result of exposure to mercury chloride, it was observed that neurons and glial cells were decomposed and the phenomenon of proliferation of cells and the collection of single-nuclei inflammatory cells. This increase is due to the reference to (Nelson, *et al.*2002), noting that glial cells contribute to many of the biological processes in the central nervous system, It is believed that the increase in the number of glial cells is due to increased cases of disease in the nervous system as well as the glial cells are more susceptible to damage and programmed death than Large Macrophage .

Its impact on the cerebellum

The histological examination showed several pathogenic lesions that cause neurological abnormalities. These abnormalities of neuronal and glial cells and the small size of glial cells also increase the glial cells diffused in the tissue. This is in line with the findings of (EI-Banhawy, 1993) that these lesions cause malfunctioning of the nervous system

And congestion is widespread in the layer of granular and molecular as well as between the cells of Berknji and this is due to blockage in the blood vessels and congestion in that region and show decomposed Berknji cells are unclear and these results consistent with the findings in the study of (Bhardwaj, *et al.* 2012) chemical and food toxicology and observed changes in cells in mice .

He said (Ajibade, *et al.* 2008) cells have many defects in the treatment of chemicals, including decomposition and small in size and sometimes are swollen and also are not clear and these cells have the main role in the cerebellum and malformations that lead to dysfunction in the cerebellum. Basket cells have also been damaged as a result of mercuric chloride. It is a heavy element that has a strong effect on the cells, causing damage, damage and degradation. This is in line with what the researcher observed (Hamori, 1969) when he studied the development of partially granular interlocking organization in cerebral cortex and cerebrum in mice .

Effect of 250 mg / kg dose and 500 mg/kg of ginkgo extract on the nervous system of rabbits

In the brain

Reduce blood circulation to the brain creates a chain reaction that energy production disruption, and ultimately leads to cell death. In his study on the effect of the ginkgo extract on the brain's blood perfusion, (Mohammed; 2016) he confirmed that the various compounds in the ginkgo can play a therapeutic role for the brain through several

mechanisms: vascular expansion of the arteries, capillaries, veins (increased blood flow) and oxygen balance .

In the cerebellum

Focal encephalitis were caused by the necrosis of neurons, glial cells and the pressure produced by the osmotic fluid (Lisk & Stoews, 1992). Summarize that exposure to mercury ultimately causes permanent damage. Efforts have been made on the use of food ingredients as therapeutic agents capable of controlling or minimizing the effects of many toxic compounds and ginkgo has been one of these factors (Teyssier, *et al.*, 1999) and (Bahia *et al.*, 2008).

The current study showed that ginkgo was responsible for minimizing or neutralizing the adverse effects of Hgcl₂ in rabbits in pathological examination.

Ginkgo flavonoids had an effect in removing the free radicals generated by the effect of mercury chloride. This is confirmed (Bahia, *et al.*, 2010) showed that flavinoids had a direct effect on glial cells by stimulating astrocytes and microglia. Furthermore flavonoids are generally thought to have antioxidants and effects to clear free radicals (Spencer *et al.*, 2003) .

That the effectiveness of flavonoids Flavonoids is by possessing the effectiveness of antioxidants Antioxidant as it works to remove the free radicals generated and directed the cell to enter the stage of programmed death

Ginkgo can remove the toxic effect of mercury and other toxic metals quickly from the central nervous system, and may be the only effective agent in the mobilization of mercury stored in space within cells (mitochondrial attachment, propolis, grease ... etc) and in the nuclear cell Mercury) Ginkgo is especially useful for removing mercury from the brain because detoxification of the brain is one of the most difficult to achieve, where mercury is excreted either by stool and urine. This makes ginkgo the first known substance to remove the toxic effect of mercury from the central nervous system (Mercola, & Dietrich Klinghardt, 2001) .

References

- Abd-Allh ,Shaima Obead., (2014). Precautionary Effect of Ginkgo biloba Against Mercury-Induced Acute Nephro-Hepatotoxicity in male Rats, Journal of Babylon University/Pure and Applied Sciences/ No.(9)/ Vol.(22) .
- Ajibade AJ; Adenowo TK;Catox-Martins ,EA. And Ekpo OE (2008). Effect of quinine administration on purkinje cells in cerebelar cortex of adult Wistar rats. WJ. Med., 27: 37-39.
- Anticonvulsants in Children with Epilepsy .Dec., 15(6),378-93.
- Aronson Jk (Feb 2000). ""Where Name And Image Meet"--The Argument For "Adrenaline"". British Medical Journal. 320 (7233): 506–9.
- Bahia, PK.; Rattray, M. and Williams, RJ. (2008). Dietary flavonoid (-)epicatechin stimulates phosphatidylinositol 3-kinase-dependent anti-oxidant response element activity and up-regulates glutathione in cortical astrocytes. J. Neurochem., 106:2194–2204 .
- Bhardwaj; Mk Srivastava; K Upasana And Lp Srivastava.(2012). Food And Chemical Toxicology .48, 1185-1190.

- Brookmeyer R., Gray S., Kawas C.,(1998) . Projections of Alzheimer's Disease in the United States and the Public Health Impact of Delaying Disease Onset, *American Journal of Public Health* .
- El-Banhawy.M; Saker. S; Sanda. S.;El-Elaimy, L. and Mahran. H. (1993).Histological studies on the effect of anticoagulant rodenticide “Brodifacoum” on the Ileum of rat.J.Egypt. Soc . Toxicol. Toxicol. 10,71-82.
- Fuller, R.W., (1990). Serotonin receptors & neuroendocrine responses, *Neruropsychopharmacology*, 3, 495-502.
- Garlic and selenium enriched garlic. *Nutr. Cancer*, 17:279-286 .
- Giangrande PL., (2003). "Six characters in search of an author: the history of the nomenclature of coagulation factors". *Br. J. Haematol.* 121 (5): 703–12.
- Hamori. J.(1969). Development of synaptic organization in the partially agranular and in the transneuronally atrophied cerebellar cortex. In R.Llinas (Ed.) .*Neurobiology of Cerebellar Evolution and Development* American Medical Association ,Chicago .pp,845-858.
- Humason,G.L.(1978).*Animal tissue techniques* , W.H. freeman Company,San Francisco.
- Jader, N.; Joice, S.; Costa, SL. and Gomes, FC. (2010). Flavonoids and Astrocytes Crosstalking: Implications for Brain Development and Pathology. *Neurochem. Res.*, 35:955–966 .
- Lewis. S.L (2008). *Medical-Surgical Nursing* (7th ed.). Vascular disorder. pp. 907–908.
- Lisk, IP. and Stoews, DJ. (1992). Mammary cancer prevention by regular
- McEvoy .G. (2002) . American hospital formulary service drug information. Bethesda. MD. American Society of Health-System Pharmacists. Inc. 960.
- Mercola, JD. and Dietrich Klinghardt, KD. (2001). Mercury Toxicity And
- Mohammed ; Mustafa T., (2016). Study the preventive effect of aqueous extract of Ginkgo biloba on cerebral damage induced by ischemia/ reperfusion in rat. *Fac Med Baghdad*, Vol.58, No.2 .
- Nelson, P.; Soma. L. and Lavi. E. (2002) Microglia in Diseases of the Central Nervous System .*Ann. Med* ..34, 491-500.
- Pappu S, Dardik A, Tagare H, Gusberg RJ (2008). "Beyond fusiform and saccular: a novel quantitative tortuosity index may help classify aneurysm shape and predict aneurysm rupture potential".
- Paul E. Gold, Larry Cahill and Gary L. Wenk, (2002). in *Psychological Science in the Public Interest*, May .
- Robbins, S. and Angell ,M. (1976) .*Basic Pathology* . 2nd ed. Philadilphia .W.B. saunders Company.
- Spencer, JP.; Rice-Evans, C. and Williams, RJ. (2003). Modulation of pro-survival Akt/PKB and ERK1/2 signalling cascades by quercetin and its in vivo metabolites underlie their action on neuronal viability. *J. Biol. Chem.*, 278:34783–34793 .
- Systemic Elimination Agents , *J of nutr. Envir. Med.*, 11:53-62.
- Teyssier, C.; Guenot, L.; Suschete, M. and Siess, MH. (1999). Metabolism of diallyl disulphide by human liver micsosomal cytochromes p-450 and flavin –containing monooxygenses . *Drug metab. Dispos.*, 27:835-841 .
- Wallace. S.J. (1996). *Acomparative Review of Adverse Effect of*

- Watanabe M, Maemura K, Kanbara K, Tamayama T, Hayasaki H (2002). "*GABA and GABA receptors in the central nervous system and other organs*". In Jeon KW. *Int. Rev. Cytol. International Review of Cytology*. 213. pp. 1–47.
- Wenzel JM ; Rauscher NA; Cheer JF; Oleson EB (2015) . "A role for phasic dopamine release within the nucleus accumbens in encoding aversion: a review of the neurochemical literature". *ACS Chem Neurosci*. 6 (1): 16–26 .
- Zeisel, Steven H. (2012). "A Brief History of Choline". *Annals of Nutrition and Metabolism*. 61 (3): 254–258 .