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# Screening for the protective efficacy of two different extracts of Pithecellobium dulce in $CCl_4$ and Isoniazid induced hepatic injury in wistar rats.

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***Abstract:*** *Hepatoprotective effect of ethanolic and hexane:benzene:acetone (1:1:1) extracts of Pithecellobium dulce (EEPD and HBAPD respectively) in carbon tetra chloride ($CCl_4$) and Isoniazid (INH) induced toxicity in wistar rats was evaluated. Two parallel sets of experiments were performed. Hepatotoxicity was induced by either single dose of $CCl_4$ or regular doses of Isoniazid (100 mg/kg b.w., i.p.). In both experiments standard drug (100 mg/kg, p.o.) and test extracts (500 mg/kg. p.o. for both) were given for ten days. Serum liver function enzymes were estimated spectrophotometrically. Both $CCl_4$ and INH treated animals showed significant increased values of serum alkaline phosphatase, serum glutamic oxaloacitic transaminase, serum glutamic pyruvic transaminase and TBil ($P<0.001$ for all). However, these were found to be restored in one or both test extracts. The decreased values of these parameters were seemed to be less or more equivalent to standard drug. Present findings suggest that the PD extracts can ameliorate hepatic injury. However, different extracts showed similar protective activities against parameter used, in both experiments. Further investigations are needed to reveal the mode of action of the same.*

***Keywords:*** *$CCl_4$, hepatoprotective, Silymarin, Pithecellobium dulce, total bilirubin, Isoniazid*

## I. Introduction

Liver diseases are caused by toxins, drugs, microbial infections, foreign particals etc. Hepatic tissues are major site of that participate in almost all the biochemical pathways of growth, defence, nutrient supply and storage, energy provision, detoxification and drug metabolism [2,3]. Therefore, abnormality in its functioning directly affects the overall health [4]. Because hepatic cells are core site of detoxification and drug metabolism, it routinely exposed by certain toxin/drug/ foreign agents [3-5].

Most of the hepatotoxins cause tissue damage via generation of excessive free radicals [4]. Carbon tetrachloride ($CCl_4$) is one of the known pro-oxidative compounds which transformed into a trichloromethyl peroxyl radical ($+OOCCl_3$) in tissues [8] and causes harms to tissue lipids and proteins [9]. On the other hand, Isoniazid (INH) is one of the most commonly used antibiotic has been found to be associated with severe hepatotoxicity. Both above mentioned toxins have been found to be associated with increased production of free radicals. Moreover, treatments with these pro-oxidatives have been reported to cause membrane lipid and protein oxidation and exert deleterious effects on enzymatic system of the cells.

Antioxidants are well known protective agents which either inhibit generation of free radicals or by enhance their removal [7,9]. A number of herbs have been known to have antioxidative property [6,10,11,12]. *Pithecellobium dulce* (PD) is reported to be used as drug in dysentery, dermatitis, inflammation, leprosy, peptic ulcer, toothache, and venereal disease etc. in addition to this, evidence of free radical scavenging potential of the same have also been reported. Though, some reports on hepato-protective efficacy of PD have been documented, but available data also showed disparity in the protective potencies of herbs in different extraction medium. Since, different extraction medium contain different active constituents so medicinal properties of the same herb vary with extraction system. In present study, ethanolic and hexane:benzene:acetone (1:1:1) leave extracts of *Pithecellobium dulce* (EEPD and HBAPD respectively) were investigated for their relative efficacies against CCl4 and INH induced systems. For this, Enzymatic activities such as serum alkaline phosphatase (ALP), serum glutamic oxaloacitic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT) and total biliruin (TBil) were taken as test parameters. In both experiments, silymarin was used as standard drug. Tissue histology was performed to validate the results.

## II. Materials and methods

### *1.1 Chemicals*

All chemicals were of the highest commercially available purity. The bark of *Syzygium cumini* were collected from D-1 University campus, Dewas road, Ujjain, M.P., India. The identification of plant was done in Dept of Botany, Safia College of science, Bhopal. (M.P.) and the voucher specimen 436/ Bot /saf/13, was deposited in the Safia College of Science Bhopal (M.P.).

***2.2 Preparation of extracts***

The leaves of PD were air dried and ground to a coarse powder. The powdered leaves were exhaustively extracted in soxhlet extractor with ethanol. Removal of solvent under reduced pressure afforded solid mass. The yield of ethanolic extract was stored for further research. To prepare HBAPD, the powdered leaves were exhaustively extracted is in soxhlet extractor with hexane: benzene: acetone, (1:1:1). Removal of solvent under reduced pressure afforded solid mass. Thus obtained extracts was stored for further research.

***2.3 Animals***

Healthy in-bred albino wistar rats of either sex (2-2.5 months old) were housed in polypropylene cages under constant temperature (27±2°C) and photo-schedule (14 h light and 10 h dark). They were provided rodent feed (Golden Feeds, New Delhi, India) ad libitum and had free access to boiled drinking water. The approval of departmental ethical committee for handling and maintenance for experimental animals was also obtained before starting the experiments.

***2.4 Induction of hepatotoxicity***

Hepatic injury was induced by single oral administration of $CCl_4$ mixed with olive oil as vehicle in 1:1 ratio (3 ml/kg of rat body weight). While in other experiment regular doses of INH (100 mg/kg b.w., i.p.) were given to healthy animals for successive ten days.

***2.5 Acute oral toxicity study***

Acute oral toxicity study was carried out in young healthy female mice using the 'Limit dose test of up and down procedure' (UDP) according to Organization for Economic Corporation and Development guidelines 425. Dose up to 2000 mg/kg body weight (bw) was given in an increasing dose order and animals were checked for general behavioural, physical and autonomic changes.

**Experimental design- the overall research work was divided into two experiments.**

In the experiment 1st, a total of 30 rats were used, which were divided into 5 groups having 6 animals in each group as follows: Group I: Normal control rats received 1ml/100gm of 0.5% CMC using an intragastric tube. Group II: Negative control rats received $CCl_4$ 3 ml/kg, p.o. only once at day 1. Group III: Rats received $CCl_4$ 3 ml/kg, p.o. and Silymarin (100mg/kg, p.o.), designated as STAND group. Group IV: Rats received $CCl_4$ 3 ml/kg, p.o. and ethanolic extract of *Pithecellobium dulce*, (500mg/kg p.o.), desinated as EEPD Group. Group V: Rats received $CCl_4$ 3 ml/kg, p.o. and hexane:benzene:acetone (1:1:1) extract of *Pithecellobium dulce*, (500mg/kg p.o.), desinated as HBAPD Group.

In the experiment 2nd, a total of 30 rats were used, which were divided into 5 groups having 6 animals in each group as follows: Group I: Normal control rats received 1ml/100gm of 0.5% CMC using an intragastric tube. Group II: Negative control group given isoniazid (100mg/kg, i.p.), designated as induced group. Group III: Rats received isoniazid 100mg/kg, i.p. and silymarin (100mg/kg, p.o.), designated as stand group. Group IV: Rats received isoniazid 100mg/kg, i.p. and ethanolic extract of *Pithecellobium dulce*, (500mg/kg p.o.), desinated as EEPD Group. Group V: Rats received isoniazid 100mg/kg, i.p. and hexane:benzene:acetone (1:1:1) extract of *Pithecellobium dulce*, (500mg/kg p.o.), desinated as HBAPD Group.

At the end of the experiments on 10th day, animals were kept on overnight fasting and blood was collected by orbital puncture method. This blood was then allowed to clot for 30 minutes at room temperature. The serum was separated by centrifugation at 3000 rpm at 30°C for 15 minutes.

***2.6 Biochemical estimation***

The serum samples were analyzed spectro-photometrically for ALP, SGOT, SGPT and total bilirubin (TBil) levels using standard kits (Span diagnostics Ltd). Activity of SGOT and SGPT were measured at 505 nm and is expressed as IU/ L of serum. In ALP assay the blue color developed which was read at 510 nm against blank and the activity is expressed as IU/L of serum. To estimate the TBil readings were taken at 540 nm, the level of total bilirubin was expressed as mg/ dL of serum [20].

***2.7 Histological study***

Histopathological study was done using protocol of Deepa and Varlakshmi [21]. Tissues were fixed in 10% formaldehyde and then dehydrated in descending grades of isopropanol, cleared in xylene, and then embedded in molten paraffin wax. These fixed tissues were subsequently cut in to 5 µm thick sections using microtome. The ribbons were then stained with hematoxylin and eosin and viewed under light microscope to study histopathological changes.

***2.8 Statistical analysis***

Data are expressed as mean ± SE. Statistical analysis was done by using one -way ANOVA followed by unpaired student's t-test and p-values of 5% and less were considered as significant.

## III. Results and discussion

In acute toxicity test, both extracts were found to be safe upto 2000 mg/kg body weight. While, In first experiment, As compared to normal control animals $CCl_4$ induced group showed significant increased values of serum ALP, SGOT, SGPT and TBil (P<0.001, for all). As already established elsewhere, here also treatment with standard drug significantly reduced the values of all above mentioned parameters (P<0.001 for all). In case of EEPD and HBAPD drug therapies significant protection were observed (at least P<0.01 for all) than $CCl_4$ induced animals, but maximum reduction of serum ALP and TBil was seen in EEPD, while, HBAPD administered animals exhibited maximum protection for SGOT and SGPT (P<0.05 than EEPD group).

In second experiment, Treatment of INH exhibited considerable increase in serum values of all test parameters P<0.001) while parallel drug therapy of silymarin or herbal extracts showed drastic decrease in almost all of the used parameters (at least P<0.05 for all). here, animals kept on EEPD therapy showed maximum restoration in ALP, SGOT (P<0.001 for both) and SGPT (P<0.01) than HBAPD group. However, for TBil no significant differences were seen among different extracts treated groups in both experiments.

**Table – 1:** Acute toxicity study of AEBM, EEBM and BAEBM bark.

| S. No. | Extract used | 100 | 200 | 500 | 1000 | 2000 |
|---|---|---|---|---|---|---|
| 1 | EEPD | All animal survived | All animal survived | All animal survived | All animal survived | All animal survived |
| 2 | HBAPD | All animal survived | All animal survived | All animal survived | All animal survived | One died and re survived |

Where, (no. of animals in each group=3) drug doses in mg/kg body weight. EEPD (ethanolic extracts of *Pithecellobium dulce*) and HBAPD (hexane:benzene:acetone (1:1:1) extract of *Pithecellobium dulce*).

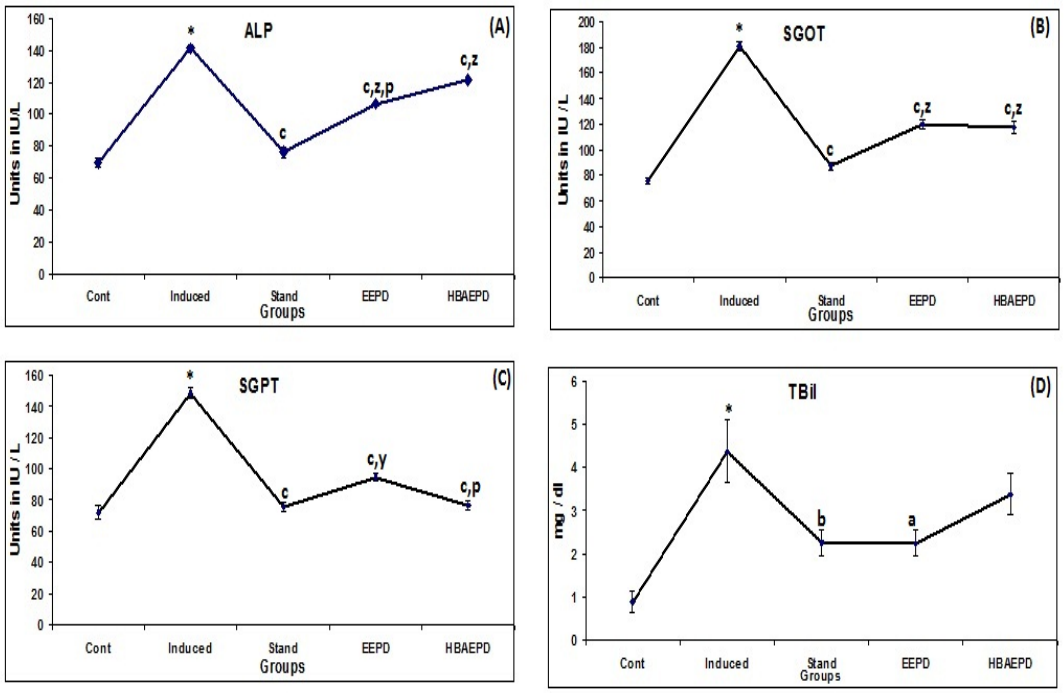


**Figure 1:** Effects on different parameters. Cont (Normal control), Induced ($CCl_4$ treated), STAND ($CCl_4$ +Silymarin), EEPD ($CCl_4$+ ethanolic extract of PD) and HBAPD ($CCl_4$+ hexane:benzene:acetone (1:1:1) extract of PD). Each bar represents the mean±SE (n=6), *P<0.001, as compared to normal control, $^{a}$P<0.05, $^{b}$P<0.01 and $^{c}$P<0.001 as compared to $CCl_4$ treated group, $^{x}$P<0.05, $^{y}$P<0.01 and $^{z}$P<0.001, as compared to standard drug while $^{P}$P<0.05 increased than other herbal extract treated group.

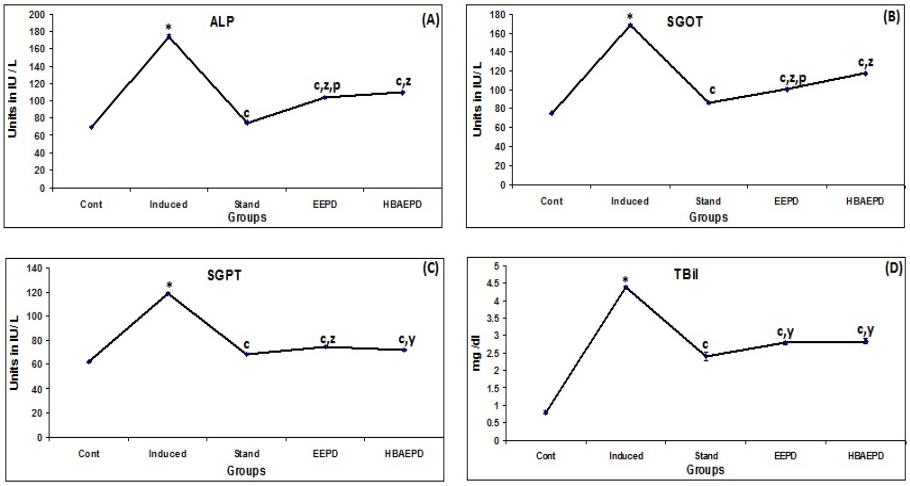


**Figure 2: 1:** Effects on different parameters. Cont (Normal control), Induced (INH treated), STAND (INH +Silymarin), EEPD (INH+ ethanolic extract of PD) and HBAPD (INH+ hexane:benzene:acetone (1:1:1) extract of PD). Each bar represents the mean±SE (n=6), *P<0.001, as compared to normal control, [a]P<0.05, [b]P<0.01 and [c]P<0.001 as compared to INH treated group, [x]P<0.05, [y]P<0.01 and [z]P<0.001, as compared to standard drug while [P]P<0.05 increased than other herbal extract treated group.

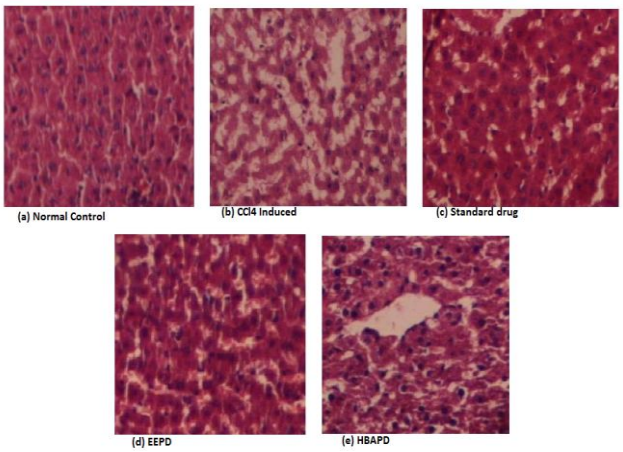


**Figure 3:** Histology of different experimental animals. Where, Cont (Normal control), Induced ($CCl_4$ treated), STAND ($CCl_4$ +Silymarin), EEPD ($CCl_4$+ ethanolic extract of PD) and HBAPD ($CCl_4$+ hexane:benzene:acetone (1:1:1) extract of PD).

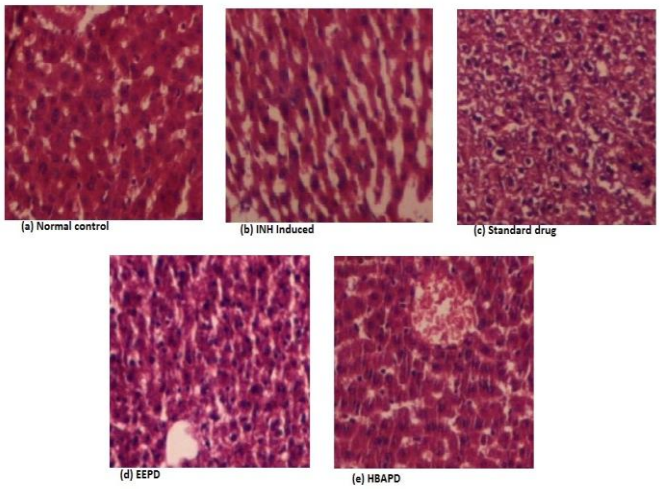


**Figure 4:** Histology of different experimental animals. Where, Cont (Normal control), Induced (INH treated), STAND (INH +Silymarin), EEPD (INH+ ethanolic extract of PD) and HBAPD (INH+ Hexane:Benzene:Acetone (1:1:1) extract of PD).

## IV. Conclusion

The leaves has been known to contain high amount of protein, crude fibre, cyclitol, dulcitol, Octa-cosanol, β - D- glucoside of a- spinastero , a- spinasterol and kaempferol-3- rhamnoside and numbers of polyphenolic and flavonoids. these chemicals are known to exert anti-oxidative, antidiabetic, antibacterial and anti-cancer activities. The present work was aimed to reveal the better protection of PD extracts against $CCl_4$ and INH induced hepato-toxicity in rats. Finding revalidate the safe and non-toxic effects of the test extracts with acute toxicity assay, as also observed earlier.

Induction of oxidative injury with the $CCl_4$ treatment has already been reported earlier [6,8,9]. This result in excessively generation of free radicals, these may cause oxidative damages to cellular lipids, proteins and DNA. Free radicals also lead the leakage of cytosolic proteins into the blood stream [18,21]. This may be uses as reliable markers of hepato-cellular damage. In this investigation also in $CCl_4$ induced rats the increased levels of SGOT, SGPT, ALP and TBil indicated severity of hepatocellular damage [8, 22]. The INH used also found to provoke oxidative injury particularly in liver cells […], also resembles with earlier investigations [..]

Clinically, enhanced level of TBil indicated hepatobiliary disease and/or severe disordered hepatocellular functions [25]. The extracts mediated control of the increased bilirubin level suggests the possibility of the extracts being able to stabilize biliary dysfunction. Parallerly, ALP is a hydrolase enzyme chiefly found in the cells lining the biliary ducts of the liver. Increased level of ALP indicates large bile duct obstruction, intrahepatic cholestasis, or infiltrative diseases of the liver [13,17]. In addition, SGOT and SGPT both are transaminase enzymes and play central role in amino acid metabolism. Both of these are found in the different body's organs such as liver, heart, skeletal muscle, kidneys, brain, and red blood cells. Serum SGOT and SGPT level, and their ratio (SGOT/SGPT ratio) are frequently measured clinically as biomarkers for liver health. Their increased level has been linked with abnormal liver functions, though these are not very specific to liver disease. Too the toxin treated animals showed increased levels of these enzymes. Conversely, extract therapies attenuated the increased level of these enzymes in serum. Recovery towards the normalization suggests that these extracts caused parenchymal cell regeneration in liver, thus protecting membrane fragility and thereby decreasing enzyme leakage [27].

Histopathological examination of liver sections of the normal control group demonstrated normal cellular architecture with separate hepatic cells and a central vein. While, both induced groups exhibited disarrangement of hepatic cells with intense necrosis. The liver sections of the rat treated with different drug therapies showed more or less recovery of the tissue damage than induced group.

Here, all three extracts were observed to be significantly effective against both toxins. Earlier studies showed that PD leave posses benenficial polyphenolic compound, flavonoids, tennins, and other protective bioactive chemicals. Thus the presence of these active ingredients could be considered responsible for the hepatoprotective activity of leave's extracts. Further, comparison of with silymarin indicated that in both experiments, both extracts were nearly equal effective as standard drug used. In addition, both EEPD and HBAPD were found to effective against different serum parameters used. These findings revealed that different extracts possess different active compounds which served via different mechanism. Therefore, to get maximum benefits, either additional alteration in the composition of extracts or mixed extracts therapy could be applied.

In conclusion, treatment $CCl_4$ and INH both responsible for drastic elevated values of liver stress markers, indicated severe hepoto-toxic events, which were observed to be completely recovered in silymarin drug therapy. Though, the three extracts posses appreciably effective protection against both toxins but for most of the parameters, these were comparatively less effective than standard drug. In addition, EEPD was seemed to more effective in some test parameters than HBAPD. Thus, assay dependent protective potencies of the test extracts possibly indicated the presence of diverse group of active compounds in them which served via different pathways. Further investigations are needed to confirm the findings I addition to this altered extraction method or mixed extracts therapy could be analysed for their possible better amelioration.

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## References

[1]. Sawant A, Mankeshwar R, Shah S, Raghavan R, Dhongde G, Raje H, et al. Prevalence of metabolic Syndrome in Urban India. Cholesterol 2011; 2011:1-7.
[2]. Pandit K, Goswami S, Ghosh S, Mukhopadhyay P, Chowdhury S. Metabolic syndrome in South Asians. Ind J Endocrinol Metab 2012; 16:44-55.
[3]. Mohan V, Deepa M, Deepa R, Shanthirani CS, Farooq S. Secular trends in the prevalence of diabetes and impaired glucose tolerance in urban south India – The Chennai urban rural epidemiology study. Diabetalogia 2006; 49:1175-78.
[4]. Agarwal N, Joshi S, Deshpande VK, Biswas DA. Correlation between glycated haemoglobin and glucose testing for diabetes mellitus screening. Indian J Med Sci 2013;67:149-54.

[5]. Durgawale P, Patil S, Shukla PS, Sontakke A, Kakade S, Yadav S. Evaluation of reference intervals of serum lipid profile from healthy population in western Maharashtra. Ind j Clin Biochem 2009; 24:30-5.
[6]. Gupta R, Sharma KK, Gupta A, Agrawal A, Mohan I, Gupta VP, et al. Persistent high prevalence of cardiovascular risk factors in the urban middle class in India: Jaipur Heart Watch-5. Indian Heart J 2012; 60:11-6.
[7]. Haffner SM. Insulin resistance, inflammation, and the prediabetic state. Am J Cardiol 2003; 18:18-26.
[8]. Sattar N. Revisiting the links between glycaemia, diabetes and cardiovascular disease. Diabetologia. 2013;56:686-95.
[9]. Parmar HS, Kar A. Protective role of Citrus sinensis, Musa paradisiaca, and Punica granatum peels against diet-induced atherosclerosis and thyroid dysfunctions in rats. Nutr Res 2007; 27:710–18.
[10]. Mohan, Pradeepa R. Epidemiology of diabetes in different regions of india. Health Administrator 2009; 22 : 1- 18.
[11]. Ashavaid TF, Todur SP, Dherai AJ. Health Status of Indian Population - Current Scenario. J Assoc Physicians of India 2004; 52 :363-69.
[12]. Das SK, Faruque ASG, Chisti MJ, Ahmed S, Mamun AA, Chowdhury AK, et al. Nutrition and Lipid Profile in General Population and Vegetarian Individuals Living in Rural Bangladesh. J Obes Wt Loss Ther 2012; 2:1-5.
[13]. Samatha P, Venkateswarlu M, Siva Prabodh V. Lipid Profile Levels in Type 2 Diabetes Mellitus from the Tribal Population of Adilabad in Andhra Pradesh, India. Clin Diagnos Res 2012; 6:590-2.
[14]. Idogun ES, Unuigbe EI, Ogunro PS, Akinola OT, Famodu AA. Assess¬ment of the serum lipids in Nigerians with type 2 diabetes mellitus complications. Pak. J. Med. Sci. (Part 1) 2007; 2:708-12.
[15]. Albrki WM, Elzouki AN Y, EL-Mansoury ZM, Tashani OA. Lipid profiles in Libian type 2 diabetes. J Sci Appls 2007; 1:18-23.
[16]. Sattar N. Revisiting the links between glycaemia, diabetes and cardiovascular disease. Diabetologia. 2013;56:686-95.
[17]. Gotto AM. Triglyceride as a risk factor for coronary artery disease. Jr Am J Cardiol 1998;82:22-5.
[18]. Lorenzo C, Hartnett S, Hanley AJ, Rewers MJ, Wagenknecht LE, Karter AJ, Haffner SM. . Impaired fasting glucose and impaired glucose tolerance have distinct lipoprotein and apolipoprotein changes: the insulin resistance atherosclerosis study. J Clin Endocrinol Metab 2013; 98:1622-30.
[19]. Penno G, Solini A, Bonora E, Fondelli C, Orsi E, Zerbini G, Trevisan R, Vedovato M, Gruden G, Laviola L,Nicolucci A, Pugliese G. Gender differences in cardiovascular disease risk factors, treatments and complications in patients with type 2 diabetes: the RIACE Italian multicentre study. J Intern Med. 2013; 274:176-91.
[20]. Durgawale P, Patil S, Shukla PS, Sontakke A, Kakade S, Yadav S. Evaluation of reference intervals of serum lipid profile from healthy population in western Maharashtra. Ind j Clin Biochem 2009; 24:30-5.
[21]. Sanyal D, Ghosh S, Mukherjee P, Mukherjee S, Chowdhury S. Dyslipidemia, metabolic syndrome, and liver enzymes in impaired glucose tolerance and new onset untreated, type 2 diabetes Indian subjects. Indian J Endocrinol Metab 2012; 16:434-5.
[22]. Saran S, Philip R, Gutch M, Tyagi R, Agroiya P, Gupta KK. Correlation between liver fat content with dyslipidemia and Insulin resistance. Indian J Endocrinol Metab 2013; 17:355-7.
[23]. Dobiasova 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 (FERHDL). Clin Biochem 2001; 34:583–8.
[24]. Das M, Saikia M. Estimation of reference interval of lipid profile in Assamese population. Ind J Clin Biochem, 2009; 24:190-93.
[25]. Malik VS, Popkin BM, Bray GA, Despres JP, Willett WC, Hu FB. Sugar-sweetened beverages and risk of metabolic syndrome and type 2 diabetes: a meta-analysis. Diabetes Care 2010; 33:2477–83.
[26]. Edwardson CL, Gorely T, Davies MJ, Gray LJ, Khunti K, Wilmot EG, et al. Association of sedentary behaviour with metabolic syndrome: a meta-analysis. Plos One 2012; 7:34916.
[27]. Tiwari S, Sadashiv, Paul BN, Kumar S, Chandra A, Dhananjai S, Negi MP. TNF-α Gene Expression in Subcutaneous Adipose Tissue Associated with HOMA in Asian Indian Postmenopausal Women. Horm Metab Res 2013 [in press].
[28]. Klop B, Elte JWF, Cabezas MC. Dyslipidemia in Obesity: Mechanisms and Potential Targets. Nutrients 2013; 5 : 1218-40;
[29]. Chopra SM, Misra A, Gulati S, Gupta R. Overweight, obesity and related non-communicable diseases in Asian Indian girls and women. Eur J Clin Nutr 2013; 67:688-96.
[30]. Misra A, Shrivastava U. Obesity and dyslipidemia in South Asians. Nutrients 2013; 15:2708-33.
[31]. Sadikot SM, Singh V. Managing diabetes in India: paradigms in care--outcomes and analysis in a comprehensive, clinical practice survey of Indian physicians. J Indian Med Assoc 2011; 109:844-8.