ejpmr, 2018,5(11), 69-74

SJIF Impact Factor 4.897

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

EFFECT OF TARTRAZINE ORALLY ADMINISTERED ON SOME ATHEROGENIC INDICES OF ALBINO RATS

[1]*Elekima Ibioku and [2]Ben-Chioma Adline Erinma

*[1,2]Department of Medical Laboratory Science, Rivers State University, Port Harcourt, Nigeria.

***Corresponding Author: Elekima Ibioku**

Department of Medical Laboratory Science, Rivers State University, Port Harcourt, Nigeria.

Article Received on 03/09/2018 **Article Revised on 24/09/2018** **Article Accepted on 14/10/2018**

ABSTRACT

In this study the effect of tatrazine on some atherogenic indices of albino rats were evaluated. Thirty (30) rats weighing approximately 0.14kg were used. The rats were divided into five (5) groups of six (6) rats per group. Lipid parameters such as Total Cholesterol (TC), triglyceride (TG), High Density Lipoprotein-cholesterol (HDL-C) and Low density Lipoprotein-cholesterol (LDL-C) were evaluated while atherogenic indices such as Atherogenic index of plasma (AIP), Non-high density lipoprotein cholesterol (Non-HDL-C), Atherogenic coefficient (AC), Castelli risk index 1 (CRI-1) and Castelli risk index 2 (CRI-2) were calculated as ratios of these lipid parameters. Results of atherogenic indices obtained were analysed using Graphpad Prism 5.1 with statistical significance considered at $P<0.05$. When atherogenic indices of tartrazine treated rats were considered, group A (control) had 0.54±0.08, 0.45±0.08, -0.09±0.08, 1.45±0.08 and 0.08±0.06 for Non HDL-C, AC, AIP, CRI-1 and CRI-11 respectively. In addition, Group B had 1.17±0.58, 0.73±0.28, -0.07±0.14, 1.72±0.28 and 0.33±0.31 for Non HDL-C, AC, AIP, CRI-1 and CRI-11 respectively while Group C had 0.74±0.26, 0.68±0.24, 0.06±0.12, 1.68±0.24 and 0.21±0.20 for Non HDL-C, AC, AIP, CRI-1 and CRI-11 respectively. More so, Group D had 0.81±0.21, 0.84±0.26, 0.09±0.06, 1.84±0.26 and 0.27±0.22 for Non HDL-C, AC, AIP, CRI-1 and CRI-11 respectively. Finally, group E had 0.81±0.10, 0.74±0.09, 0.10±0.08, 1.74±0.09 and 0.21±0.14 for Non HDL-C, AC, AIP, CRI-1 and CRI-11 respectively. The comparison of group A and B showed significant increases in Non-HDL-C, AC and CRI-1 of Group B tartrazine treated rats at $p<0.05$ while significant increases were observed in AC, AIP and CRI-1 when group A and C were compared at $p<0.05$. Also, when Group A and Group D as well as Group A and Group E were compared, significant increases were seen in Non-HDL, AC, AIP, CRI-1 and CRI-11 (table 3.5) except that the comparison between Group A and D showed non-significant increase in CRI-11 at $p<0.05$. The one-way analysis of variance (ANOVA) indicated significant differences in all of the indices considered except CRI-11. However, when post-analysis was done using the turkey's comparison test, significant differences were not observed between treated groups except between Group A and Group D as well as Group A and Group E.

KEYWORDS: Tartarzine, Atherogenic Indices, Cholesterol, Triglycerides, HDL-C, LDL-C.

1. INTRODUCTION

Tartrazine is a synthetic azo food dye that reflects a lemon-yellow colouration[1],[2],[1],[3],[4], stated it is commonly found in edibles and food products such as ice creams, powdered drinks, cereals, soft drinks, cakes, biscuits, rice, energy drinks et cetera. However, as a food additive, tartrazine has been reported to induce hepatocellular, renal and haematological disorders[1],[2] as well as hypersensitive and allergic reactions when consumed in high dosages in human especially in children.[2],[3] Similarly, tartrazine has also been reported to cause aberration of mammalian chromosomes, carcinogenic and mutagenic effects, loss of memory and learning capacity.[4][5]

However, the aim of this study is to evaluate the effect of tartrazine orally administered on atherogenic indices of albino rats.

Athrogenic indices are ratios derived from the combination of two or more lipid parameters such as total cholesterol (TC), triglyceride (TG), HDL-C, LDL-C and VLDL-C used in predicting risks of cardiovascular diseases.[6][7] Though, one or more lipid parameters are used to predict an individual's risk for cardiovascular disease but lipid ratios have been reported to be more useful in assessment of cardiovascular risk.[8] In 2013,[9] reported that atherogenic ratios predicts cardiovascular risk especially when absolute values of lipid ratios are not markedly deranged.

Atherogenic indices or ratios considered in this study were Atherogenic Index of Plasma (AIP), Non-High Density Lipoprotein Cholesterol (Non-HDL-C), Atherogenic Coefficient (AC), Castelli Risk Index 1 (CRI-1) and Castelli Risk Index 2 (CRI-2).

AIP is a very sensitive predictor for the diagnosis and prognosis of the risk of cardiovascular diseases.[10] AIP reveals the relationship between protective and atherogenic lipoprotein particle and is linked with the size of pre and anti- atherogenic lipoprotein particles.[10] The TG and HDL-C ratio is a significant atherogenic index that can be used as a strong predictor of cardiovascular disease and determine the presence and degree of coronary artery disease by non-invasive method[11],[12],[13],[14] defined AIP as the logarithm of the ratio of TG to HDL-C. That is, AIP = Log (TG/ HDL-C). This is a very useful and better marker because the logarithmical ratio of TG to HDL-C negatively and positively correlate with LDL-C size and Fractional Esterification Rate of HDL (FERHDL) respectively.[15],[16]

Non-HDL-C is derived by subtracting the HDL-C fraction from TC fraction. That is, Non-HDL-C = TC – HDL-C. Therefore, from the calculation, the major cholesterol fraction remaining is specifically LDL cholesterol which is an atherogenic lipid fraction.[17]

AC is a measure of Low-Density Lipoprotein Cholesterol (LDL-C), Very-Low-Density Lipoprotein Cholesterol (VLDL-C) and Intermediate Density Lipoprotein Cholesterol (IDL-C) lipoprotein fractions in relation to High-Density Lipoprotein Cholesterol (HDL-C).[18] AC was also defined by[19] as the ratio of Non-HDL-C to HDL-C. That is, AC = (TC – HDL-C)/ HDL-C. Where non-HDL-C = TC – HDL-C. Thus, AC is used as a marker for the assessment of the risk of cardiovascular disease and as AC value increases, the risk of developing cardiovascular disease increases and vice versa.[6] It correlates positively with HDL-C in the prognosis and diagnosis of cardiovascular disease.[6]

Castelli risk index (CRI) is dependent on TG, LDL-C and HDL-C which are independent risk factors for cardiovascular disease. Studies have also reported that increase in TG, TC and LDL-C fractions as well as reduced HDL-C fractions are predictors of atherogenicity which correlated positively with several cardiovascular risk factors.[17] CRI is made up of two ratios, which are; Castelli Risk Index-1 (CRI-1): Castelli Risk Index-1 which is also called Coronary Risk Index (CRI) or Cardiac Risk Ratio (CRR) is = TC/(HDL-C).[20] Castelli Risk Index-2 (CRI-2) is a ratio of LDL-C to HDL-C. That is, CRI-II = LDL-C/ HDL-C. These indices are considered to be more sensitive and specific index of cardiovascular risk particularly in individuals with hypertriglyceridemia of >300mg/dl.

3. MATERIALS AND METHODS

3.1 Materials

Materials used include centrifuge, chloroform, weigh balance, spectrophotometer, cholesterol (TC), triglyceride (TG), high density lipoprotein (HDL-C) reagents purchased from Randox Diagnostics, United Kingdom.

3.2 Animals

A total of 30 rats of average weight of 0.14kg were used for this study. The rats were randomly separated into five (5) groups namely group A, B, C, D and E. Each group had a total of six (6) rats. The rats were purchased from the animal farm of University of Port Harcourt and were transported to the animal house of the Department of Medical Laboratory science, Rivers State University, Port Harcourt. The rats were acclimatized for 10 days (alternate day and night) at normal room temperature and were fed with chicken growers mash and water *ad libitum*.

3.4 Administration of Tartrazine Dye

The rats were administered with varying concentration of tartrazine dye for 30 days orally using gavage technique. Group A (control), B, C, D and E were treated daily with 1.0ml of 0.0% (water), 1.0%, 1.5%, 2.0% and 2.5% of tartrazine dye respectively.

3.5 Specimen Collection and Laboratory Analysis

Blood samples were collected using cardiac puncture (after anesthetising with chloroform) into lithium heparinised bottles. The whole blood was spun at 4000rpm for five (5) minutes to obtain plasma for the analysis of total cholesterol (TC), triglyceride (TG) and High Density Lipoprotein (HDL). TC and TG were analysed based on modified enzymatic methods as described by[21] and[22] respectively. More so, the analysis of HDL-C involved two steps procedure: firstly, the precipitation of lipoprotein fractions of LDL and VLDL in the samples using phosphotungstic acid and magnesium ions, with HDL remaining in the supernatant. The second step involves HDL cholesterol quantitation contained in the supernatant using the cholesterol (reagents) modified enzymatic method as described.[21] Low Density Lipoprotein (LDL) concentration was determined from other lipoproteins fraction mathematically as described by[23] using the Friedwald equation: LDL–C (mmol/l) = TC – (TG/2.2 + HDL).

3.6 Calculation of Atherogenic Indices

After determining the concentration in mmol/L of the TC, TG, HDL-C and LDL-C fractions, the atherogenic indices were calculated using the following arithmetic formulae. AIP = Log (TG/ HDL-C); Non-HDL-C = TC – HDL-C; AC = (TC – HDL-C)/ HDL-C; CRI-1= TC/HDL-C and CRI-II = LDL-C/ HDL-C as described by[11],[17],[19] and[20] respectively.

3.6 Statistical Analysis

Data obtained were statistically analysed using Graphpad prism 5.03 package. Mean, standard deviation, One-Way ANOVA and inferential statistics using the statistical t-test were the statistical parameters considered and results obtained were expressed as Mean±SD. Statistical significance were seen at $p<0.05$.

3. RESULTS

Table 3.1 shows the concentration of lipid parameters of the various groups were athrogenic indices were calculated using the appropriate formulae as stated above.

When atherogenic indices of tartrazine treated rats were considered, Group A had 0.54±0.08, 0.45±0.08, -0.09±0.08, 1.45±0.08 and 0.08±0.06 for Non HDL-C, AC, AIP, CRI-1 and CRI-11 respectively (table 3.2). In addition, Group B had 1.17±0.58, 0.73±0.28, -0.07±0.14, 1.72±0.28 and 0.33±0.31 for Non HDL-C, AC, AIP, CRI-1 and CRI-11 respectively while Group C had 0.74±0.26, 0.68±0.24, 0.06±0.12, 1.68±0.24 and 0.21±0.20 for Non HDL-C, AC, AIP, CRI-1 and CRI-11 respectively. More so, Group D had 0.81±0.21, 0.84±0.26, 0.09±0.06, 1.84±0.26 and 0.27±0.22 for Non HDL-C, AC, AIP, CRI-1 and CRI-11 respectively and finally, group E had 0.81±0.10, 0.74±0.09, 0.10±0.08, 1.74±0.09 and 0.21±0.14 for Non HDL-C, AC, AIP, CRI-1 and CRI-11 respectively.

The comparison of group A and B showed significant increases in Non-HDL-C, AC and CRI-1 of Group B tartrazine treated rats at $p<0.05$ (table 3.2) while significant increases were observed in AC, AIP and CRI-1 when group A and C were compared at $p<0.05$ (table 3.3). Also, when Group A and Group D as well as Group A and Group E were compared, significant increases were seen in Non-HDL, AC, AIP, CRI-1 and CRI-11 (table 3.5) except that the comparison between Group A and D showed non-significant increase in CRI-11 at $p<0.05$ (table 3.4).

The analysis of variance (ANOVA) indicated significant differences in all of the indices considered except CRI-11. However, when post-analysis was done using the turkey's comparison test, significant differences were not observed between treated groups except between Group A and Group D as well as Group A and Group E (table 3.6).

Table 3.1: Concentration (Mmol/L) Of Lipid Parameters of various Groups.

Parameter (Dose)	TG (mmol/L)	TC (mmol/L)	HDL (mmol/L)	LDL(mmol/L)
Group A (0.0%)	0.994± 0.175	1.745±0.063	1.203±0.065	0.0910±0.067
Group B (1.0%)	1.316 ±0.078	2.791 ±0.895	1.616 ±0.453	0.577±0.583
Group C (1.5%)	1.113±0.371	1.812±0.283	1.076±0.078	0.230±0.225
Group D (2.0%)	1.215±0.117	1.784±0.152	0.979±0.065	0.253±0.198
Group E (2.5%)	1.269±0.191	1.900±0.111	1.092±0.029	0.231±0.149

Table 3.2: Comparative Analysis of Group A and Group B Treated Rats.

Parameters	Non-HDL-C (mmol/L)	AC	AIP	CRI-1	CRI-11
Group A (0.0%)	0.54±0.08	0.45±0.08	-0.09±0.08	1.45±0.08	0.08±0.06
Group B (1.0%)	1.17±0.58	0.73±0.28	-0.07±0.14	1.72±0.28	0.33±0.31
Pvalue	0.0242	0.0451	0.7861	0.0451	0.0847
Tvalue	2.653	2.289	0.2787	2.289	1.914
Remark	S	S	NS	S	NS

Table 3.3: Comparative Analysis of Group A and Group C Treated Rats.

Parameters	Non-HDL-C (mmol/L)	AC	AIP	CRI-1	CRI-11
Group A (0.0%)	0.54±0.08	0.45±0.08	-0.09±0.08	1.45±0.08	0.08±0.06
Group C (1.5%)	0.74±0.26	0.68±0.24	0.06±0.12	1.68±0.24	0.21±0.20
Pvalue	0.1091	0.0496	0.0380	0.0496	0.1595
Tvalue	1.759	2.233	2.389	2.233	1.520
Remark	NS	S	S	S	NS

Table 3.4: Comparative Analysis of Group A and Group D Treated Rats.

Parameters	Non-HDL-C (mmol/L)	AC	AIP	CRI-1	CRI-11
Group A (0.0%)	0.54±0.08	0.45±0.08	-0.09±0.08	1.45±0.08	0.08±0.06
Group D (2.0%)	0.81±0.21	0.84±0.26	0.09±0.06	1.84±0.26	0.27±0.22
Pvalue	0.0157	0.0068	0.0018	0.0068	0.0634
Tvalue	2.906	3.399	4.207	3.399	2.087
Remark	S	S	S	S	NS

Table 3.5: Comparative Analysis of Group A and Group E Treated Rats.

Parameters	Non-HDL-C (mmol/L)	AC	AIP	CRI-1	CRI-11
Group A (0.0%)	0.54±0.08	0.45±0.08	-0.09±0.08	1.45±0.08	0.08±0.06
Group E (2.5%)	0.81±0.10	0.74±0.09	0.10±0.08	1.74±0.09	0.21±0.14
Pvalue	0.0005	0.0002	0.0036	0.0002	0.0494
Tvalue	5.109	5.721	3.785	5.720	2.235
Remark	S	S	S	S	S

Table 3.6: Analysis of Variance of the various Groups.

Parameters	Non-HDL-C (mmol/L)	AC	AIP	CRI-1	CRI-11
Group A (0.0%)	0.54 ± 0.08^{a}	0.45 ± 0.08^{a}	-0.09 ± 0.08^{a}	1.45 ± 0.08^{a}	0.08 ± 0.06^{a}
Group B (1.0%)	1.17 ± 0.58^{bc}	0.73 ± 0.28^{bc}	-0.07 ± 0.14^{ac}	1.72 ± 0.28^{bc}	0.33 ± 0.31^{ac}
Group C (1.5%)	0.74 ± 0.26^{ade}	0.68 ± 0.24^{bcd}	0.06 ± 0.12^{bde}	1.68 ± 0.24^{bcd}	0.21 ± 0.20^{acd}
Group D (2.0%)	0.81 ± 0.21^{bdef}	0.84 ± 0.26^{bcde}	0.09 ± 0.06^{bdef}	1.84 ± 0.26^{bcde}	0.27 ± 0.22^{acde}
Group E (2.5%)	0.81 ± 0.10^{bdef}	0.74 ± 0.09^{bcde}	0.10 ± 0.08^{bdef}	1.74 ± 0.09^{bcde}	0.21 ± 0.14^{bcde}
pvalue	0.0230	0.0495	0.0051	0.0496	0.3301
Fvalue	3.425	2.766	4.811	2.766	1.213
Remark	S	S	S	S	NS

Non-HDL-C & AIP: Values in each column with different superscript letter (a, b) differ significantly ($p<0.05$) when comparing Group A with other groups. Values in each column with different superscript letter (c, d) differ significantly ($p<0.05$) when comparing Group B with others groups. Values in each column with same superscript letter e and f do not differ significantly ($p<0.05$) when comparing C with other groups and Group D with E respectively.

AC & CRI-1: Values in each column with different superscript letter (a, b) differ significantly ($p<0.05$) when comparing Group A with other groups. Values in each column with same superscript letter c, d, and e do not differ significantly ($p<0.05$) when comparing Group B with others groups, Group C with other groups and Group D with group E respectively.

CRI-11: Values in each column with different superscript letter (a, b) differ significantly ($p<0.05$) when comparing Group A with other groups. Values in each column with different superscript letter c, d and e do not differ significantly ($p<0.05$) when comparing Group B with others groups, group C with other groups and Group D with E respectively. S=Significant, NS= Not Significant at $p<0.05$

4. DISCUSSION

Cardiovascular diseases have been a major cause of death especially in developing countries.[24][25][26] Several studies have indicated that there is an increased rate of death due to cardiovascular complications in developing countries especially in Africa and Asia.[24][26] Similarly, it has also been reported that several factors can trigger cardiovascular risks viz-a-viz genetic deposition, lifestyle, dieting and environmental exposures.[26][27] However, in this work, the influence of tartrazine food dye on cardiovascular risk indices was evaluated.

From the result when group A was compared with group B of tartrazine treated rats, there was a significant increase in Non HDL-C, AC and CRI-1 while non-significant increases were observed in AIP and CRI-11. Also, the comparison of group A and group C indicated significant increases in AC, AIP and CRI-1 while group A and D comparison showed significant increases in all the atherogenic indices except CRI-11. Finally, the comparison of Group A and E indicted significant increases in all the atherogenic indices considered.

The significantly increased atherogenic indices compared to control suggests an overall increase in atherogenic lipid fractions such as TC, LDL-C and VLDL-C which in turn are implicated in obesity and dyslipidemia as cardiovascular risk factors. This finding is in line with the reports of[25][26][26], reported increases in atherogenic indices in post menopausal women in which similar increases were seen in their atherogenic lipid parameters.

The significant increases observed in the atherogenic indices especially at high doses compared to the control could also be as a result of increased lipid peroxidation in the liver due to oxidative stress induced by the azo dye tartrazine. This finding is concurring with the report of.[1] They reported increases in oxidative markers when rats were treated with tartrazine dye at low and high doses. According to[28], oxidative stress due to azo dyes arises from the metabolism of azo bonds by the liver and intestinal microbial interactions yielding oxidative reactive species such as aromatic amine, aryl amine, n-hydroxyl groups and superoxides.[2], reported that reactive oxygen species are associated with oxidative damages such as cell membrane distortion and leakages, lipid peroxidation, chromosomal aberration, mutagenic and carcinogenic effects. Peroxidation of lipids leads to hyperlipidaemias which has been linked with

atherosclerosis which serves as one of the major precursors of coronary heart diseases.

AIP is an important marker of cardiovascular disorders which could serve as an indicator of the lipoprotein atherogenicity and a risk of developing cardiovascular disease such as myocardial infarction.[15][16] The increases seen in AIP when compared to control group probably indicates an increased in TG particles and a probably reduced HDL-C fractions in the plasma. An increase in TG (hypertriglyceridaemia) has been implicated in cardiovascular disturbances. This is also in line with the reports of.[11] They reported that increase in TG is an individual indicator of cardiovascular complication. These increases seen in AIP could be due to activities of reactive oxygen species having a retrogressive effect on hepatocellular enzymes such as lecithin cholesterol transferases and hepatic lipoprotein lipase which are useful enzymes in the catalysis of cholesterol and triglycerides respectively. This report is also in accordance with the findings of.[1][28][29] They also reported that deficiencies of these enzymes could induce dyslipidaemia which is a major risk factor for cardiovascular diseases.

The observed increase in AC directly reflects atherogenic potential of the entire line of lipoprotein fractions which are strongly associated with cardiovascular diseases. The increase in AC seen especially at higher doses reflects an increase in TC and especially LDL-C and reduced HDL-C. Increased TC and LDL-C as well as reduced HDL-C are atherogenic pattern of lipid fractions implicated in cardiovascular disease. This finding is in agreement with the findings of.[26]

The significant increases seen in CRI especially that of CRI-1 in the tartrazine treated rats compared to control could also be tied to increases seen in TC and LDL-C in the treated rats as a result oxidative induced lipid peroxidation probably leading to increase TC and LDL since CRI-1 and CRI-2 are ratio of TC to HDL-c and LDL-C to HDL-C respectively. This finding is also supported by the reports of.[1][26][30] According to[31], the risks of developing cardiovascular disorder correlates with increase in the plasma levels of lipid fractions such as TC, TG and LDL-C and probably a normal or decrease HDL-C concentration.

5. CONCLUSION

The study revealed that tartrazine orally administered at various high concentrations beyond its acceptable daily intake (ADI) induced elevated levels of atherogenic indices which are indicators of cardiovascular risks. Similarly, the study also showed that though the individual lipid parameters might appear normal but calculation of atherogenic indices could reveal risks of cardiovascular diseases. Therefore, the use of tartrazine azo dye in food especially in high doses beyond its acceptable daily intake should be avoided.

REFERENCES

1. Amin H, Abdel Hameid II AH, Abd Elsttar KA. Effect of food azo dyes Tartrazine and Carmoisine on biochemical parameters related to renal, hepatic function and oxidative stress biomarkers in young male rats. Food and Chemical Toxicology, 2010; 48: 2994–2999.
2. Sasaki YF, Kawaguchi S, Kamaya A, Ohshima M, Kabasawa K, Iwama K, Taniguchi K, Tsuda S. The comet assay with 8 mouse organs: Results with 39 currently used food additives. Mutation Research, 2002; 519: 103-119.
3. Devlin J, David TJ. Tartrazine in atopic eczema. Archives of Disease in Childhood, 1992; 6(2): 119-129.
4. Borzelleca JF, Hallagan JB. A chronic toxicity/carcinogenicity study of FD & C Yellow No.5 (Tartrazine) in mice. Food Chemical Toxicology, 2002; 26: 189-194.
5. Patterson RM, Butler JS. Tartrazine induced chromosomal aberrations in mammalian cells. Food and Cosmetics Toxicology, 1982; 20(4): 461-465.
6. Usoro CAO, Adikwuru CC, Usoro IN, Nsonwu AC. Lipid profile of postmenopausal women in Calabar, Nigeria. Pakistan Journal of Nutrition, 2006; 5: 79-82.
7. Superko HR, King S. Lipid management to reduce cardiovascular risk: a new strategy is required. Circulation, 2008; 117: 560 –568.
8. Yusuf S, Hawken S, Öunpuu S. Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the interheart study): Case-control study. Lancet, 2004; 364: 937– 952.
9. Bhardwaj S, Bhattacharjee J, Bhatnagar MK, Tyagi S. (2013). atherogenic index of plasma, castelli risk index and atherogenic coefficient- new parameters in assessing cardiovascular risk. International Journal of Pharmacology and Biological Science, 2013; 3(3): 359-364.
10. Dobiásová M, Frohlich J. The Plasma Parameter Log (TG/HDL-C) as an Atherogenic Index: Correlation with Lipoprotein Particle Size and Esterification Rate in apoB-Lipoprotein-Depleted Plasma. Clinical Biochemistry, 2001; 34: 583 – 588.
11. Gaziano JM, Hennekens CH, O' Donnell CJ, Breslow JL, Buring JE. Fasting triglycerides, high density lipoprotein, and risk of myocardial infarction. Circulation, 1997; 96: 2520 - 2525.
12. Bambi AB, Rochitte CE, Favarato D, Lemos PA, da Luz PL. Comparison of non-invasive methods for the detection of coronary atherosclerosis. Clinics, 2009; 64(7): 675-682.
13. Suman U, Umeshchandra DG, Awantis SM. Atherogenic index of plasma (AIP) in postmenopausal women. Research Journal Pharmacology, Biological Chemical Science, 2012; 3(1): 519.
14. Dobiasova M. Atherogenic Index of Plasma [log triglyceride/high density lipoprotein-cholesterol]):

Theoretical and practical implications. Clinical Chemistry, 2004; 50: 1113-1115.
15. Veerendra KA, Nutakki V, Ramarao J. Dose-dependent impacts on the diagnostic efficacies of atherogenic lipids in adult Indian smokers. Journal of Clinical and Diagnostic Research, 2011; 5(7): 1352 - 1355.
16. Priya K, Desigamini K, Kavitha G, Rita-Mary A. (2011). Coronary artery disease. Journal of Clinical and Diagnostic Research, 2011; 5: 516-518.
17. Ascaso J, González P, Hernández A. Management of dyslipidemia in the metabolic syndrome: Recommendations of the Spanish high density lipoprotein Forum. American Journal of Cardiovascular Drugs, 2007; 7: 39–58.
18. Nimmanapalli HD, Ambika DK, Prabath KD, Vani N. Lipid ratios, atherogenic coefficient and atherogenic index of plasma as parameters in assessing cardiovascular risk in type 2 diabetes mellitus. International Journal of Research in Medical Science, 2016; 4(7): 2863 - 2869.
19. Brehm A, Pfeiler G, Pacini G, Vierhapper H, Roden M. Relationship between serum lipoprotein ratios and insulin resistance in Obesity. Clinical Chemistry, 2004; 50: 2316 - 2322.
20. Martirosyan DM, Miroshnichenko LA, Kulokawa SN, Pogojeva AV, Zoloedov VI. Amaranth oil application for heart disease and hypertension lipid health. Cardiovascular Disease, 2007; 6: 1-3.
21. Flegg HM. An Investigation of the Determination of Serum Cholesterol by an Enzymatic Method. Annals of Clinical Biochemistry, 1973; 10: 79 - 80.
22. Stavropoulous WS, Crouch RD. A new Colourimetric Procedure for the determination of Serum Triglycerides. Clinical Chemistry, 1975; 20: 857 – 858
23. Friedewald WT, Levy RI, Fredrickson DS. Estimation of concentration of low density lipoprotein cholesterol in plasma without the use of the preparative ultracentrifugation. Clinical Chemistry, 1972; 18: 499 – 502.
24. Anderson TJ, Grégoire J, Hegele RA. Update of the Canadian cardiovascular society guidelines for the diagnosis and treatment of dyslipidemia for the prevention of cardiovascular disease in the adult. Canadian Journal of Cardiology, 2013; 29(2): 151-67.
25. Lafta AM. A comparative study for some atherogenic indices in sera of myocardial infarction, ischemic heart diseases patients and control. Journal of Natural Sciences Research, 2014; 4 (8): 96 – 103.
26. Ranjit PM, Guntuku G, Pothineni BR. New atherogenic indices: Assessment of cardiovascular risk in postmenopausal dyslipidemia. Asian Journal of Medical Science, 2015; 6(6): 25-31.
27. Bray GA, Nielsen SJ, Popkin BM. Consumption of high-fructose corn Syrup in beverages may play a role in the epidemic of obesity. American Journal of Clinical Nutrition, 2016; 79(6): 537 – 543.
28. Chung KT, Stevens SE Jr., Cemiglia CE. The reduction of azo dyes by intestinal microflora. Critique Review Microbiology, 1992; 18: 175-190.
29. Helal EGE. The protective role of Royal jelly against Sodium nitrite and Sunset yellow toxicity in Albino rats. Egyptian Journal of Hospital Medicine, 2001; 2: 121-137.
30. Aboel-Zahab H, El-Khyat Z, Sidhom G, Awadallah R, Abdelal W, Mahdy K. Physiological effects of some food coloring additives on rats. Lancet, 1997; 136(10): 615–627.
31. Chatterjea MN, Shinde R. Metabolism of lipids. In Textbook of Medical Biochemistry, 7th Edn. New Delhi: Jaypee Brothers: 2007.