Journal of Advances in Medicine and Medical Research

23(5): 1-7, 2017; Article no.JAMMR.35540
ISSN: 2456-8899
(Past name: British Journal of Medicine and Medical Research, Past ISSN: 2231-0614, NLM ID: 101570965)

The Glutathione S-Transferase Activities in the Evaluation Acute Viral Hepatitis B and Chronic Alcoholic Liver Disease

S. D. Sawant[1*] and M. R. Mogarekar[2]

[1]Department of Biochemistry, Dr. V. M. Government Medical College, Solapur, Maharashtra, India.
[2]Department of Biochemistry, Swami Ramanand Tirth Rural Government Medical College, Ambajogai, Beed, Maharashtra, India.

Authors' contributions

This work was carried out in collaboration between both authors. Authors SDS designed the study, managed the analyses of the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author MRM guided the whole process of this research project. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JAMMR/2017/35540
Editor(s):
(1) Georgios Tsoulfas, Assistant Professor of Surgery, Aristoteleion University of Thessaloniki, Thessaloniki, Greece.
Reviewers:
(1) Ukegbu Chimere Young, University of Nigeria Nsukka, Nigeria.
(2) Igor Iskusnykh, University of Tennessee Health Science Center, USA.
Complete Peer review History: http://www.sciencedomain.org/review-history/20555

Original Research Article

Received 18th July 2017
Accepted 12th August 2017
Published 19th August 2017

ABSTRACT

Glutathione S-Transferase is an important hepatic detoxifying enzyme. Half-life of GST alpha in plasma is about1hr; its concentration follows the changes in the hepatocellular damage more rapidly than aspartate amino transferase (AST) or alanine amino transferase (ALT).
Aim and Objectives: To validate chronic alcoholic liver disease and acute viral hepatitis B could produce any significant changes in serum glutathione S-transferase activities.
Materials and Methods: A total of 120 samples were used in the present research. 20 hepatitis B patient 40 chronic alcoholic hepatitis patients and 60 age and sex matched control subjects. Serum GST activity and standard LFT (Liver Function Test) parameters done.
Results: GST activity showed a significantly increase (p value< 0.001) in chronic alcoholic liver disease patients (22.293±4.159 IU/L vs 20.127±4.789 IU/L) and in acute viral hepatitis (23.685±6.751 IU/L vs 20.127±4.789 IU/L). In multiple logistic regression analysis when GST

**Corresponding author: E-mail: drswatitalekar@gmail.com;*

measurement was added to a LFT, the diagnostic significance increased from R^2=0.792 to 0.884 in acute viral hepatitis B and from R^2= 0.843 to 0.849 in chronic alcoholic cirrhosis. Standard LFT parameters are significantly different in both groups. Area under ROC (Receiver Operative Characteristic Curve) is 0.952 for model having albumin+ ALT which is increased to 0.992 after addition of GST in previous model in acute viral Hepatitis B. While in alcoholic Hepatitis Area under ROC is 0.966 for model having albumin+ ALT which is increased to 0.974 after addition of GST in previous model.

Conclusion: GST activity may provide additional LFT measurement to the current battery of tests to differentiate acute hepatitis B and alcoholic liver disease from normal population and may improve the evaluation of Hepatitis B virus and alcoholic cirrhosis when added to routine LFT parameters.

Keywords: Glutathione S Transferase; hepatitis B; alcoholic liver cirrhosis; liver function test.

1. INTRODUCTION

Nowadays the alcoholic liver disease is a major cause of morbidity and mortality in India [1]. Glutathione S-Transferase (GST) is a hepatic detoxifying enzyme [2]. Wide hepatic distribution and short plasma half-life of this enzyme make its monitoring more useful than conventional biochemical liver function tests as a marker of hepatocellular damage [3]. It is released quickly in large quantities into the blood in hepatocellular damage. Half-life of GST alpha in plasma is about 1hr and its concentration follows the changes in the hepatocellular damage more rapidly than aspartate amino transferase (AST) or alanine amino transferase (ALT) [4].

Chronic liver diseases are classified as chronic alcoholic liver disease, chronic viral hepatitis and non alcoholic steatohepatitis and are characterised by the concomitant presence of oxidative stress and a severe inflammatory response [5]. Oxidative stress is thought to play a major role in the pathogenesis of both alcoholic and non-alcoholic fatty liver disease [6]. Oxidative stress precedes and is stakeholder in the evolution of ALD (Alcoholic liver disease) [7]. Hepatitis B is a viral infection that attacks the liver and can cause both acute and chronic disease HBV infection is one of the commonest infections in the world. According to WHO, a third of the world's population (2 billion people) has been infected with HBV and about 5% are chronically infected [8].

One study throws the focus on the pathophysiological link between HBV infection and hepatic inflammation, and this chain of events might contribute to early steps in HBV-associated liver carcinogenesis [9]. Free oxygen radicals take part in pathogenesis of chronic hepatitis B and C type in children as they decrease the antioxidant barrier efficiency [10].

Glutathione S-Transferase (GST) is a liver enzyme which showed properties making it useful in assessment of liver cell damage [11]. A number of studies demonstrated its early elevation in different hepatic insults, but its pattern in HCV was controversial [12]. Whereas no study was done in patients with HBV Hepatitis and ours is the first study showing the association of GST activity and HBV Hepatitis.

2. MATERIALS AND METHODS

Case group consist of patients with a diagnosis of acute liver disease consisted of 20 patients of hepatitis B, and 40 patients of chronic alcoholic liver disease based on clinical evidence, laboratory data and echography to evaluate splenomegaly or portal vein dilatation of either sex and age greater than 18 years. Control group consist of 60 healthy age and sex matched subjects.

Exclusion criteria included the use of supplemental vitamins, history of diabetes mellitus, coronary artery disease, renal disease, rheumatoid arthritis, cancer, systemic or local infection, the existence of alcohol intake, smoking habit, pregnancy, and non-alcoholic steatohepatitis. Blood samples were obtained following an overnight fasting state. Samples were withdrawn from a cubital vein with all aseptic precautions into a plain bulb. The serum was separated by centrifugation and were analysed for the estimation of following parameters. Biochemical parameters of liver function evaluated such as serum AST by Reitman & Frankel method.

ALT by Reitman & Frankel method, ALP by King and Kind method, albumin by bromocresol green method, total bilirubin by modified Jendrassic grof method were . Serum GST activity was done by method of Habig et al. [13]. This study was approved by the Human Research Ethics Committee S.R.T.R. Govt. Medical College and Hospital Ambajogai, Maharashtra India Project approval code- REC 9/13.

2.1 Statistical Analysis

Statistical analysis was performed with the MYSTAT statistical package.

Results were expressed as mean±SD. Student's *t* test used for group comparisons. Multiple linear regression analysis was used between GST and possible determinants, such as AST, ALT, ALP albumin, total bilirubin.

3. RESULTS

Demographic and clinical data of the subjects are shown in Table 1. There were no differences in age, sex between the patients and controls. There was no significant difference in the age, gender. The patients with chronic alcoholic liver impairment had, as expected, a significant increase in serum aminotransferases and alkaline phosphatase activities together with a significant decrease in albumin; they also showed a profound decrease in serum GST activity. Statistically significant differences in all the given parameters were observed between chronic alcoholic liver disease patients and control group. In acute liver disease except for albumin all other parameter shows statistically significant difference with respect to control group. Results from the present study suggest that GST activity is significantly increased in chronic alcoholic liver disease patients (22.293±4.159 IU/L vs 20.127±4.789 IU/L) and in acute viral hepatitis (23.685±6.751 IU/L vs 20.127±4.789 IU/L) Table 1.

Results are also depicted in Fig. 1. A multiple logistic regression analysis in acute viral hepatitis B showed that when GST measurement was added to a standard liver function tests Model 1= albumin + ALT, Model 2= Model1+ GST, the diagnostic significance increased from R^2=0.792 to 0.884 and from R^2= 0.843 to 0.849 in chronic alcoholic cirrhosis in above models respectively. Standard LFT parameters are significantly different in both groups (Table 1). Area under ROC is 0.952 for model having albumin+ ALT (Fig. 2) which is increased to 0.992 after addition of Glutathione S-Transferase (GST) in previous model (Fig. 3) in acute viral Hepatitis B. While in alcoholic Hepatitis Area under ROC is 0.966 for model having albumin+ ALT (Fig. 4) which is increased to 0.974 after addition of GST in previous model (Fig. 5).

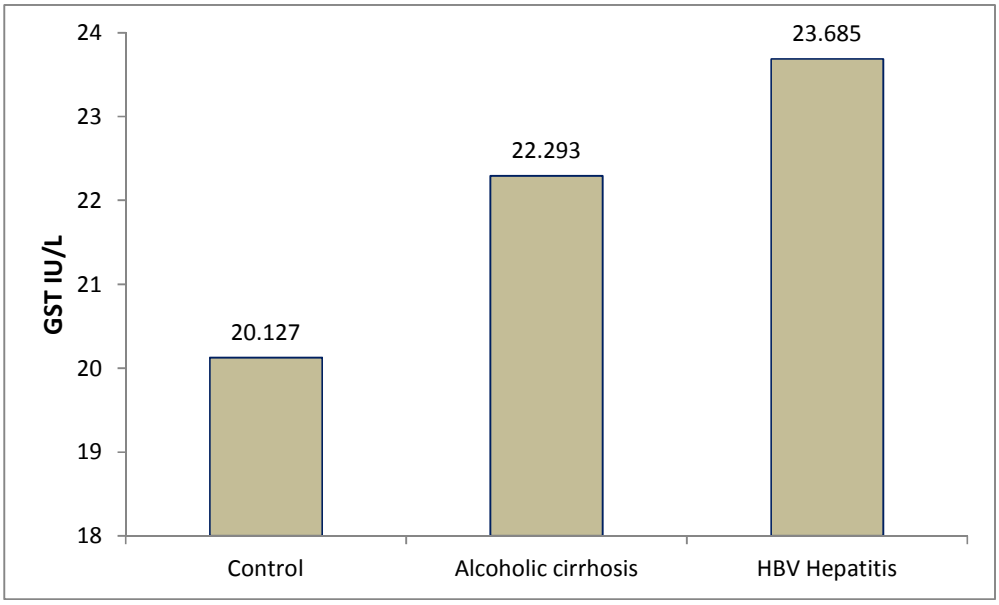

Fig. 1. Activity of GST in Cases and Control groups

Table 1. Standard LFT parameters

Parameter	Control	ALD	Viral
Bilirubin (mg/dL	1.193±0.548	3.135±1.33**	3.085±1.085**
Albumin (g/dL)	2.962±0.271	2.663±0.421**	2.820±0.324
AST (U/mL)	25.5±6.769	66.7±37.303**	74.6±52.936*
ALT (U/mL)	23.633±4.109	53.75±31.09**	61.5±45.837**
Alkaline phosphatase (KA U/L)	5.903±2.492	12.388±3.36**	13.565±3.09**
GST (IU/L)	20.127±4.789	22.293±4.159**	23.685±6.751**

*** = p < 0.001, * = p < 0.05 with respect to the control group*

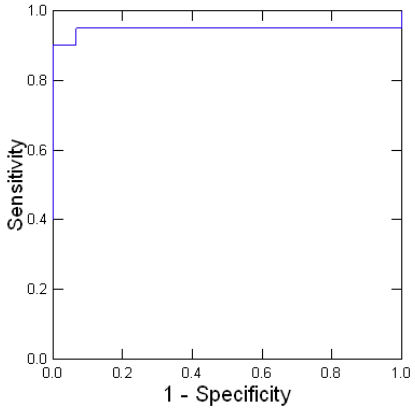

Fig. 2. Area under ROC Curve: 0.952

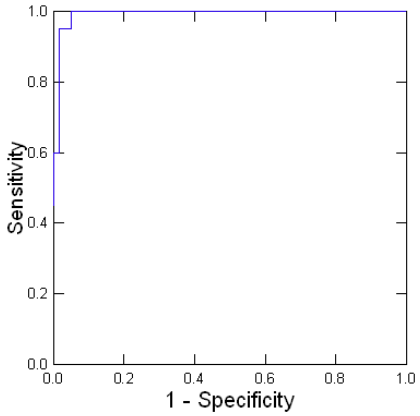

Fig. 3. Area under ROC Curve: 0.992

4. DISCUSSION

Several authors have reported the role of serum GST activity as a marker of hepatocellular damage [14-16]. In plasma of patients with alcoholic cirrhosis, glutathione S-transferase activities are often raised, even when transaminases are normal indicating chronic injurious stimulus of hepatocytes [17]. In the present study, we have found significant difference (p value < 0.001) in the serum GST activity in patients with acute viral hepatitis, when compared to healthy controls. Glutathione S-transferase is found to be a sensitive marker for the assessment of hepatocellular damage compared with transaminases [18]. It is distributed uniformly throughout the liver compared with the transaminases, which are found predominantly in the periportal hepatocytes [19].

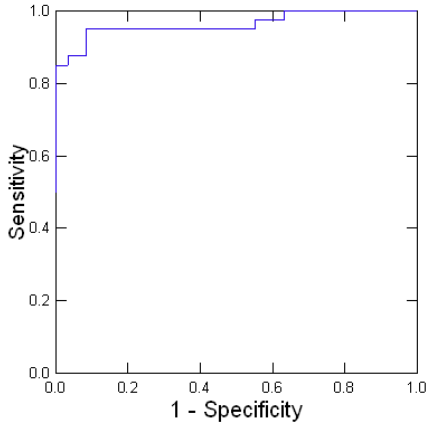

Fig. 4. Area under ROC Curve: 0.966

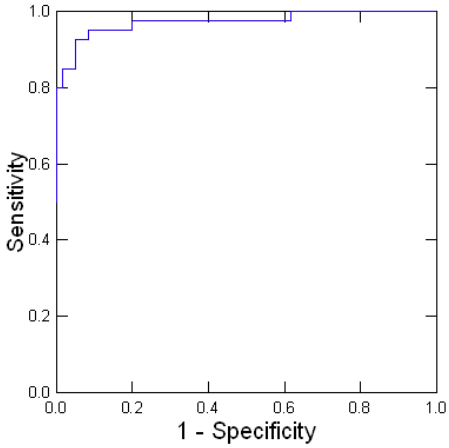

Fig. 5. Area under ROC Curve: 0.974

Ethanol is known as an inducer of the GST activity in the hepatocytes [20] and the determination of this enzyme in humans has been suggested as a useful monitor of cellular induction [21]. One study showed significantly GST higher in the chronic alcoholics as compared to that inthe healthy controls (p value < 0.05) [22]. Loguercio V [23] (and Muttigi et al. [24] have also reported the increased GST activity. In the present study We observed significant increase (p value < 0.05) in the levels of GST in the chronic alcoholic patients. This could be due to the release of GST from the hepatocytes into the circulation following hepatic damage by oxidative stress, which was produced due to the generation of ROS during alcohol metabolism [2]. It has been well established that GST is primarily involved in the cellular detoxification processes and that the elevated, circulating GST activity is considered to be an early index of the increased load on the hepatocytes in detoxifying toxins [2]. It is indicated by the increased presence of oxidative stress refected by the elevated circulating GST activity [25]. GST has been found to be unaffected by muscle damage, extra-hepatic inflammation, and hemolysis, and is therefore presumed to be more specific than transaminases [26].

Several authors have reported the role of serum GST activity as a marker of hepatocellular damage [27-29]. One study showed that association of plasma alpha-GST with ALT may improve the biochemical assessment of liver damage in patients with chronic hepatitis C, [30]. In the present study, we have found significant difference (p value < 0.001) in the serum GST activity in patients with acute viral hepatitis B, when compared to healthy controls. Several authors havereported the presence of oxidative stress in patients with HBV hepatitis [31,32]. Recent study showed that decrease in expression of endogenous antioxidant enzymes including GST, and up-regulated oxidative defense mechanisms against inflammation plays an important role in host defence mechanism [33]. This significantly decrease (< 0.05 p value) in antioxidant defence to survival against inflammation plays a key role in hepatocellular damage in these patients. However, in agreement with findings reported by Adachi Y et al. [34] in our study there was significant increase in serum GST activity in patients with acute viral hepatitis. However, in animal models decreased glutathione and increased activity of glutathione dependent antoxidant enzymes GST was found in cirrhotic liver tissue [35] Similarly, Erh-Hao L et al. have shown significantly increased in GST in animals during recovery from cirrhosis [36].

The glutathione system participates not only in the antioxidant defence system but also plays an important role in many processes on a molecular, cellular and organism level; Therefore, disturbances in glutathione system homeostasis are involved in pathogenesis and the progression of cancer and liver diseases [37,38]. GSTs are a family of detoxification enzymes that catalyse the conjugation of reduced glutathione (GSH) to a wide variety of endogenous and exogenous compounds, making them less biologically active, more water soluble and more quickly eliminated from an organism. Hence, GSTs are important in controlling toxic products by generating lipid oxidation and oxidative stress [39,40].

5. CONCLUSION

The current study indicates that the relatively simple GST activity measurement could significantly improve the laboratory's current efficiency in evaluating patients with suspected acute viral hepatitis B and chronic alcoholic liver disease to a new high.

CONSENT

All authors declare that written informed consent was obtained from the patient for publication of this paper.

ETHICAL APPROVAL

As per international standard or university standard, written approval of Ethics committee has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Basra S, Aand BS. Definition, epidemiology and magnitude of alcoholic hepatitis. World J Hepatol. 2011;3(5):108-13.
2. Hayes JD, McLellan LI. Glutathione and glutathione-dependent enzymes represent a co-ordinately regulated defence against

oxidative stress. Free Radic Res. 1999; 31:273–300.

3. Helaly GF, Mahmoud MM. Diagnostic value of alpha-glutathione S-transferase as a sensitive marker of increased risk for hepatocellular damage in hepatitis C virus (HCV) infection: Relation to HCV viraemia. J Egypt Public Health Assoc. 2003;78(3-4):209-223.
4. Beckett GJ, Hayes JD. Glutathione S-transferases: Biomedicalapplications. Adv Clin Chem. 1993;30:282–380.
5. Ribeiro RT, Marinho RT, Sanches JM. Classification and staging of chronic liver disease from multimodal data. IEEE Trans Biomed Eng. 2013;60(5):1336-44.
6. Tsukamoto H, Lu SC. Current concepts in the pathogenesis ofalcoholic liver injury. Faseb J. 2001;15:1335–49.
7. Meagher EA, Barry OP, Burke A, Lucey MR, Lawson JA, Rokach J, Fitz Gerald GA. Alcohol-induced generation of lipid peroxidation products in humans. J Clin Invest. 1999;104(6):805-13.
8. WHO. Hepatitis B fact sheet N°204; 2000. Available:http://www.who.int/mediacentre/factsheets/fs204/en/ (Accessed August 2008)
9. Lim W, Kwon SH, Cho H, Kim S, Lee S, Ryu WS, Cho H. HBx targeting to mitochondria and ROS generation are necessary but insufficient for HBV induced cyclooxygenase-2 expression. J Mol Med (Berl). 2010;88(4):359-69.
10. Chrobot AM, Szaflarska-Szczepanik A, Drewa G. Antioxidant defense in children with chronic viral hepatitis B and C. Med Sci Monit. 2000;6:713-718.
11. Thorburn D, Bird GL, Spence E, Mac Sween RN, Mills PR. alpha-Glutathione S-transferase levels in chronic hepatitis C infection and the effect of alpha-interferon therapy. Clin Chim Acta. 1996;253(1-2):171-80.
12. Abraham R, Ramakrishna B, Balekuduru A, Daniel HD, Abraham P, Eapen CE, et al. Clinicopathological features and genotype distribution in patients with hepatitis C virus chronic liver disease. Indian J Gastroenterol. 2009;28:53–8.
13. Habig, WH, Pabst MJJakoby WB. Glutathione *S*-transferases: The first enzymatic step in mercapturic acid formation. J. Biol. Chem. 1974;249:7130–7139.
14. Matsumoto R, Watanabe S, Beppu T, Futagawa S. Serum alpha-glutathione S-transferase: A new marker of hepatocellular damage associated with hepatectomy. Hepatol Res. 2000;18(1):10-18.
15. Karahalil B, Yağar S, Ozin Y. Release of alpha-glutathione S-transferase (alpha-GST) and hepatocellular damage induced by Helicobacter pylori and eradication treatment. Curr Drug Saf. 2007;2(1):43-6.
16. Ozturk G, Ozturk N, Aksoy H, Akcay MN, Atamanalp SS, Acemoglu H. Hepatocellular damage following burn injury demonstrated by a more sensitive marker: Alpha-glutathione S-transferase. J Burn Care Res. 2009;30(4):711-6.
17. Harrison DJ, May L, Hayes PC, Haque MM, Hayes JD. Glutathione S-transferases in alcoholic liver disease. Gut. 1990; 31(8):909-12.
18. Koo DJ, Zhou M, Chaudry IH, Wang P. Plasma alpha-glutathione S-transferase: A sensitive indicator of hepatocellular damage during polymicrobial sepsis. Arch Surg. 2000;135(2):198-203.
19. Hiley C, Fryer A, Bell J, Hume R, Strange RC. The human glutathione S-transferases. Immuno histochemical studies of the developmental expression of Alpha- and Pi-class isoenzymes in liver. Biochem J. 1988;254(1):255-9.
20. David RM, Nerland DE. Induction of mouse liver glutathione S-transferase by ethanol. Biochem Pharmacol. 1983; 32(18):2809-11.
21. Hayes PC, Bouchier IA, Beckett GJ. Glutathione S-transferase in humans in health and disease. Gut. 1991;32(7):813–818.
22. Mamta Singh, HariKrishan Aggarwal, Surinder Kumar Aggarwal. Significance of The Glutathione s transferase activity and the total thiols status in chronic alcoholics. Journal of Clinical and Diagnostic Research. 2013;6:31-33.
23. Loguercio V, Degirolamo A, Cuomo F, Argenzio C, Iannotta D, Disalvo A, et al. Determination of Plasma α-Glutathione-S-Transferase in chronic alcohol abusers: Relationship with alcohol intake and liver involvement. Alcohol Alcoholism. 1998; 33:366-72.
24. Muttigi MS, Prabhu LS, Kedage V, Prakash M, Shetty JK, Devaramane V, et al. Glutathione-S Transferase activity and total thiol status in chronic alcohol abusers before and 30 days after alcohol

abstinence. Online J Health Allied Scs. 2009;8:1-5.
25. Prakash M, Kedage V, Muttigi MS, Nataraj K, Baig WW, Attur RP. Glutathione-S-Transferase and thiol stress in patients with acute renal failure. Online J Health Allied Scs. 2010;9(2):10.
26. Narkewicz MR: Markers of cystic fibrosis-associated liver disease. J Pediatr Gastroenterol Nutr. 2001;32:421-422.
27. Beckett GJ, Foster GR, Hussey AJ, Plasma glutathione S-transferase and F protein are more sensitive than alanine aminotransferase as indicators of paracetamol (acetaminophen) induced liver damage. Clin Chem. 1989;35:2186-9.
28. Beckett GJ, Hussey AJ, Laing I, Measurement ofglutathione S-transferase B1 in plasma after birth asphyxia:an early indication of hepatocellular damage. Clin Chem. 1989;35:995-9.
29. Hussey AJ, Howie JJ, Allan LG, Drummond H, Hayes JD, Beckett GJ. Impaired hepatocellular integrity during general anaesthesia as assessed by measurement of plasma glutathione S-transferase. Clin Chim Acta. 1986;161:19-28.
30. M Vaubourdolle O, Chazouillères I, Briaud C, Legendre L, Serfaty, R Poupon et al. Plasma alpha-glutathione S-transferase assessed as a marker of liver damage in patients with chronic hepatitis C. Clin Chem. 1995;41(12):1716-9.
31. Duygu F, Karsen H, Aksoy N, Taskin A. Relationship of oxidative stress in hepatitis B infection activity with HBV DNA and fibrosis. Ann Lab Med. 2012;32(2):113-8.
32. Severi T, Ying C, Vermeesch JR, Cassiman D, Cnops L, Verslype C, et al. Hepatitis B virus replication causes oxidative stress in HepAD38 liver cells. Mol Cell Biochem. 2006;290(1-2):79-85.
33. Sohail M, Kaul A, Raziuddin M, Adak T. Decreased glutathione-Stransferaseactivity: Diagnostic and protective role in vivax malaria. Clin Biochem. 2007;40:377-82.
34. Adachi Y, Horii K, Takahashi Y, Tanihata M, Ohba Y, Yamamoto T. Serum glutathione S-transferase activity in liver diseases. Clin Chim Acta. 1980;106(3): 243-55.
35. Erh-Hao L, Miin-Fu C, Ta-Sen Y. A useful model to auditliver resolution from cirrhosis in rats using functional proteomics. J Surg Res. 2007;138(2):214-23.
36. Czeczot H, cibior D, Skrzycki M, Podsiad M. Glutathione and GSH-dependent enzymes in patients with liver cirrhosis and hepatocellular carcinoma. Acta Biochemica Polonica. 2006;53:237-41.
37. Czuczejko J, Zachara BA, Staubach-Topczewska E, Halota W, Kedziora J. Selenium, glutathione and glutathione peroxidases in blood of patients with chronic liver diseases. Acta Biochim Pol. 2003;50:1147-54.
38. Raza H. Dual localization of glutathione S-transferase in the cytosol and mitochondria: Implications in oxidative stress, toxicity and disease. FEBS J. 2011; 278:4243-51.
39. Traverso N, Ricciarelli R, Nitti M, et al. Role of glutathione in cancer progression and chemoresistance. Oxid Med Cell Longev. 2013;2013:972913.
40. Townsend DM, Tew KD. The role of glutathione-S-transferase in anti-cancer drug resistance. Oncogene. 2003;22:7369-75.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/20555