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IMPACT OF ARUMUGA CHENDOORAM ON LIVER MARKERS IN EXPERIMENTAL HYPOTHYROIDISM

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ABSTRACT

The aim of the study was to investigate the liver markers on the therapeutic efficacy of Arumuga chendooram in experimental hypothyroidism. In the present study, methimazole treated rats increased liver enzymes and decreased protein, albumin and globulin content.. Hypothyroid rats treated with Arumuga chendooram (Hypothyroid + Arumuga chendooram group) exhibited a remarkable restored in liver marker enzymes and protein profile. It can be concluded that in this study methimazole induced a model of hypothyroid associated with hepatic function markers disturbance in rats were observed. The hypothyroid and its associated problems in this study could be ameliorated by supplementation of Arumuga chendooram. The study showed that herbo-mineral drug significantly restored the activity of liver enzymes. Thus, the Arumuga chendooram possesses potential hepatoprotective effect

Keywords: Methimazole , Liver markers, Arumuga chendooram, Protein, Hypothyroid

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INTRODUCTION

Methimazole inhibits the synthesis of thyroid hormones and thus is effective in the treatment of hyperthyroidism. The drug does not inactivate existing thyroxine and triiodothyronine that are stored in the thyroid or circulating in the blood nor does it interfere with the effectiveness of thyroid hormones given by mouth or by injection. Methimazole is readily absorbed in the gastrointestinal tract, metabolized in the liver, and excreted in the urine. The methimazole reactive metabolite, glyoxal, is a well-known cytotoxic agent with capability of inducing oxidative stress and cellular dysfunction. It has been found that in addition to N- methylthiourea, as the proposed toxic metabolite of methimazole, glyoxal might also has a great role in methimazole-induced cytotoxicity. Glyoxal detoxification process is involved the effect of glyoxalase enzyme, which is a glutathione (GSH)-required process. GSH depleted cells and/or liver are reported to be very susceptible to methimazole adverse effects. The higher susceptibility of GSH-depleted cells to methimazole might be expectable by considering the role of GSH in detoxification of methimazole reactive metabolites such as glyoxal. The bioactivation of this drug in liver is

a proposed mechanism by which methimazole caused liver damage in contribution with other potential factors (Shangari N, O'Brien, 2004; Banach et al., 2009; Heidari et al., 2013).

MATERIALS AND METHODS

Animals

Male albino rats of Wistar strain approximately weighing 180-190g were used in this study. They were healthy animals purchased from the Indian Institute of Science, Bangalore. The animals were housed in spacious polypropylene cages bedded with rice husk. The animal room was well ventilated and maintained under standard experimental conditions (Temperature 27 ± 2º C and 12 hour light/dark cycle) throughout the experimental period. All the animals were fed with standard pellet diet and water were provided *ad libitum*. They were acclimatized to the environment for one week prior to experimental use. The experiment was carried out according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India.

Chemicals

Nitroblue tetrazolium (NBT), ethylenediaminetetra acetic acid (EDTA), Trichloro acetic acid (TCA), Thiobarbituric acid (TBA), 5,5'-dithio-bis (2-nitrobenzoic acid), glutathione (reduced), glutathione (oxidized) and Nicotinamide adenine dinucleotide phosphate ($NADP^{+}$/NADPH) were purchased from Sigma Chemical Company (St. Louis, MO, USA). All other chemicals used were of analytical grade and were obtained from Glaxo Laboratories, Mumbai, India, and Sisco Research Laboratories, Mumbai, India.

Preparation of Arumuga chendooram

The Siddha medicine Arumuga chendooram was prepared at its different stages of preparation in departmental laboratory with the help of a traditional siddha medical practioners as per the IMCOPS method.

In the first stage of the preparation of Arumuga chendooram. Five parts of purified mercury (Suththi seitha rasam), nine parts of purified sulphur (Suththi seitha kanthakam), seven parts of purified lode stone (Suththi seitha kantham), twelve parts of purified iron filing (Sutht.hi seitha ayapodi), four parts of rock salt (Induppu) and eight parts of desiccated borax (Poriththa venkaram) were ground with sufficient quantity of aloe juice (Kumari charu for five days continuously. This was then made into small cakes and dried. It was then sealed in discs and burnt for 24 hours. If the colour of the chendooram does not appear as dark purple the grinding and burning are usually repeated equal to pH and then attractive particle interations predominate which may influence the drug delivery.

Experimental design

Body weights of the animals were recorded and they were divided into **4** groups of 6 animals each as follows. First group was normal rats fed with standard diet and served as a control which received saline. Second group was negative control administered Methimazole (40mg/kg) induced experimental hypothyroidism for 40 consecutive days Third group was treatment group treated with Methimazole (40mg/kg) along with Arumuga chendooram (10mg/kg) for 40 days. Fourth group was positive control treated with Methimazole (40mg/kg) along with standard throxine sodium (20µg/kg) for 40 days.

Collection of samples

On completion of the experimental period, animals were anaesthetized with thiopentone sodium (50mg/kg). The blood was collected with or without EDTA as anticoagulant. Blood, plasma and serum were separated for the estimation of various biochemical parameters. The Liver, heart and adipose tissues were dissected out, washed in ice-cold saline, and weighed. A known weight of them was used for homogenate preparation and used for various biochemical analyses.

Biochemical estimation

The serum GOT and GPT were estimated by the method of Reitman and Frankel (1957). The activity of serum lactate dehydrogenase was measured by the method of King (1965). Acid phosphatase activity was measured by the method of Annon (1963). The serum alkaline phosphatase activity was estimated by the method of Kind and King's (1954). Protein was estimated by the method of Lowry *et al.* (1951).

Albumin was estimated by the method of Rodkey (1965).Serum creatine phospho kinase estimated by the method of Ochei & Kolhatkar (2000).

RESULTS AND DISCUSSION

Liver function tests help in the diagnosis of any abnormal/normal condition of liver. Leakage of cellular enzymes into plasma indicates the sign of hepatic tissue damage. Generally measurement of alanine aminotransferase (ALT), LDH, CK, ACP, ALP and GGT are used as an important diagnostic marker to indicate liver injury due to hepatotoxins. Hepatic injury caused by methimazole treated generally reflects instability of liver metabolism which leads to distinctive changes in the serum enzyme activities. Intracellular enzymes, such as transaminases, ALP, ACP, LDH, CK, GGT and Protein are useful indicators for liver and thyroid functions their increased levels are indicators of liver and thyroid dysfunction. In the present results demonstrated that Arumuga chendooram treatment significantly attenuated the increased activities of these enzymes (Table 1 and Fig.

1 and 2). Arumuga chendooram helps in regeneration in liver and thus protecting membrane integrity and thereby minimizing enzyme leakage. This result suggested that Arumuga chendooram possess potential hepato-protective activity.

Table 1 Effect of Arumuga chendooram on liver markers in experimental rats

Parameters	Group I	Group II	Group III	Group IV
Protein (gm/dl)	6.19±0.43[a]	5.39±0.37[b]	5.59±0.39[a]	6.52±0.45[a]
Albumin gm/dl)	3.02±0.21	2.54±0.17	2.97±0.20	3.18±0.22
Globulin gm/dl)	3.17±3.35	2.85±0.19	2.62±0.18	3.34±0.23
SGOT (IU/dl)	47.92±3.35	68.42±4.78	52.28±3.65	58.46± 4.09
SGPT (IU/dl)	29.55±2.06	42.88±3.00	32.66±2.50	35.77± 2.50
ALP (IU/dl)	73±5.11	98±6.86	71±4.97	74± 5.18
ACP (IU/dl)	31±2.17	47±3.29	32± 2.24	39±2.73
CK (IU/dl)	126.32±8.82	182.35±12.74	132.45±9.32	130.55±9.05
LDH (IU/dl)	223.54±14.32	370.62±25.40	235.44±15.67	234.12±14.12
GGT (IU/dl)	23.95±1.67	30.20±2.11	24.47±1.71	26.56±1.85

Each value is expressed as mean ± SD for six rats in each group
[a]As compared with group II, [b]As compared with group I, III and IV. [*]p<0.05.

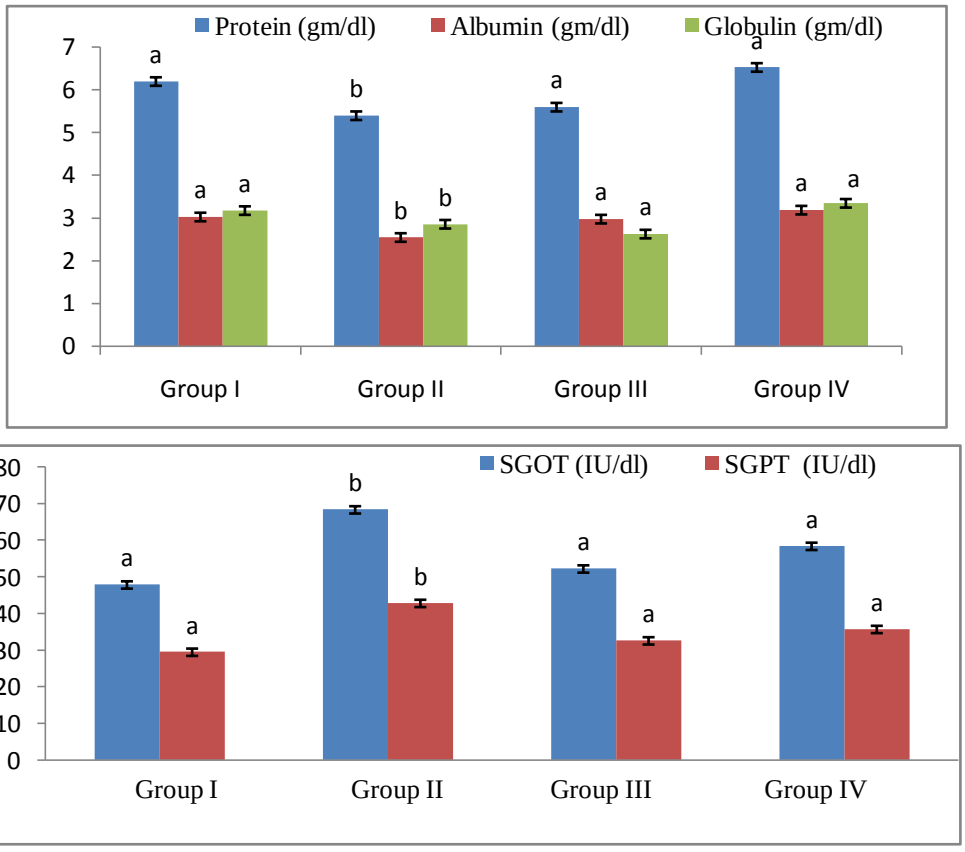

Figure 1 Effect of Arumuga chendooram on liver markers in experimental rats

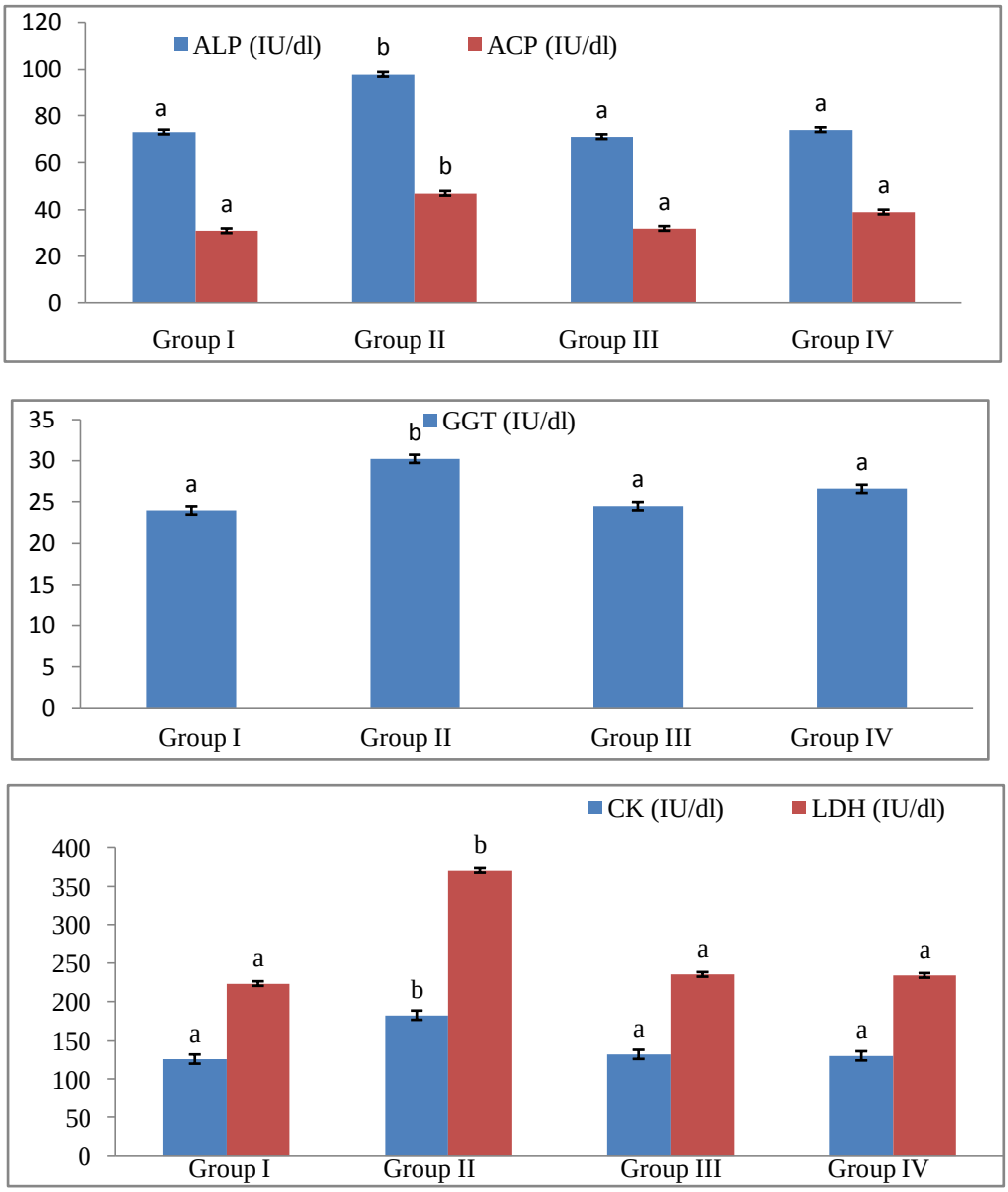

Figure 2 Effect of Arumuga chendooram on liver markers in experimental rats

Each value is expressed as mean ± SD for six rats in each group. [a]As compared with group II, [b]As compared with group I, III and IV. *p<0.05.

The liver is the key organ regulating homeostasis in the body. It is involved with almost all the biochemical pathways related to growth, fight against disease, nutrient supply, energy provision and reproduction (Ward and Daly, 1999). The liver is expected not only to perform physiological functions but also to protect against the hazards of harmful drugs and chemicals. Inspite of tremendous scientific advancement in the field of hepatology in recent years, liver problems are on the rise. Jaundice and hepatitis are two major hepatic disorders that account for a high death rate (Pang et al., 1992). Presently, a few hepatoprotective drugs and that too from natural sources, are available for the treatment of liver disorders. Hence, people are looking at the traditional systems of medicine for remedies to hepatic disorders

Studies suggest that metabolic activation of methimazole by cytochrome P450 and Flavin mono-oxygenase to N-methylthiourea and glyoxal is a necessary step for generation of liver damage. These reactive metabolites disrupt cellular function resulting in glutathione depletion and adduct formation (Reza Heidari et al., 2014). Metabolism dependent

hepatotoxicity of compounds like methimazole can be influenced by coadministration of enzyme inducers and inhibitors by altering reactive metabolite formation. This is exemplified by augmented toxicity of paracetamol by phenobarbitone induced hepatic enzymes while simultaneous administration of cimetidine with paracetamol proved to be hepatoprotective (Banu et al., 2007). Similarly, methimazole hepatotoxic potential is largely dependent upon the amount and activity of metabolizing enzymes and can be modified depending upon the nature of concomitantly used drug.

Protein metabolism is a major project of liver and a healthy functioning liver is required for the synthesis of the serum proteins. Hypoproteinemia is a feature of liver damage due to significant fall in protein synthesis. Serum albumin, the major plasma protein synthesized in the liver, is a clinically useful marker of hepatic synthetic function (Friedman *et al*., 1996). Albumin's protective effect has been attributed to its nonspecific binding of redox-active transition metal ions capable of catalyzing reactions that yield hydroxyl or hydroxyl-like radicals (Strubelt and Younes, 1994). Some evidence suggests that albumin may act more directly as a free radical scavenger or as a participant in scavenging reactions. Joe *et al.* (1999) reported that serum albumin acts as a major physiologic antioxidant. Studies with the albumin suggested that low serum albumin concentration was associated with a greater loss of muscle mass (Castaneda *et al*., 1993). In the present study, we observed the decreased level of protein and albumin in methimazole treated rats as compared with control rats. This may be due to impairment of kidney functions. Administration of Arumuga chendooram significantly increased in the level of albumin in methimazole treated rats.

Enzymes catalyze specific biochemical reactions in the body. Changes in their levels and properties alter the functional ability of an organism. The diagnosis of organ disease/damage is aided by measurement of a number of non-functional plasma enzymes characteristic of that tissue or organ. The amount of enzyme released depends on the degree of cellular damage, the intracellular concentration of the enzymes and the mass of affected tissue. The concentration of the enzymes released reflects the severity of the damage. ALP, ACP, GGT, SGOT and SGPT are enzymes normally present in the liver, heart, muscles and blood cells. They are basically located within hepatocytes. So when liver cells are damaged or die transaminases are released into blood stream, where they can be measured they are therefore of index of liver injury (Reitman and Frankel, 1957). Mild inflammatory conditions are also likely to release cytoplamic enzymes (Vasudha et al., 2006). In the present study, the increased activity of ALP, ACP, GGT, SGOT and SGPT in methimazole treated rats as compared with control rats. This elevated activity of these enzymes in methimazole treated rats due to inflammation in the liver. Administration of Arumuga chendooram significantly restored the activity of enzymes in methimazole treated rats.

Results obtained in this work revealed that methimazole caused biochemical alterations in liver of rats. In accordance with these results, Tashkandi et al., (2014) showed that methimazole induced hepatotoxicity in albino rats. They added that methimazole induced degenerative changes including congestion of blood vessels, appearance of inflammatory infiltrative cells, cytoplasmic vacuolization of the hepatocytes and necrosis. Moreover, ALP, ACP, GGT, SGOT and SGPT are levels increased in sera of the methimazole treated animals. It is known that the ALP, GGT, AST and ALT serum levels are indicative for hepatic function and their increase is correlated with the hepatic injury (Zhao et al., 2014). Other studies revealed that methimazole treatment caused liver damage (Cano-Europa et al.2011; Gallelli et al. 2015). It has been documented that methimazole treatment has induced liver damage and showed abnormality in the enzymes liver, in accordance with this statement the obtained results have confirmed that methimazole is a hazard material that cause damage and abnormality to the liver function and structure. The elevation of serum enzymes in hypothyroidism results from increased permeability of cell membranes, slower catabolism and hypothermia (Gaede, 1977; Jenkins, 1978; Giampietro, 1981). These are expected to increase progressively with the advancement of the disease.

Creatine kinase (CK) was first used as a diagnostic aid in progressive muscular dystrophy. It has since then become important clinical marker for muscle damage. The serum CK levels in healthy individuals depend on age, race, lean body mass and physical activity. Musculoskeletal disorders often accompany thyroid dysfunction. In addition to well-known observation that musculoskeletal disorders are common in patients with hypothyroidism, they are also observed in thyrotoxicosis and level of CK is altered in both these conditions (Saha et al., 2009).

The serum creatine kinase (CK) activity in healthy individuals depends on age, race, lean body mass and physical activity (Meltezer et al., 1971). It has since become an important clinical marker for muscle damage. Musculoskeletal disorders often accompany thyroid dysfunction. The association of myopathy with

both myxedema and thyrotoxicosis is well known (Rainsay, 1971) Concentrations of CK in serum are often increased in patients with primary hypothyroidism (Graig and Ross, 1963; Griffiths, 1963). The findings of this study confirm that elevated plasma CK activity is frequently increased in hypothyroidism. This study also indicates that CK activity correlates with the degrees of hypothyroidism as evident by the magnitude of the TSH. Elevated serum CK activity was observed in hypothyroid patients, and was higher in patients with overt hypothyroidism. The finding of decreased CK activity in Administration of Arumuga chendooram as compared with controls is in accordance with other studies (Aquaron et al., 1971; Griffiths, 1985).

Lactate dehydrogenase catalyses the pyruvate lactate concomitant of NADH and NAD+. has been that can raise LDH levels hypothyroidism a highly disorder. As thyroid hormones for normal development basal metabolic cells, can entire metabolism and can alter activity enzymes. There is regarding levels in thyroid disorders with studies stating that LDH can a parameter thyroid disorder (McGrowder, 2011). It noted various conditions the including which is prevalent are essential or organ growth, function and regulate the rate of all its alteration affects the the of serum negotiation the increased of LDH roid some be used as for screening the thyroid disorder. Liver, muscle and kidney metabolizes thyroid hormones and regulates their systemic endocrine effects; which suggests thyroid dysfunction may disturb liver, muscle, other organ function and vice versa (Biondi, 2005). Also the elevated LDH levels could reflect increased release and/or decreased clearance from the liver (Klein, 1984).

Studies have shown that LDH activity was increased in the hypothyroid states (Roti, 1980; Doran, 1978) The study found increase in LDH activity in hypothyroidism which correlates with the degree of hypothyroidsm. LDH activity have been reported to be increased in hypothyroidism (Griffiths, 1985; Fleisher et al., 1965). Treatment with Arumuga chendooram and thyroxine resulted not only in lowering of CK and LDH from an elevated level to normal.

It can be concluded that in this study methimazole induced a model of hypothyroid associated with hepatic function markers disturbance in rats were observed. The hypothyroid and its associated problems in this study could be ameliorated by supplementation of Arumuga chendooram. The study showed that herbo-mineral drug significantly restored the activity of liver enzymes. Thus, the Arumuga chendooram possesses potential hepatoprotective effects.

References

Annon K. (1963) The colorimetric determination of phosphatase- Sigma Technical Bulletin, *Sigma chemicals co., St Louis*, USA. Pp104-108.

Aquaron R, Boeuf G, Codacciona J.L. (1971) Serum creatinine in thyroid disorders. Ann Endocrinol. 32:142-56.

Banach M.S, Dong Q, O'Brien P.J. (2009) Hepatocyte cytotoxicity induced by hydroperoxide (oxidative stress model) or glyoxal (carbonylation model): prevention by bioactive nut extracts or catechins. *Chem Biol Interact* 178(1-3): 324-31.

Banu L. A, Begum H. A and Choudhury S. (2007) Effects of Cimetidine and Phenobarbitone on Paracetamol Induced Hepatotoxic Rats. *Bangladesh Journal of Physiology and Pharmacology*, 23(1): 13-15.

Biondi B, Palmieri E.A, Klain M. (2005) Subclinical hyperthyroidism: Clinical features and treatment options. *Eur. J. Endocrin*, 152: 1-9.

Cano-Europa, E., Blas-Valdivia, V., Franco-Colin, M., Gallardo-Casas, C.A., and Ortiz-Butron,R. (2011). Methimazole-induced hypothyroidism causes cellular damage in the spleen, heart, liver, lung and kidney, Acta Histochem, 113(1): 1-5.

Castaneda C, Charnley J.M, Evans W.J and Gim M.C. (1993) Elderly women accommodate to a low protein diet with losses of body cell mass, muscle function and immune response. *American Journal of Clinical Nutrition*, 62: 30-39.

Fleisher G.A, McConahey W.M, Pankow M. (1965) Serum creatine kinase, lactic dehydrogenase and glutamic-oxaloacetic transaminase in thyroid diseases and pregnancy. *Mayo Clin Proc*, 40: 300-11.

Gaede J.T. (1977) Serum enzyme alteration in hypothyroidism before and after treatment. *J Am Geriatr Soc*, 25: 199–201.

Gallelli,L., Staltari O, Palleria C, De Sarro G. and Ferraro M. (2015).Hepatotoxicity Induced by Methimazole in a Previously Health Patient. Current Drug Safety, 4(**3):** 204-206.

Giampietro O, Boni C, Carpi A, Buzzigoli. (1981) Monitoring of the serum levels of muscle enzymes during replacement therapy in hypothyroidism with myopathy. *J Nucl Med All Sci*, 25: 211–8.

Graig F.A, Ross G. (1963) Serum Creatine-phoaphokinase in thyroid disease. *Metabolism* 12; 57-9.

Griffiths P.D. (1963) Creatine-phosphokinase levels in hypothyroidism. *Lancet* 1: 894.

Heidari R, Babaei H and Eghbal M. (2013) Mechanisms of methimazole cytotoxicity in isolated rat hepatocytes. *Drug Chem Toxicol*, 36(4): 403-11.

Jenkins D.J. (1978) An investigation into creatine kinase and other plasma enzymes in thyroid disorders. *Clin Chim Acta*, 85:197–204.

Joe G.G, Vethanayagam, Green E.H, Rose R.C and Bode A.M. (1999) Glutathione-dependent ascorbate recycling activity of rat serum albumin. Free Radical Biology & Medicine. 26(11/12): pp. 1591–1598.

Kind E.J and King R.P.N. (1954) Determination of alkaline phosphatase activity by colorimetric method.*J. Clin. Path*, 7: 322.

King J. (1965) In practical clinical enzymology. D. Van Nostrand Co., London. Pp83-93.

Klein I and Levey G.S. (1984) Unusual manifestations of hypothyroidism. *Arch. Intern. Med*, 144: 123-128.

Lowry O.H, Rosenbrough N.J, Farr A.L and Randall R.J, (1951) Protein measurement with the Folin's reagent. *Journal of Biological Chemistry* 193: 265-276.

McGrowder D.A, Fraser Y.P, Gordan L, Crawford T.V and Rawlins J.M. (2011) Serum creatine kinase and lactate dehydrogenase activities in patients with thyroid disorders. Niger. *J. Clin. Pract*, 14 (4) : 454-459.

Meltezer H.Y. (1971) Factors affecting creatine phosphokinase levels in the general population: The role of race, activity and age. *Clin Chem Acta*, 33:165-72.

Ochei J and Kolhatkar K. (2000) Medical laboratory science. Theory and practice. *Tata McGraw-Hill publishing company lit. New Delhi*, p234-36.

Pang S, Xin X and Stpierre M.V. (1992) Determinants of metabolic disposition. *Ann Rev Pharmacol Toxicol*, 32: 625–626.

Rainsay I. (1974)Thyroid disease and muscle dysfunction. London: *Heinemann Medical Books Ltd*.

Rodkey FL., 1965. Direct spectrophotometric determination of albumin in human serum. Clinical Chemistry 11, 478-9.

Reitman S and Frankel S. (1957) A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminase. *Am J Clin Path* 25, pp: 56.

Reza Heidari H.N, Akram Jamshidzadeh, Mohammad Ali Eghbal and Narges Abdoli. (2014) An Overview on the Proposed Mechanisms of Antithyroid Drugs-Induced Liver Injury. *Advanced Pharmaceutical Bulletin*.

Roti E, Bandini P, Robuschi G, Emanuele R, Bolognesi R, Ciarlini E. (1980) Serum concentrations of myoglobin, creatine kinase, lactate dehydrogenase and cardiac isoenzymes in euthyroid, hypothyroid and hyperthyroid subjects. *Ric Clin Lab*, 10: 609-17.

Saha M, Sarkar P, Chattopadhyay R, Mukhopadhyaya M and Bhowmick K. (2009) Role of creatine kinase and its coenzymes as surrogate markers of thyroid function. IJMB 13(2): 10-4.

Shangari N, O'Brien P.J. (2004) The cytotoxic mechanism of glyoxal involves oxidative stress. *Biochem Pharmacol*, 68(7): 1433-42.

Strubelt O, Younes M and Li Y. (1994) Protection by albumin against ischaemia- and hypoxia-induced hepatic injury. *Pharmacology and Toxicology*, 75: 280-284.

Tashkandi, B., Saleh, H. Abdul Salam, H. (2014).The protective effect of vitamin E and selenium on methimazole-induced hepato-renal toxicity in adult rats. Life Science Journal. 11(10):893-899.

Vasudha KC, Nirmal Kumar A, Venkatesh T. (2006) Studies on the age dependent changes in serum adenosine deaminase activity and its changes in hepatitis. *Indian J Clin Biochem*, 21: 116-120.

Ward F.M, Daly M.J. (1999) Hepatic disease. In. Clinical Pharmacy and Therapeutics. Walker R, Edward C, Eds, Churchill Livingston, New *York*, 195-212.

Zhao J, Peng L, Geng C, (2014). Preventive effect of hydrazinocurcumin on carcinogenesis of diethylnitrosamine-induced hepatocarcinoma in male SD Rats. Asian Pac J Cancer Prev, 15 (5): 2115-21.