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Efficacy trials of Indian bread developed from sorghum based composite flour mix on blood lipid profile of experimentally induced hyperlipidemic rats

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Abstract
The aim of this study was to compare the effect of different experimental diet model on experimentally induced hyperlipidemic rats. Hyperlipidemia has been ranked as one of the greatest risk factors contributing to the prevalence and severity of coronary heart diseases. Hyperlipidemia is characterized by elevated serum total cholesterol (TC), low density lipoprotein (LDL), and very low density lipoprotein (VLDL) and decreased high density lipoprotein (HDL) levels. Inducing hypercholesterolemia in rats is done through a high fat, high cholesterol diet, with the fat source varying from lard to canola, coconut, soybean or palm oil. The present study was carried out to evaluate the efficacy of Indian bread developed from unprocessed cereals and legumes and other functional ingredients in reducing the risk of CVD's as they are rich source of dietary fiber, phytonutrients and with potent antioxidant activity. Besides medication, diet also plays an important role in the management of lipid and lipoprotein concentrations in blood. In the present study an approach was made to assess the functional efficacy of Indian bread developed sorghum based composite flour mix for a period of 21 days on the blood lipid profile of the experimentally induced hyperlipidemic rats.

Keywords: Indian bread developed, sorghum based, flour mix, blood lipid

Introduction
In 21st century the major challenges to development is the non-communicable diseases that are a serious public health and socio-economic problem. According to World Health Organization (WHO) fact sheets, lifestyle diseases like cardiovascular diseases, diabetes, obesity, cancer, osteoporosis, respiratory diseases and gastro-intestinal diseases account for 59 percent of the 56.5 million deaths annually and 45.9 percent of the global burden of disease. The rise of functional foods has occurred at the convergence of several critical factors, such as: awareness of personal health deterioration, led by busy lifestyles with poor choices of convenience foods and insufficient exercise; increased incidence of self-medication; increased level of information from health authorities and media on nutrition and the link between diet and health; scientific developments in nutrition research; and a crowded and competitive food market, characterized by pressurized margins. During the last decades, knowledge of the dietary influence on health and well being has highly increased and often related to specific food components. Hyperlipidemia and related cardio vascular diseases are now the first leading cause of death and in 2013 World Health Organization (WHO) estimated that over 23 million people will die from cardiovascular diseases before 2030. Prevention and management of this disease is now first priority of the research field among scientist in the world (Chen C.Y. 2015) [1]. Vegetable protein present in cereals and legumes elevates plasma HDL level along with lowering the LDL, Triglycerides and cholesterol level in experimented rats and mice by stimulating the liver or in small intestine also the plant sterol present in selected cereals and legumes are beneficial for hypercholesterolimic patients and also prevent progression of increasing plasma cholesterol in the experimentally induced hyperlipidemia rats (Stevenson D.G. 2007) [4]. This effect could be due to presence of dietary fibre in cereals and legumes, confirmed by several epidemiological studies, showing that independent of the fat intake, the millet fibres are the dietary components which are important in preventing

cardiovascular disease (Nijjar P.S. 2010) [5]. This is a unique action in cholesterol metabolism among vegetable proteins. The present study aimed to find out the efficacy of Indian bread on the blood profile level of the experimentally induced hyperlipidemia rats.

Materials and Methods

White Sorghum (*Sorghum bicolor moench*) grains, Khesari dal (*Latyrus Sativus*), whole wheat flour, sweet potato and flax seeds were selected for the present study due to their easy availability, accessibility, high therapeutic value and nutraceutical properties.

Processing of ingredients

Indian bread was prepared following the guidelines outlined by AACC method (Anon., 1990). The Indian bread was prepared using composite flour from cereals and legumes that include sorghum grains flour, khesari dal flour, whole wheat flour, sweet potato flour and flaxseed flour at three different level of incorporation. For roti's 120 ml of water was used in each preparation.

Formulation of sorghum based composite flour mix from unmalted cereals and legumes for development of functional foods and product categorization

Formulations	Level of incorporation				
	Sorghum Grains flour	Whole wheat flour	Khesari dal flour	Sweet potato flour	Flaxseed flour
TS I	80%	5%	5%	5%	5%
TS II	70%	7.5%	7.5%	7.5%	7.5%
TS III	60%	10%	10%	10%	10%
TS IV	50%	12.5%	12.5%	12.5%	12.5%

TS I: Test sample 1
TS II: Test sample 2
TS III: Test sample 3
TS IV: Test sample 4

List of ingredients outlined by American Association of Cereal Chemists, AACC method (Anon., 1990) for formulation of Indian bread

Ingredients	Quantity (in gms)
Flour	100
Water	120

Supplementation of developed functional foods along with high fat diet:

The study was conducted after the approval of Institutional animal ethics committee and the approval no. is 770/ac/CPCSEA/FVSc/AAU/IAEC/17-18/602dated 09.08.2017. For 21 days supplementation studies albino rats weigh between 150 to 250 grams were selected and divided into 6 groups namely Group A, Group B, Group C, Group D, Group E and Group F. The feed of the Experimental rats were replaced with 40% Sorghum based composite mix flour (SBCMF) and 20% coconut oil. Each group comprises of 6 animals, notably, with no statistical differences.

Table 1: Proportion of supplementation of developed functional foods along with high fat

Group	Number of animals	Diet
A	6	Normal control: 100% RR
B	6	Reference group (High fat diet): 80% RR+ 20% CO
C	6	40% RR+40% TS I+20%CO
D	6	40% RR+40% TS II+20%CO
E	6	40% RR+40% TS III+20%CO
F	6	40% RR+40% TS IV+20%CO

CO: Coconut oil RR- Rat ration
Group C – Indian bread developed from 40% TS I
Group D- Indian bread developed from 40% TS II
Group E- Indian bread developed from 40% TS III
Group F- Indian bread developed from 40% TS IV

Blood collection

Blood samples were collected individually at heparinized sterile centrifuge tubes by puncture from the inner retro-orbital of the rat eye every week on 0^{th}, 7^{th}, 14^{th} and 21^{st} days of experimentation. The samples were then centrifuged at 3000 rpm for 15 minutes to obtain serum. The serum was stored in micro centrifuge tube for further biochemical investigation.

Estimation of blood lipid profile in experimentally induced hyperlipidemia rats

Parameters like plasma HDL (High Density lipoprotein), plasma LDL (Low density lipoprotein), Total Cholesterol and triglyceride level were estimated to determine the Hypocholestrolemic and Hypolipidemic effect of TS I, TS II, TS III and TS IV with the use of standard commercial kits (coral: HDL-D-160ml, LDL-D-160 ml, Cholesterol 250 ml, Triglycerides 250ml).

Impact of supplementation of Indian bread on experimentally induced hyperlipidemia rats

All the experimental groups supplemented with the test diets shows a significant improvement ($p \leq 0.05$) in the plasma HDL levels as compared to the experimental group A (control) and B (reference group). The experimental groups supplemented with the test diets shows a significant decrease ($P < 0.05$) in the plasma LDL, Triglyceride and total cholesterol levels as compared to the experimental group A (control) and B (reference group).

The initial plasma HDL level of Group A fed with rat ration showed no significant improvement i.e from 59±0.57mg/dl to 60±1.31 mg/dl at the end of the supplementation period. Group B fed with rat ration along with 20% coconut oil showed significant decrease ($p \leq 0.05$) in plasma HDL level with an initial level of 55±1.09 mg/dl to 50±0.63 mg/dl. Among the Experimental Groups fed with test diet TS I Group C showed a significant increase ($p \leq 0.05$) in the plasma HDL levels from an initial value of 62±0.93 mg/dl to 75±1.31 mg/dl. Group D supplemented with TS II showed a significant increase from an initial value of 57±0.81 mg/dl to 66±1.06 and Group E supplemented with TS III showed a significant increase from an initial value of 58±0.57 mg/dl to 65±1.31. Group F supplemented with TS IV showed a significant increase from an initial value of 55±0.65 mg/dl to 61±1.31 when compared with the reference group at the end of the supplementation period.

All the experimental groups, supplemented with test diets showed significant ($p \leq 0.05$) increase in the plasma HDL level but the highest was observed in Group C fed with TS I.

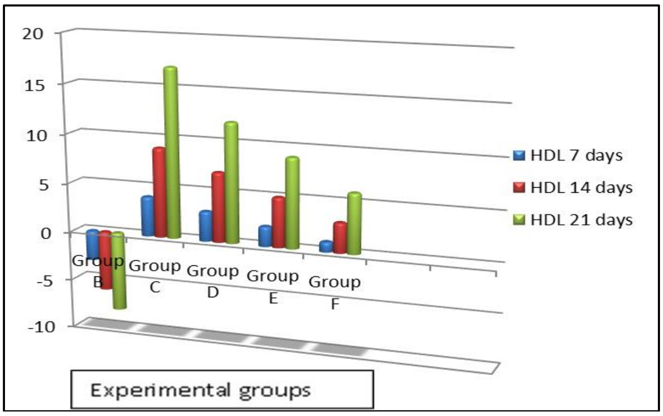

Fig 1: Mean Increment in Plasma HDL Level (Mg/Dl) After Supplementation of Test Diets In Comparison To Reference Group

All the experimental groups supplemented with the test diets shows a significant ($p \leq 0.05$) decrease in the plasma LDL levels as compared to the experimental group A (control) and B (reference group).

The Experimental Group A supplemented with rat ration showed no significant change in the plasma LDL level with an initial value of 126±1.06 mg/dl to 128±0.58 mg/dl at the end of the supplementation period. Group B fed with rat ration along with 20% of coconut oil showed significant ($p \leq 0.05$) increase in plasma LDL level with an initial level of 135±1.31mg/dl to 154±0.57 mg/dl at the end of the supplementation period.

Among the Experimental Groups fed with test diets developed from sorghum based composite flour, Group C supplemented with TS I along with 20% coconut oil showed a significant increase ($p \leq 0.05$) from an initial value of 128±0.57 mg/dl to 141±1.57 mg/dl. Group D supplemented with TS II showed a significant increase ($p \leq 0.05$) in the plasma LDL level from an initial value of 138±1.69 mg/dl to 150±1.06. Similarly, Group E supplemented with TSIII

showed a significant increase ($p \leq 0.05$) in the plasma LDL level from an initial value of 133±0.57 mg/dl to 152±3.41 at the end of the supplementation period and Group F supplemented with TS IV showed a significant increase ($p \leq 0.05$) in the plasma LDL level from an initial value of 143±1.01 mg/dl to 165±2.45 at the end of the supplementation period.

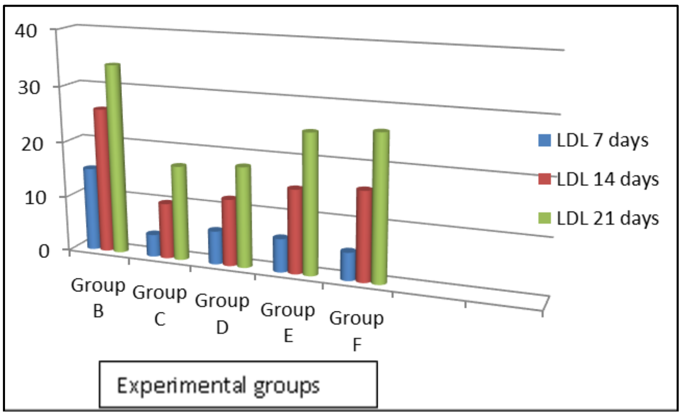

Fig 2: Mean Changes of Plasma LDL Level (Mg/Dl) After Supplementation of Developed Functional Foods In Comparison To Reference Group

The Experimental Group A supplemented with rat ration showed no significant improvement in the total cholesterol level with an initial value of 164±1.06 mg/dl to 166±1.06 mg/dl at the end supplementation period. Group B fed with rat ration along with 20% of high fat showed significant ($p \leq 0.05$) increase in total cholesterol level with an initial level of 176±1.06 mg/dl to 248±0.57 mg/dl at the supplementation.

Among the Experimental Groups fed with test diets developed from sorghum based composite flour there was a significant increase ($p \leq 0.05$) in the total cholesterol level. Group C supplemented with TS I showed a significant increase ($p \leq 0.05$) in the total cholesterol level from an initial value of 178±0.57 mg/dl to 187±0.81 at the end of the supplementation period. Group D supplemented with TS II showed a significant increase ($p \leq 0.05$) in the total cholesterol level from an initial value of 181±0.57 mg/dl to 194±1.06 and Group E supplemented with TS III showed a significant increase ($p \leq 0.05$) in the total cholesterol level from an initial value of 173±0.81 mg/dl to 195±0.57 at the end of the supplementation period. Group F fed with TS IV showed a significant increase ($p \leq 0.05$) in the total cholesterol level from an initial value of 158±1.24 mg/dl to 190±2.57 at the end of the supplementation period

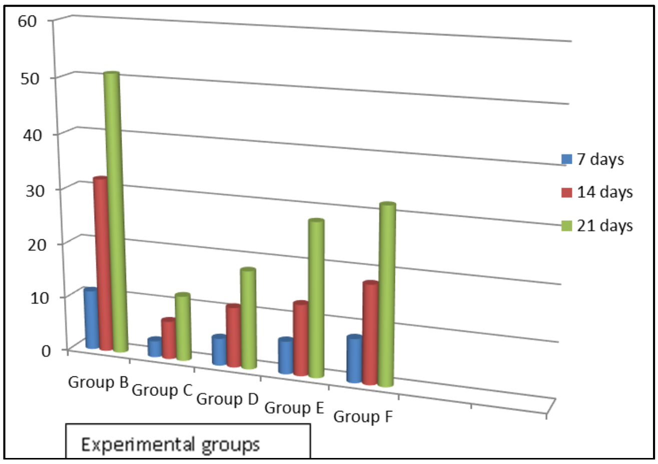

Fig 3: Mean Changesof Plasma Total Cholesterol Level (Mg/Dl) After Supplementation of Developed Functional Foods In Comparison To Reference Groups

The Experimental Group A supplemented with rat ration showed no significant improvement in the Triglyceride level with an initial value of 75±1.31 mg/dl to 76±1.06 mg/dl at the end of the supplementation period. Group B fed with rat ration along with 20% coconut oil showed significant increase ($p \leq 0.05$) in Triglyceride level with an initial level of 78±2.26 mg/dl to 94±1.65 mg/dl at the end of supplementation period

Among the Experimental Groups fed with functional foods developed from sorghum based composite flour, Group C supplemented with TS I showed a significant decrease ($p \leq 0.05$) in the Triglyceride level from an initial value of 73±1.23 mg/dl to 63±1.81 at the end of the supplementation period. Group D supplemented with TS II along with showed a significant decrease ($p \leq 0.05$) in the Triglyceride level from an initial value of 73±2.93 mg/dl to 66±0.63 and Group E supplemented with TS III showed a significant decrease ($p \leq 0.05$) in the Triglyceride level from an initial value of 74±1.52 mg/dl to 70±1.75 at the end of the supplementation period. Group F supplemented with TS IV showed a significant decrease ($p \leq 0.05$) in the Triglyceride level from an initial value of 69±1.52 mg/dl to 65±1.75 at the end of the supplementation period.

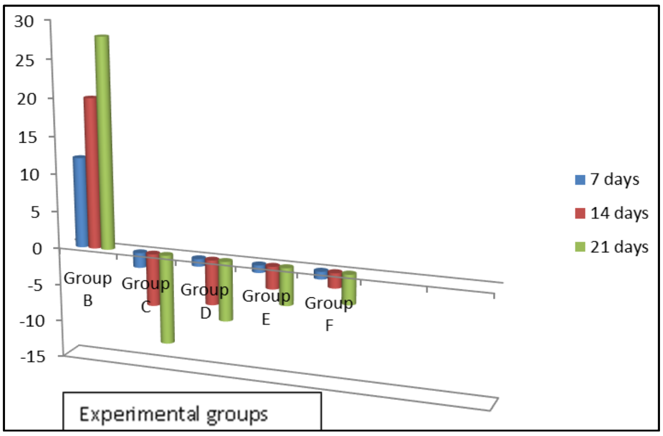

Fig 4: Mean Changes of Plasma Triglyceride Level (Mg/Dl) After Supplementation of Developed Functional Foods In Comparison To Reference Groups

Nishizawa *et al.* (2005) [7] reported that protein from millets is effective for improving lipid metabolism. He found that the feeding of millet protein elevates plasma HDL cholesterol level in rats and mice by stimulating the synthesis of HDL in liver or in small intestine. This effect could be due to presence of dietary fibre in millets, confirmed by several epidemiological studies, showing that independent of the fat intake, the millet fibres are the dietary components which are important in preventing cardiovascular disease. This is a unique action in cholesterol metabolism among vegetable proteins.

In 2010, Yanai *et al*. revealed that the high-density lipoprotein (HDL) plays a important role in reverse cholesterol transport and suppresses cholesterol accumulation in the peripheral tissues. Various epidemiological studies have shown an inverse association between the concentration of plasma HDL level and the risk of developing atherosclerotic cardiovascular disease. The findings of the present study was in conformity with the research finding by Manso *et al.*(2008) [3] who reported that the serum HDL cholesterol level increased significantly ($p \leq 0.01$) by 23% by incorporating malted sorghum flour in baked products because malted sorghum flour have a higher proportion of non-starchy polysaccharides and dietary fiber. He also reported that supplementation of foxtail millet based biscuits showed a significant reduction ($p \leq 0.01$) in initial value of serum LDL cholesterol by 20%.Another factor that enhances the health benefit properties of sorghum based composite flour mix are vegetable proteins, which are present in higher content in malted flour and are present abundantly in malted legumes possesses hypocholestrolemic properties.

Another studied by Mohanraj and Sivasankar, 2014 [6] revealed that the sweet potato has a high nutritional value and phytochemical composition along with a number of bioactive constituents such as phenolic compounds, saponins, bioactive proteins, glycoalkaloids, and phytic (like Proteins, sugars, polyphenols and water- soluble polysaccharides (Zhao *et al.* 2006) [11]. Peptides isolated from proteins of sweet potato have shown various health benefits, such as antioxidation (Zhang *et al*. 2010) [10], amelioration of glucose tolerance (Otani *et al*. 2009) [8], and hypocholesterolemic effects (Liu *et al.* 2010) [2]. According to a study conducted in 2008 FNRI National Nutrition Survey the significant increase in HDL cholesterol levels of all study participants after consumption of sweet potato observed in this study was a good result as it was found to have a very low plasma HDL cholesterol effect. The decrease in the LDL-cholesterol observed in the present study could be due to presence of beneficial dietary fibre and abundance of phytochemicals with potent antioxidant activity in cereals like millets as reported by Chethan *et al.* (2008).

Khoury *et al.* (2010) indicated that a 10% dose of dietary fibre rich food particularly oats had potential hypolipidemic, hypotriglyceridemic and hypocholesterolemic effect in serum of hypercholesterolemic rats with a reduction of serum LDL-cholesterol. This can be due to the presence of ß-glucan in oats (David *et al*. 2005).

Knoop *et al*. (2001) investigated various aspects of enterohepatic bile acid cycling in rats adapted to fibre-free diets (supplemented with 0.25% cholesterol) and concluded that the enterohepatic cycling of steroids, especially bile acids reabsorption in portal blood is reduced. The improvement in LDL-cholesterol level seen in the present study may be due to the similar mechanisms as sorghum is rich source of dietary fibres such as water soluble gum and ß-glucans (Vijayalakshmi and Radha, 2016).

The findings of the present study revealed that sorghum have the potential to lower down the level of plasma total cholesterol in experimental rats. This decrease in plasma total cholesterol may be due to the presence of beneficial dietary fibre and phytochemicals with potent antioxidant capacity in sorghum grain.In 2017 Thilagavathi and Kanchana reported that when experimental animals were supplemented with different incorporations of millet based diet along with high fat diet showed significant reduction in total cholesterol, triglycerides and low density lipoprotein (LDL).

whereas increased level of high density lipoprotein (HDL) at the end of 28 days of supplementation period compared to normal control rats fed with only high fat diet. Studies (Liu *et al.* 2000; Isken *et al.* 2010; Maki *et al.* 2016) [2] have also evidenced that both soluble and insoluble dietary fibers have the potentiality to lower total cholesterol, LDL and triglyceride level in blood by binding bile acids or cholesterol during the intraluminal formation of micelles in intestinal wall during absorption.

From the present study, it was concluded that the functional foods developed from sorghum based composite flour mix showed health benefit properties in experimental animals by increasing the plasma HDL level and significantly decreasing the plasma LDL, triglyceride and total cholesterol level and this may be due to the presence of phytonutrients like phenolic acids, anthocyannins, phytosterols and policosanols, dietary fibre, vegetable protein, antioxidants etc.

The main mechanism responsible for the Triglyceride and cholesterol lowering effect of plant proteins is to inhibit the intestinal triglyceride and cholesterol absorption. There are several sites where intestinal absorption of triglyceride and cholesterol takes place within the intestinal tract. Different mechanisms of plant proteins, such as competition with triglyceride and cholesterol for solubilisation in dietary mixed micelles, co-crystallisation with triglyceride and cholesterol to form insoluble mixed crystals, and interference with the hydrolysis process by lipases and cholesterol esterases are believed to contribute to the lowering of serum triglyceride and cholesterol concentrations by plant proteins (Jenkins *et al* 2010). Fernandez *et al.* (2015) revealed that vegetable proteins affects serum lipid levels and presents a significant relationship with decreases in total and LDL cholesterol and the risk of coronary heart disease.

Conclusion

The present study provides substantial evidence that millets and cereals along with pulses and oil seeds improves the nutritional and physico-chemical characteristics of formulated sorghum based composite flour mix. The food products developed using sorghum based composite flour mix has proven to possess immense nutritional and functional properties in terms of protein, crude fibre, phytonutrients particularly phenolic compounds like total phenolics, total flavonoids and minerals with potent antioxidant capacity and phenomenol improvement in plasma high density lipoprotein (HDL). Low density lipoprotein (LDL), total cholesterol of experimental animals. The outcomes of the present research can be used as valuable information for emphasizing the significance of sorghum based composite flour mix as a functional food ingredient in management of risk associated non-communicable disease (NCD's) as well as to prevent the onset of degenerative disease and help in management of metabolic disorder associated with today's changing lifestyles and environment and thus improving the overall health of the population.

Recommendation

Further in depth studies can be undertaken to develop novel value added products and evaluation of the protein quality as well as finding therapeutic potential of the developed products for the management of non-communicable diseases.

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Reference

1. Chen CY, Holbrook M, Duess MA, Dohadwala MM, Hamburg NM, Asztalos BF. Effect of almond consumption on vascular function in patients with coronary artery disease: randomized, controlled, cross-over trial. Nutr J. 2015; 14:61.
2. Liu Y, Yan X, Lin S, Li X. Serum levels of FGF-21 are increased in coronary heart disease patients and are independently associated with adverse lipid profile. 2010; *5*(12):e15534.
3. Manso MA, Miguel M, Even J, Hernández R, Aleixandre A, López-Fandiño R. Effect of the long-term intake of an egg white hydrolysate on the oxidative status and blood lipid profile of spontaneously hypertensive rats. Food Chemistry, 2008; 109(2):361-367.
4. Stevenson DG, Eller FG, Wang L, Jane JL, Wang T, Inglett GE. Oil and tocopherol content and composition of pumpkin seed oil in12 cultivars. J Agric. Food Chem, 2007; 55:4005-4013,
5. Nijjar PS, Burke FM, Bloesch A, Rader DJ. Role of dietary supplements in lowering low density lipoprotein cholesterol: a review. J Clin Lipidol, 2010; 4:248-258.
6. Mohanraj R, Sivasankar S. Sweet Potato (Ipomoea batatas [L.] Lam)-A valuable medicinal food: A review. Journal of medicinal food. 2014; 17(7):733-741.
7. Nishizawa M, Segawa K, Tanaka M, Kishimoto K, Watanabe E. Visfatin: a protein secreted by visceral fat that mimics the effects of insulin. Science. 2005; 307(5708):426-430.
8. Otani H, Kimura K, Nishikawa O, Yamada Y. Effect of vitamin E on lipid metabolism and atherosclerosis in ESRD patients. Kidney International. 2009; 56:S126-S129.
9. Yanai H, Ito K, Tomono Y, Koikeda T, Tsukahara H, Tada N. Administration of natural astaxanthin increases serum HDL-cholesterol and adiponectin in subjects with mild hyperlipidemia. Atherosclerosis. 2010; 209(2):520-523
10. Zhang XM, Shulman GI. Apolipoprotein C3 gene variants in nonalcoholic fatty liver disease. New England Journal of Medicine, 2010; 362(12):1082- 1089.
11. Zhao Y, Jia G, Chai Z. Acute toxicological effects of copper nanoparticles *in vivo*. Toxicology letters. 2006; 163(2):109-120.
12. World Health Organization. The Global Burden of Disease: 2004 Update, World Health Organ: Geneva, 2013, 2-5.