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Haematological studies on the effect of antioxidants vitamin C, E and garlic on tramadol-induced toxicity in wistar rats

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Abstract
The effect of antioxidants, vitamin C, E and garlic on tramadol-induced toxicity was studied in wistar rats. Thirty five (35) rats were used and designated into five (5) study groups with seven (7) rats each. There was a significant decrease in the body weight of the negative control group when compared with the positive control group. However, there was a significant increase in the body weight of the experimental groups administered antioxidants, vitamins C, E and garlic when compared to the negative control group at ($P<0.05$). The weight of the liver in the negative control group increases significantly when compared to the weight of the liver in the positive control group. This shows the metabolic changes in the liver as it metabolizes the drug which results in inflammation of the liver. The weights of the heart and kidney also showed similar variations in the graphs. The RBC's, Hb, PCV, MCV, MCH and MCHC levels were all decreased in the negative control group when compared to the positive control group. This is largely due to the effect of drug on erythropoiesis and nutritional deficiencies caused by lack of appetite. There was a significant increase in RBC's Hb, PCV, MCV, MCH and MCHC levels in the experimental groups administered antioxidants, vitamin C, E and garlic when compared with the negative control group at ($P<0.05$). The WBC's level was increased in the negative control group when compared to the positive control group. This is due to the activation of defense mechanism and immune system of the wistar rats. There was a significant increase in the WBC's Level In The Experimental Groups Administered Antioxidants, Vitamin C, E And Garlic When compared to the negative control group at ($P<0.05$).

Keywords: tramadol, haematological indices and antioxidant vitamins C, E and garlic

Introduction
Tramadol is a synthetic centrally acting analgesic drug with opioid and non-opioid properties. It is used parentally and orally for the treatment of moderate to severe pains due to its relatively lower risk of respiratory depression and generally better safety profile when compared with other opiates (Atici *et al*., 2005) [3]. It is rapidly absorbed after oral administration and reaches its peak blood level at 2-3 hours thereafter. But their active metabolites (0-desmethylTramadol) have a higher affinity for the mu-opioid receptors and exhibit twice the analgesic effect of the parent drug (Raffa *et al.*, 2012) [13]. It is often combined with acetaminophen as this known to improve the efficacy of Tramadol in relieving pains (Brayfield, 2013) [6].
Tramadol is commonly available as tablets, oral drops, solution for injection and suppository (Shadnia *et al*., 2008) [14], with the oral route being the most common route of administration. However, therapeutic dose of Tramadol is 50mg to 100mg (50mg oral, 50-100mg intramuscular (IM) and 100mg rectal; 1.5mg/kg/day in a 60kg patient) three to four times a day. Doses higher than 400mg/day are generally not necessary and toxic (Shadnia *et al*., 2008) [14]. Tramadol is considered to be a safe drug. Toxicity can happen accidentally. Patients with the previous history of addiction are at extreme danger for such toxicity and according to FDA warnings Tramadol administration should be performed cautiously in these patients (Afshari *et al*., 2011) [1]. Toxicity of Tramadol can be predicted by P450 polymorphism (Johansson, 2011). Recently, young adult addicts "typically substituted Tramadol for heroin". Repeated Tramadol administration in such patients might lead to accumulation of toxic metabolites in their blood and thus increase its potential for toxicity (Vazzana *et al*., 2015).
Garlic (*Allium sativum)* has long been a common seasoning worldwide. It is a plant in the Allium (onion) family with a history of several thousand years of human consumption (Block, 2010) [4]. It was known to ancient Egyptians and has been used both as a food flavoring agent and as a traditional medicine (Block, 2010) [4]. Scientists now know that most of the health benefits of *Allium sativum* are caused by sulphur compounds formed when a garlic clove is chopped, crushed (Borlinghaus *et al*., 2014) [5]. Perhaps the most famous of those compounds is known as allicin. However, allicin is an unstable compound that is only briefly present in a freshly crushed garlic sample (Borlinghaus *et al, 2014)* [5]. Other compounds that may play a role in garlic's health benefits include diallyldisulfide and s-allyl cysteine (Zarezadch *et al*., 2017) [16]. The sulphur compounds from garlic enter the body from the digestive tract and diffuse to parts of the body, where it exerts its antioxidant role.
Many studies have shown that the health benefits of garlic including – Human studies have found garlic supplements to

have significant impact on reducing blood pressure in people with high blood pressure (Dhawan and Jain, 2005) [8]. For those with high cholesterol, garlic supplements appear to reduce total and LDL cholesterol by about 10-15% (Silagy and Veil, 1994) [15]. Garlic also contains antioxidants that support the body's protective mechanisms against oxidative damage (Amagase *et al*., 2001) [2]. High doses of garlic supplements have been shown to increase antioxidant enzymes in humans, as well as significantly reduce oxidative stress in those with high blood pressure (Amagase *et al*., 2001) [2]. Studies have shown that eating garlic may help detoxify heavy metals in the body – A four-week study in employees of a garlic car battery plant that was exposed to lead reported that garlic reduced lead levels in the blood by 19%. It also reduced many clinical signs of toxicity, including headaches and blood pressure (Kianosouch *et al.*, 2012) [11].

Vitamins C and E are powerful antioxidants and their role in the testament of various toxicity conditions in the body cannot be overemphasized because of their ability to scavenge free radicals that promotes toxicity. However, the nature of interaction between these vitamins in vivo is still controversial (Burton *et al,* 1990) [7]. It has been recently suggested that the administration of Ascorbic acid in combination with Vitamin E can exert synergistic antinociceptive effects (Lu *et al.,* 2011) [12] and it was reported that vitamin C exerted its antinociceptive effects primarily as a result of its antioxidant properties. Several investigations have been carried out on physiological benefits of ascorbic acid and its applications as therapeutic agent in various disease states. Furthermore, the beneficial effects of ascorbic acid as a cost-effective, convenient and safe supplement for detoxifying narcotic drugs had been identified for many years (Free and Sanders, 1979) [9]. Vitamin E is a fat-soluble vitamin that acts as an antioxidant. The crucial function played by vitamin E that makes it a vitamin is poorly understood, but may involve antioxidant functions in cell membranes (Galli *et al*., 2017) [10].

Materials and methods

Experimental Animals

Thirty five (35) *wistar* rats, weighing between 120g to 140g were purchased from the animal house, College of Medical Sciences, University of Calabar, Calabar, Cross River State, Nigeria. They were housed in wooden cages with sawdust beddings and placed in a well-ventilated and aerated room at a laboratory temperature of 22 ± 3^0c, maintained for two weeks for acclimatization period and fed standard laboratory rat feed and water *ad-libitum*. All aspects of animal care treatment were carried out according to the local guidelines of the ethical committee of Faculty of Basic Medical Sciences, College of Medical Sciences, University of Calabar, Calabar.

Chemicals and equipments: Diethyl ether, hydrochloric acid and methylated spirit, Beakers, saw dust, wooden cages, electronic weighing scale, latex gloves, hand gloves, plastic, EDTA tubes, syringes and needles, tissue, cotton wool, sample containers, dissecting set/kit, permanent markers, feed, distilled water, tap water, sysmsex automated haematological analyzer.

Source of the Drugs: Tramadol, vitamins E and E, and Garlic (syrup) were purchased under the brand names Tramadol, vitamin E, Embite C syrup and Garlic respectively from Benz pharmacy, Calabar, Cross River State.

Preparation of Dugs

Tramadol – a total of 1025mg of Tramadol was measured out (based on weight and number of rats) to be used for the preparation of the stock solution of Tramadolto last for 28 days of administration. The 1025mg of Tramadol was dissolved in 100ml of distilled water to prepare the required stock solution. It is put in an air tight container and stored in a temperature of 4^0C to be administered to the rats at 0.2ml of stock solution per rat. Garlic was purchased as a syrup and 130mg was weighed out and diluted with 40ml of distilled water to prepare the stock solution which was also stored at appropriate temperature and administered to the rats at 0.2ml per rat. Vitamin C–A total of 419.2mg was weighed out and diluted with 40ml of distilled water to prepare a stock solution which was stored under a temperature of 4^0C and administered at 0.2ml of stock solution per rat. Vitamin E – A total of 419.2mg was weighed out and diluted with 40ml of distilled water to prepare a stock solution which was stored under a temperature of 4^0C and administered at 0.2ml of stock solution per rat.

Experimental design

After two weeks of acclimatization, the 35 *wistar* rats were divided into 5 study groups of 7 rats each on the basis of their weight before the commencement of experimental treatment. The groupings andtreatment given to the rats were as follows: Group 1 (Control group): Designated NC. The rats in this group were fed with a standard laboratory diet (feed) and water for twenty eight (28) days. Group 2 (Negative control group): Designated TM.The rats in this group were administered 0.2ml of dissolved Tramadol (at a dose of 1.9/130g body weight of rat)orally for 28 days, including standard laboratory feed and water daily for this duration. Group 3 (Experimental group): Designated TmVC. The rats in this group were given 0.2ml of Tramadol and 0.2ml of vitamin C orally for 28 days. Feed and water were also given daily for this duration. Group 4 (Experimental group): Designated Tm VE. The rats in this group were administered 0.2ml of Tramadol and 0.2ml of vitamin E orally for 28 days. Feed and water were also given daily for this duration. Group 5 (Experimental group): Designated Tm G. The rats in this group were administered 0.2ml of Tramadol and 0.2ml of diluted garlic syrup (at a dose of 0.6mg/130g body weight of rat) orally for 28 days. Feed and water were also given daily for this duration.

At the end of the administration and experimental period, rats were anesthetized with light diethyl ether and sacrificed. All approved conditions used for animal housing and handling were considered. The experimental protocol used followed the regulations for administration and painless sacrifice of the experimental animals.

At the end of the experimental period, the rats in each study group were fasted overnight and sacrificed under anesthesia by cervical dislocation. After sacrificing the rats, 2-4ml of blood was collected from the rats by cardiac puncture using 2ml syringes. The collected blood samples were placed in sterile EDTA tubes for onward transportation to the laboratory for haematological analysis.

Collection of Organ samples
After sacrificing the rats, the liver, heart and kidney organ samples were carefully removed and placed in clean sample containers for weighing.

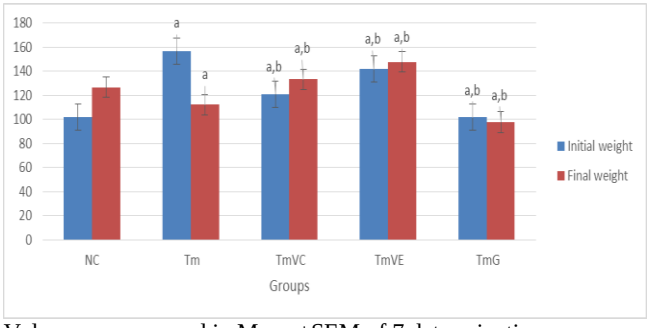
Measurement of organ Weight
The organ samples collected were measured by placing them in a beaker and using the electronic weighing balance. The weight of the beaker was subtracted to get the weight of the organs measured.

Measurement of body weight
The body weight of the rats used in this work was measured before the commencement of administration and treatment (initial weight) and before sacrifice (final weight) using the weighing scale.

Estimation of Haematological Parameters
Three (3) ml of blood sample collected by cardiac puncture were used to determine some haematological parameters including; Red Blood Cell Count (RBC), White Blood Cell Count (WBC), Packed Cell Volume (PCV), Mean Corpuscular Volume (MCV), Haemoglobin (Hb), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC) using automated cell counter, sysmsex automated haematological analyzer (KX-2IN, sysmex corporation Koba-Japan) (Halim *et al*., 2011).

Statistical Analysis
The data will be expressed as mean value ± SEM (Standard error of mean). All results will be the mean of 7 data samples and the statistical analysis will be carried out using students `t`-test.

Results

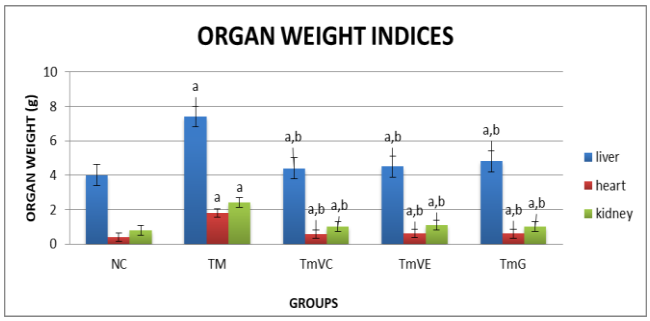


Values are expressed in Mean ±SEM of 7 determination

Fig 1: A graph showing the effect of Tramadol and antioxidants vitamin C, E and garlic interactions on the body weight.

NC: Group I, positive control, no Tramadol administered
TM: Group II, negative control, only Tramadol administered
TmVC: Group III, Tramadol and vitamin C administered
TmVE: Group IV, Tramadol and vitamin E administered
TmG: Group V, Tramadol and garlic administered

a. Shows significant diffence when compared with positive control at (P<0.05)
b. Shows significant difference when compared with negative control at (P<0.05)

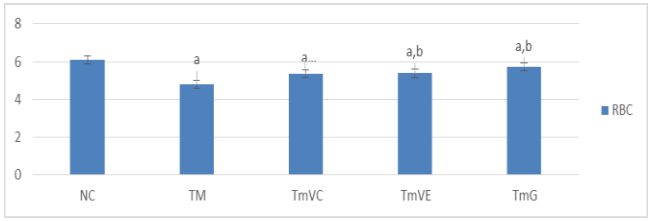


Values are expressed in Mean ±SEM of 7 determination

Fig 2: A graph showing the effect of Tramadol and antioxidants vitamin C, E and garlic interactions on organ weight.

NC: Group I, positive control, no Tramadol administered
TM: Group II, negative control, only Tramadol administered
TmVC: Group III, Tramadol and vitamin C administered
TmVE: Group IV, Tramadol and vitamin E administered
TmG: Group V, Tramadol and garlic administered

a. Shows significant diffence when compared with positive control at (P<0.05)
b. Shows significant difference when compared with negative control at (P<0.05)

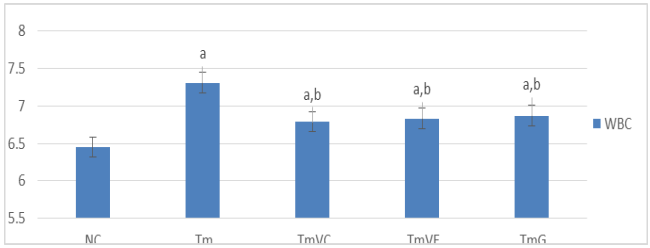


Values are expressed in Mean ±SEM of 7 determination

Fig 3: A graphical illustration showing the effect of Tramadol vitamins C & E and garlic interactions on Red Blood Cell Count

NC: Group I, positive control, no Tramadol administered
TM: Group II, negative control, only Tramadol administered
TmVC: Group III, Tramadol and vitamin C administered
TmVE: Group IV, Tramadol and vitamin E administered
TmG: Group V, Tramadol and garlic administered

a. Shows significant diffence when compared with positive control at (P<0.05)
b. Shows significant difference when compared with negative control at (P<0.05)

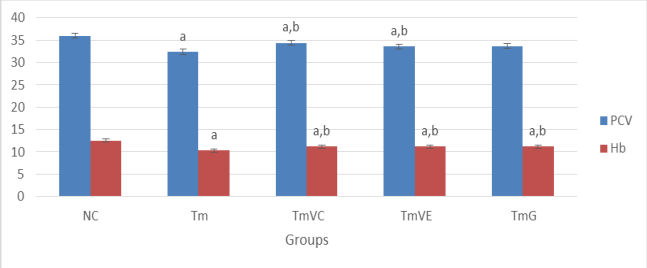


Values expressed in Mean ±SEM of 7 determination

Fig 4: A graph showing the effect of Tramadol vitamins C & E and garlic interactions on White Blood Cell Count

NC: Group I, positive control, no Tramadol administered
TM: Group II, negative control, only Tramadol administered
TmVC: Group III, Tramadol and vitamin C administered
TmVE: Group IV, Tramadol and vitamin E administered
TmG: Group V, Tramadol and garlic administered

a. Shows significant diffence when compared with positive control at (P<0.05)
b. Shows significant difference when compared with negative control at (P<0.05)

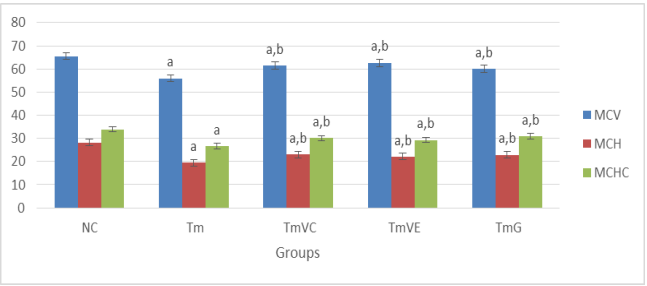


Values were expressed in Mean ±SEM of 7 determination

Fig 5: Graph showing the effect of Tramadol vitamins C & E and garlic interactions on Haemoglobin Concentration and Packed Cell Volume.

NC: Group I, positive control, no Tramadol administered
TM: Group II, negative control, only Tramadol administered
TmVC: Group III, Tramadol and vitamin C administered
TmVE: Group IV, Tramadol and vitamin E administered
TmG: Group V, Tramadol and garlic administered

a. Shows significant Diffence when compared with positive control at (P<0.05)
b. Shows significant difference when compared with negative control at (P<0.05)



Values expressed in Mean ±SEM of 7 determination

Fig 6: A graph showing the effect of Tramadol vitamins C & E and garlic interactions on MCV, MCH and MCHC.

NC: Group I, positive control, no Tramadol administered
TM: Group II, negative control, only Tramadol administered
TmVC: Group III, Tramadol and vitamin C administered
TmVE: Group IV, Tramadol and vitamin E administered
TmG: Group V, Tramadol and garlic administered

a. Shows significant diffence when compared with positive control at (P<0.05)
b. Shows significant difference when compared with negative control at (P<0.05)

Discussion

This research work was carried out to evaluate and compare the effects of vitamin C, vitamin E and garlic on some haematological parameters of Tramadol Induced *Wistar* rats. In this work, garlic, vitamins E and E were administered to the animals over a period of twenty eight (28) days.

There was a significant (P<0.05) decrease in the body weight of the negative control group in which Tramadol toxicity was induced. The final body weight of the rats in the negative control group decreased significantly from the initial body weight, also, there was a significant decrease when compared to the body weight of the rat in the positive control groupat (p>0.05). this is attributed to lack of appetite, and the effect of Tramadol toxicity which also leads to weight loss. However, there was an increase in the final body weight of the other groups (including the TmVC group, TmVE group and TmG group) when compared to the final weight of the negative control group which indicates the antioxidant activities of garlic and vitamins C & E to mop up the free radicals produced during Tramadol toxicity.

The weight of the liver in the negative control group increased significantly at (P<0.05) when compared to the weight of the liver in the positive control group. This shows the oxidative stress undergone by the liver as the liver strives to metabolize and detoxify the Tramadol to a less armful substance (metabolite), this results in the inflammation of the liver. The weight of the liver also increases in the other groups significantly when compared to the positive control group. But when the liver weights of these other groups are compared to the negative control group at (P<0.05) there is a significant decrease, once again highlighting the effect of the antioxidants garlic, vitamins C and E. the weight of the heart and kidney also showed similar variations in the graphs. This is because all three organs including liver, kidney and heart are affected to an extent by the induction of Tramadol toxicity.

The Red Blood Cells count of the negative control group decreased significantly when compared to the positive control group at (P<0.05). This finding may be explained on the basis of inhibitory effect on Tramadol on a erythropoiesis and also the effect of Tramadol on the appetite of the rats. Also the RBC's counts increased significantly in the other groups when compared to the negative control group, this can be attributed to the introduction of the antioxidants.

The study also revealed that the total White Blood Cell (WBC) counts in the negative control group was significantly elevated when compared to the positive control group at (P<0.05). The significant increase in the WBC's count indicated the activation of defense mechanism and the immune system of the rats. This induction of White Blood Cell is a positive response for survival due to cell mediated immune response of the animals. Also, the WBC counts decreased significantly in other groups when compared with the negative control group indicating the activities of the antioxidants but the WBC counts in these other groups showed a significant increase when compared to the positive control group.

The haemoglobin concentration and Packed Cell Volume in the negative control group were significantly decreased when compared to the positive control group at (P<0.05). This can be attributed to the decreased levels of RBC's caused by lack of appetite and nutritional deficiencies. This decrease was also noticed in the other groups (TmVC, TmVE and TmG) when compared to the positive control group but there was an increase (although not pronounced) in the Hb and PCV of these other groups when compared to the negative control group.

The MCV, MCH and MCHC in the negative control groups were significantly decreased when compared to the positive control group at (P<0.05). This is as a result of the low

RBCs and haemoglobin levels in the Tramadol administered group which is due to malnutrition, and effect of Tramadol toxicity. This decrease was also noticed in the other groups when compared to the positive control group, but there was a significant increase in the MCV, MCH and MCHC of these other groups when compared to the negative control group at ($P<0.05$) which can be attributed to the antioxidant activities of garlic, vitamins C and E.

Conclusion

In conclusion, the result obtained from the study showed that oral administration of Tramadolat 29mg/kg body weight for 28 days induces Tramadol toxicity in wistar rats. In the Tramadol induced rats, the RBC's, Hb, PCV, MCV, MCH and MCHC levels were all decreased while the WBC level was increased. However, garlic, vitamins C and E had varying effects on the haematological parameters and have modulatory potentials by increasing the RBC's, Hb, PCV, MCV, MCH and MCHC levels while decreasing the WBC level of wistar rats in tramadol induced toxicity due to the presence of antioxidants in these compounds.

References

1. Afshari R, Afshar R, Mergarbane B. Tramadol overdose: review of the literature. Journal of Reanimation. 2011; 20(5):41-43.
2. Amagase H, Petesch BL, Matsuura H, Kasuga S, Itakura Y. Intake of garlic and its bioactive components. Journal of Nutrition. 2001; 131(3):62-955.
3. Atici S, Cinel S, Doruk N, Eskandari G, Oral U. Liver and kidney toxicity in chronic use of opioids: an experimental long term treatment model. Journal of Biosciences. 2005; 30:245-252.
4. Block E. Garlic and other Alliums: The core and the science. Royal society of Chemistry, 2010, 5-6.
5. Borlinghaus J, Albrecht F, Gruhlke MC, Nwachukwu ID, Slusarenko AJ. Chemistry and biological properties.U.S. National library of medicine. 2014; 19(8):618-12591.
6. Brayfield A. Tramadol hydrochloride. The complete drug reference. Pharmaceutical Press. Retrieved, 2013-2018.
7. Burton GW, Wronska U, Stone L, Foster DO, Ingold KU. Biokinetics of dietary RRR-alpha-tocopherolin the male guinea pig at three dietary levels of vitamin C and two levels of vitamin E. evidence that vitamin C does not "spare vitamin E in vivo. Journal of Pubmed Central: Lipids. 1990; 25(4):199-210.
8. Dhawan V, Jain S. Garlic supplementation prevents oxidative DNA damage in essential hypertension. Journal of molecular cell Biochemistry. 2005; 275:85-94.
9. Free V, Sanders P. The use of ascorbic and mineral supplements in the detoxification of narcotic addicts. Journal of Phychedelic Drugs. 1979; 11(3):2-217.
10. Galli FA, Birringer A, Cook-mills M, Eggersdorfer JM, Frank M, Cruciani J. Vitamin E: Emerging aspects and new direction Journal of Free Radicals. Biomed. 2017, 102.
11. Kianosouch S, Balali-mood M, Mousavi SR, Moradi V, Sadeghi M, Dadpour B, Rajabi O. Comparison of therapeutic effects of garlic and d-penicillamine in patients with chronic occupational lead poisoning. Journal of Basic Clinical Pharmacology and Toxicology. 2012; 110(5):81-476.
12. Lu R, Kallenborn Gerhardt W, Geisslinger G, Schmidko A. Addictive effects of a combination of vitamin C and vitamin E after peripheral nerve injury. Pubmed Journal. 2011; 6:12.
13. Raffa RB, Buschmann H, Christoph T. Mechanistic and functional differentiation of tapentadol and Tramadol. Expert opinion on pharmacotherapy. 2012; 13(10):1437-1449.
14. Shadnia S, Soltaninejad K, Heydari K, Sasanian G, Abdollahi M. Tramadol and experimental toxicology. 2008; 27(3):5-201.
15. Silagy C, Neil A. Garlic as a lipid lowering agent: a Meta-analysis. J. R. Coll Physicians London. 1994; 28(1):39-45.
16. Zarezadch M, Baluchnejadmojarad T, Kiasalari Z, Afshin-majd S, Roghani M. Garlic active constituent S-allyl cysteine protects against lipopolysaccharide-induced cognitive defects in the rat: possible involved mechanisms. Pubmed Central Journal. 2017; 795:13-21.