Research Article

Effects of DL-Methionine at various doses *per se* on liver in albino rats: An experimental study

Ervilla Dass[1]*, Swapan Goswami[2], Maulin Mehta[1]

[1]*Department of Pharmacology, Smt. Bhikhiben Kanjibhai Shah Medical Institute & Research Centre, Sumandeep Vidyapeeth an Institution Deemed to be University, At. & PO. Piparia, Tal. Waghodia, Dist. Vadodara -391760 (Gujarat), India*

[2]*Department of Pathology, Smt. Bhikhiben Kanjibhai Shah Medical Institute & Research Centre, Sumandeep Vidyapeeth an Institution Deemed to be University, At. & PO. Piparia, Tal. Waghodia, Dist. Vadodara -391760 (Gujarat), India*

Received: 17 April 2020 Revised: 10 May 2020 Accepted: 16 May 2020

Abstract

Objective: Oxidative stress and diminished glutathione play critical roles in the pathogenesis of liver disease. Synthesis of glutathione, the most abundant mammalian antioxidant, is regulated at the substrate level by cysteine, which is synthesized from homocysteine via the trans-sulfuration pathway. Hence, the research study was aimed to explore the effects of single oral dose per se DL-Methionine. **Material and Methods**: Albino rats were separated into three groups for the experimental study. After overnight fasting, the albino rats belonging to Group I Control Group were administered Distilled Water 10 ml/kg orally; Group II Met. 700 mg/kg & Group III Met. 1400 mg/kg were administered DL-Methionine as a single oral dose by intragastric cannula. After 24-hours of post-treatment, serum level was evaluated to demonstrate the per se effects of DL-Methionine on liver. Also, the liver samples from each group were examined for the histopathological study. **Results**: The histopathological findings of DL-Methionine per se treated rats were compared with the control group. Gross examination revealed near to normal appearance & morphologic changes of liver tissue showed normal morphology and normal hepatic parenchymal cells. We observed that DL-Methionine as a single oral dose of 700 mg/kg and 1400 mg/ kg although caused alteration in levels of liver enzymes the alteration was not statistically significant. **Conclusion**: DL-Methionine per se although showed alteration in the biochemical parameters, they were not found to be significant so as to be considered as hepatotoxic agent. Also, Methionine is commonly used nutritional, essential amino acid, is preferred in the treatment of Paracetamol poisoning.

Keywords: DL-Methionine, liver function tests, biochemical changes, serum transaminase enzymes, histopathology

Introduction

DL-Methionine (2-amino-4-methylsulfanylbutanoic acid) also known as Methionine is considered as sulphur containing essential amino acid that is important for many of the body functions is required for growth and tissue repair and is also involved in many detoxifying processes; also an important sulphur donor protects cells from pollutants and slows cell aging (Brosnan et al., 2006). It is used in protein synthesis, including the formation of S-adenosyl-L-Methionine (SAMe), L-homocysteine, L-cysteine, taurine, and sulphate. It is also considered as a component of enkephalins and various endorphins which are pain-relieving peptides, coenzyme A, heparin, biotin, and tripeptide glutathione which are important antioxidant and detoxifying agents. Also essential trace element selenium needs Methionine for its absorption, transportation and bioavailability. It also acts as a lipotropic agent and prevents excess fat build-up in the liver. Low levels of Methionine are known to cause temporary folic acid deficiency by trapping the folate in the liver (Brosnan et al., 2006; Kamat et al, 1989).

Nutrient supplements like taurine, methionine, S-Adenosylmethionine, Arginine, Polyenylphosphatidylcholine, A-Lipoic Acid, Vitamin B,

Address for Corresponding Author:
Dr. Ervilla Dass
Associate Professor,
Department of Pharmacology, Smt. Bhikhiben Kanjibhai Shah Medical Institute & Research Centre, Sumandeep Vidyapeeth an Institution Deemed to be University, At. & PO. Piparia, Tal. Waghodia, Dist. Vadodara -391760 (Gujarat), India
Email: ervilladass@gmail.com

DOI: https://doi.org/10.31024/ajpp.2020.6.3.3

Antioxidant Vitamins [A,C,E] and Methylsulfonylmethane that support phase I and phase II activities also serve as hepatoprotective agents. Synthesis of glutathione, the most abundant mammalian antioxidant, is regulated at the substrate level by cysteine, which is synthesized from homocysteine via the trans-sulfuration pathway. Cysteine is often rate-limiting for Glutathione (GSH) synthesis hence methionine metabolism *via* SAM and trans-sulfuration is very important in regulating GSH levels in the liver. In high doses of Paracetamol, the hepatic glutathione levels decreases causing increased oxidative stress and hepatic injury. L-methionine, a precursor of L-cysteine, which is considered to have antioxidant activity, is found to be a precursor to glutathione as well (Brosnan et al., 2006; Kamat et al., 1989). Antioxidant activity of L-Methionine and its metabolites are therefore, attributed for their possible hepatoprotective activity. There are also evidences suggesting that Methionine by itself has the free radical scavenging activity due to the sulphur moiety and the chelating ability, although its mechanism of action to produce hepatoprotection is not clear (Mato and Lu, 2007; Arthur, 2010).

Analyses of certain enzyme activities in blood serum give valuable diagnostic information for a number of disease conditions. Hepatonecrosis induced by Paracetamol per se, Chloroquine alone and both their combinations and its protection with Methionine was revealed by histopathological study & Methionine significantly prevented the rise in transaminases levels produced by hepatotoxic doses of Paracetamol and Chloroquine (Dass and Shah, 2000).

The study was taken up to evaluate the haemato-biochemical alterations and histopathology in healthy Albino rats, due to orally administered DL-Methionine per se at different doses.

Materials and methods

The present research study was accepted & approved by the Institutional Animal Ethics Committee (IAEC), of S.B.K.S.M.I. & R.C., Sumandeep Vidyapeeth Deemed to be University, Piparia, Vadodara; which is registered under Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment, Forest & Climate change, Govt. of India.

Chemicals and reagents

The drug and chemicals used were DL-Methionine 100 gm extrapure from Aatur Instru Chem, Vadodara. Other materials included the Diagnostic kit reagents for the estimation of Liver Function Tests (LFTs), Distilled water & Ether. The chemicals used were 10% Formalin, Xylene, Hemotoxylin and Eosin stains, for preparation of histopathology slides. The wax blocks & glass slides were used for studying the histopathology studies.

To evaluate the levels of liver enzymes, serum Glutamic-Pyruvic Transaminase (SGPT), Serum Glutamic-Oxaloacetic Aminotransferases (SGOT), Serum Alkaline Phosphatase, Serum bilirubin – Direct and Indirect Bilirubin, Total Bilirubin; Serum Gamma-Glutamyl Transpeptidase (GGTP) the diagnostic kit reagents (Erba Diagnostics, Manheim) was used. Standard Erba estimation kit was used by using auto analyzer (Erba, Chem 7, Germany). Standard procedure as specified in the kit literature was followed.

Animal Protocol

Young healthy albino rats of either sex weighing 150-400g body weight were separated before starting the experimental study. They were housed in poly-propylene rat-cages, acclimatized and were given free access to food and purified drinking water ad libitum. Rice husk was used as bedding and all the animals were housed and were allowed to acclimate under controlled environmental conditions of temperature 24° ± 2°C and 55% ± 5% relative humidity in a 12-hour light-/dark rhythm, Light and dark cycles of 12 hr and 12 hr respectively 7 am to 7 pm light; throughout the experiment.

Demonstration of effect on healthy Albino rats with orally administered DL-Methionine per se on haemato-biochemical parameters & histopathology

Three groups (*n=6)* of healthy Albino rats were separated for the experimental study as indicated in **table 1**. Each animal was used only once. After overnight fasting, the albino rats belonging to Group I Control Group (*n=6*) were administered Distilled Water 10 ml/kg orally; Group II (Met. 700 mg/kg) (*n=6*) were administered DL-Methionine 700 mg/kg *per se* as a single oral dose & Group III (Met. 1400 mg/kg) (*n=6*) were administered DL-Methionine 1400 mg/kg *per se* as a single oral dose; by intragastic cannula; and the animals were re-housed in separate individual polypropylene cages. All the drug solutions were freshly prepared before use and were administered orally with the volume of 10 ml/kg (Ferguson, 1962). The volume administered was maintained constant at 10 ml/kg, since the study of Ferguson has shown that the drug toxicity can be increased with increasing volume of distilled water as vehicle (Ferguson, 1962).

After 24-hour of post-treatment with Distill Water & DL-Methionine as indicated in table 1, whole blood was collected in labeled collecting glass tubes, under light ether anaesthesia, from retro-orbital plexus of eye by capillary method technique using the glass capillary tube, which was centrifuged at 3000 rpm for 10 minutes to obtain the serum. Serum was stored at −20°C until analyzed & was used for estimation of haemato-biochemical alterations, to record the observations of various serum liver enzyme levels; for determination of alanine amino transferase, aspartate

amino transferase, alkaline phosphatase, Gamma-Glutamyl Transpeptidase (GGTP) or Gamma-Glutamyl Transferase (GGT), & total bilirubin (Thomas, 1998; Gerhard, 2011; Pearlman and Lee, 1974; Persijn and Vander, 1976; Moss and Henderson, 1999).

Histopathological examination

From the same Group of Albino rats, after collection of serum, liver from each albino rat was immediately dissected out and washed with normal saline in glass petridish and preserved in 10% formalin for fixation for histopathological studies in separately labeled specimen collection jars. The livers were excised quickly and fixed in 10% formalin and paraffin embedded. Sections of about 4- 6 µm were stained with haemotoxylin and eosin (H&E) for histopathological evaluation. In brief, 4-6 µm thick section of paraffin embedded albino rat liver was dewaxed with distilled water for 2 min. After that the section was stained with haemotoxylin for 5 min at room temperature. After 15 min, the section was counterstained with eosin for 2 min, dehydrated with alcohol, washed with xylene and blocked by eosin. Hemotoxylin and eosin stained studies were observed under microscope, in the Department of Pathology. The sections were observed and desired areas were photographed in photomicroscope. The sections were viewed under 40x or 100 x magnifications (Humason, 1979).

Statistical analysis

All experimental data was entered in computerised Microsoft excel worksheet, & were subjected for statistical analysis and the results were expressed as Mean ± SEM, in terms of International Units Litre^{-1}. The statistical calculations were performed using computer-based statistical software SPSS version 21.0. Values were considered to be significant when P values were less than or equal to 0.05 ($p \leq 0.05$).

Results

Albino rats (*n=6*) that were administered Distilled Water (10 ml/kg) was considered as control group (Group I). The albino rats from the Group II and Group III were treated with DL-Methionine as a single dose each of 700 mg/kg and 1400 mg/kg, respectively, to demonstrate their *per se* effects on the liver enzymes as indicated in table 1.

It was observed that, DL-Methionine as a single oral dose of 700 mg/kg and 1400 mg/ kg although caused alteration in the levels of liver enzymes; the alteration was not statistically significant, which suggests that *per se* DL-Methionine has no toxic effect on the liver as indicated in table 1. Also, the control group administered with Distilled Water 10 ml/kg showed no significant alteration in the level of liver enzymes (Figure 1 and Figure 2).

Histopathological findings

Control group of animal's liver appeared pale with no changes in the texture (figure 1).

The light microscopic evaluation of livers, in the control rats, showed normal morphology of hepatic parenchyma (figure 1).

Following were the observations of the histopathological findings on DL-Methionine (700 mg/kg and 1400 mg/kg) *per se* Group II & Group III respectively, that were compared with the Group I control group.

Treated group of animal's liver appeared normal as similar to control treated rats (figure 1).

The liver tissue showed normal morphology and normal hepatic parenchymal cells (figure 2).

Discussion

Liver being a principle organ for maintenance and regulation of the internal milieu is involved for the structural alterations of the administered drugs. It is the target organ, which gets exposed to the drugs in higher concentration, than other organs of the body, when they are

Table 1. Changes in the levels of liver enzymes following oral administration of DL-Methionine

Groups	Biochemical Parameters of Liver Function Tests (Mean ± SEM values)				
(n=6)	SGPT (IU/L)	SGOT (IU/L)	Total Serum Bilirubin (µmol\L)	ALP (IU/L)	GGTP (IU/L)
Control (DW 10 ml/kg)	32.83±2.91	126.00±15.07	0.70±0.08	106.17±23.15	2.33±0.56
DL-Methionine (700 mg/kg)	50.33±7.06	184.50 ± 6.58	0.83±0.03	92.00±16.19	6.25±1.72
DL-Methionine (1400 mg/kg)	57.00 ± 7.01	225.75 ±19.14	0.87± 0.02	106.75 ±24.04	3.10±1.25

Note: *p value < 0.05 = significant, values are presented as Mean ± SEM; Serum Glutamic-Pyruvic Transaminase (SGPT), Serum Glutamic-Oxaloacetic Aminotransferases (SGOT), Total serum bilirubin, Alkaline Phosphatase (ALP) and Gamma Glutamyl Transpeptidase (GGTP) or γ-Glutamyl Transferase (GGT), DW = Distilled Water

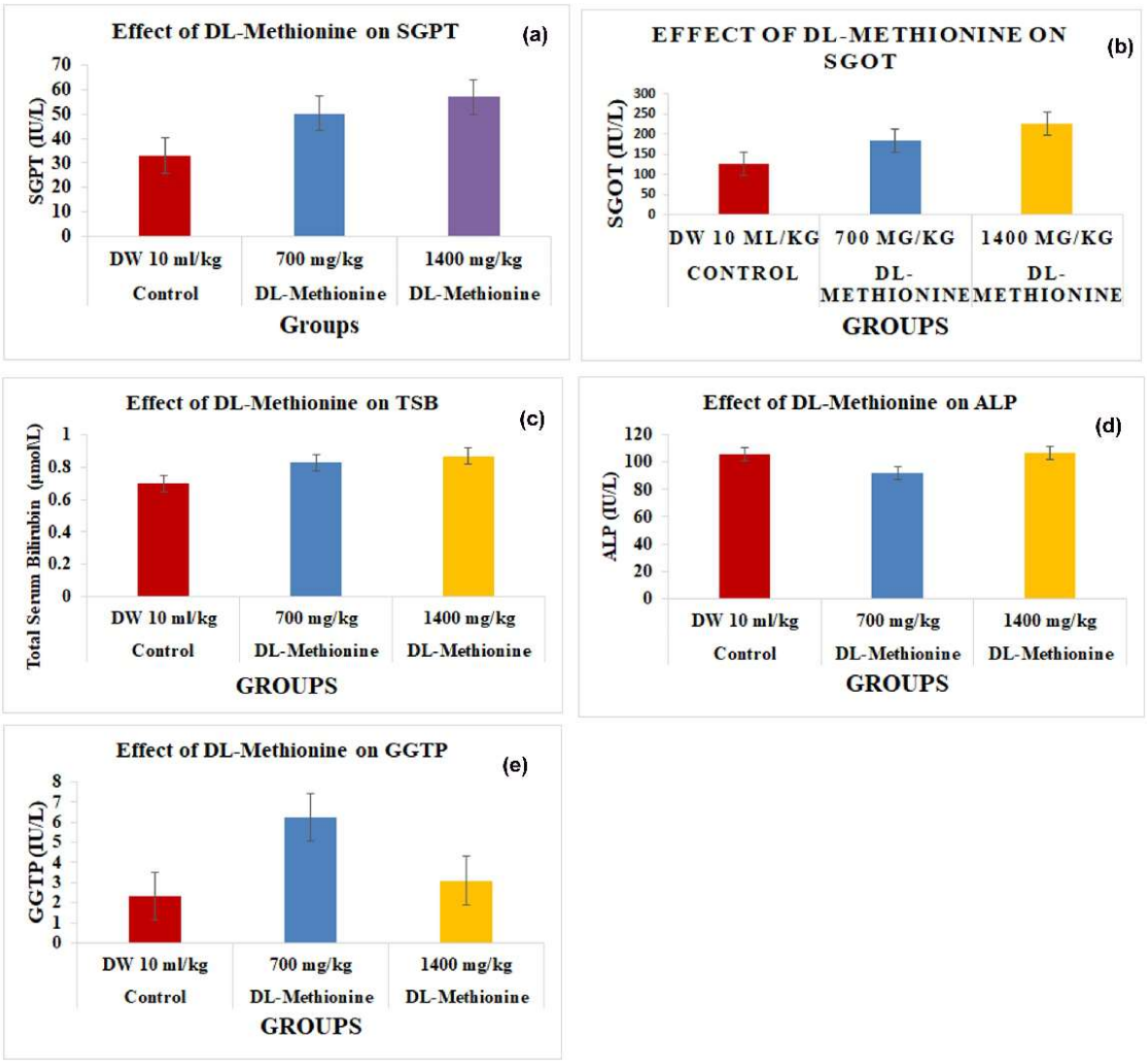

Figure 1. Effect of DL-Methionine (700 and 1400 mg/kg) per se in the Serum parameters: (a) Serum Glutamic-Pyruvic Transaminase (SGPT) levels; (b) Serum Glutamic-Oxaloacetic Aminotransferases (SGOT) levels; (c) Total Serum Bilirubin (TSB) levels; (d) Alkaline Phosphatase (ALP) levels (e) Gamma Glutamyl Transpeptidase (GGTP) levels

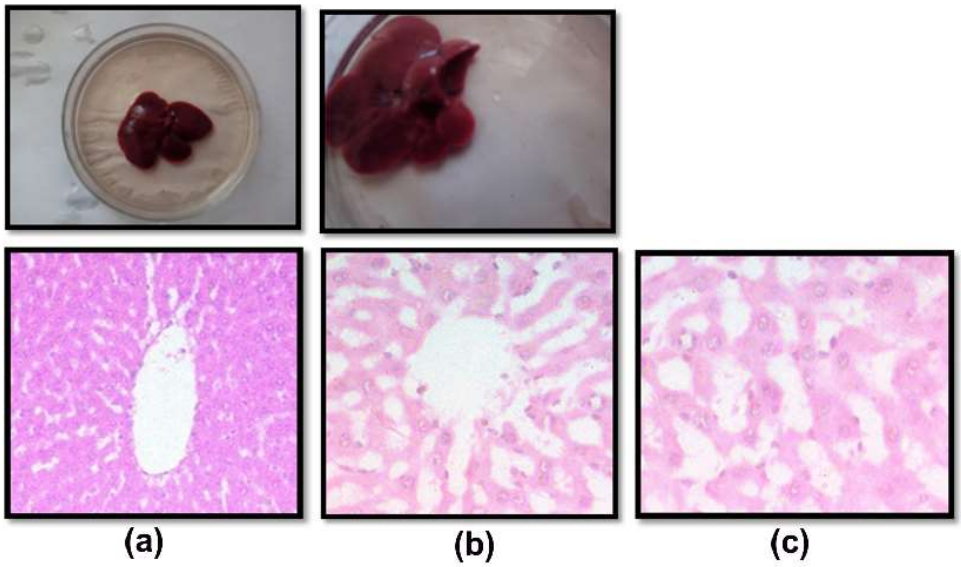

Figure 2. Histological observations of liver section of different treatment groups: (a) control group; (b) DL-Met 700 mg/kg per se ; (c) DL-Met 1400 mg/kg per se

orally administered. Hence, it is the most vulnerable organ to be injured by the chemicals and the drugs, which leads to hepatic dysfunction (Dass et al., 2018; Dass & Patel et al., 2018).

In the present research study, DL-Methionine per se effect, although showed alterations in the serum enzyme levels, which were not statistically significant and also there was no gross significant changes in the liver morphology as well through the histopathological findings. Moreover, it has been proposed (Anstee and Day, 2012)' that supplementation of DL-Methionine can improve the liver injury and reduce the development of hepatic cell carcinoma in chronic liver disease, by abolishing the oxidative stress and the cellular damage that would be caused by the hepatotoxic drugs (Quentin et al., 2012). This is being achieved by the generation of antioxidant enzymes such as Superoxide Dismutase (SOD), glutathione peroxidase and catalase, along with the ability to scavenge the free radicals (Copple, 2010). Further, inhibition of apoptosis, and reduction of cholestasis may further contribute to the hepatoprotective effect of DL-Methionine.

DL-Methionine has also been used in the treatment of Paracetamol poisoning and it has been demonstrated to be comparatively effective in decreasing the hepatotoxicity induced by Paracetamol. Similarly, Dass and Shah (2000), in their study have showed the hepatoprotective effect of DL-Methionine against Chloroquine and Paracetamol-induced hepatotoxicity (Dass and Shah, 2000).

Conclusion

It can be concluded that, DL-Methionine *per se*, although showed alteration in the serum biochemical parameters, they were not found to be statistically significant so as to be considered as toxic to liver. Moreover, in our histopathological findings, DL-Methionine *per se* on the liver tissue showed normal morphology of hepatic parenchymal cells compared to the control group.

Acknowledgments

Work is a part of author's Ph D thesis. Author is grateful to Smt. Bhikhiben Kanjibhai Shah Medical Institute & Research Centre, Sumandeep Vidyapeeth an Institution deemed to be University, Piparia, Vadodara, for permitting to conduct the review study. Author would like to thank Dr. BM Sattigeri, Ex-Professor & Head, Department of Pharmacology, SBKS MI & RC, Piparia, & Ph. D. Guide, for valuable contribution and guidance pertaining to the Ph. D. study. Author is also grateful to Mr. Devendra Waghasia, Statistician, Department of Community Medicine, SBKS MI & RC, for his valuable guidance pertaining to the statistical analysis in the present study.

Author contributions

The authors have accepted responsibility for the entire content of this submitted manuscript and approved submission.

Research funding: No funding involved.

Ethical approval

The conducted research study is related to small laboratory animal, albino rat use. The present research study was accepted & approved by the Institutional Animal Ethics Committee (IAEC), of S.B.K.S.M.I. & R.C., Sumandeep Vidyapeeth Deemed to be University, Piparia, Vadodara; which is registered under Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment, Forests & Climate Change, 5th Floor, Vayu Wing, Indira Paryavaran Bhawan, Ali Ganj, Jor Bagh Road, New Delhi-110 003, INDIA.

Conflict of interest

None

References

Brosnan JT, Brosnan ME. 2006. The sulfur-containing amino acids: an overview. The Journal of Nutrition, 136(6):1636S-1640S.

Cederbaum AI. 2010. Hepatoprotective effects of S-adenosyl-L-methionine against alcohol-and cytochrome P450 2E1-induced liver injury. World Journal of Gastroenterology, 16(11):1366.

Copple BL, Jaeschke H, Klaassen CD. 2010. Oxidative stress and the pathogenesis of cholestasis. Seminars in Liver Disease, 30(02): 195-204.

Dass E, Patel M, Patel S, Patel D, Patel G, Pobaru U. 2018. A prospective study of liver cirrhosis: An overview, prevalence, clinical manifestation & investigations in patients admitted to the medicine ward in a rural teaching hospital. International Journal of Scientific Research, 7(2): 39-41.

Dass E, Sattigeri B, 2018. Diclofenac-Induced Liver Toxicity in Albino Rats; Dose Dependent Study. Paripex-Indian Journal of Research, 7(1): 64-66.

Dass EE, Shah KK. 2000. Paracetamol and conventional antimalarial drugs induced hepatotoxicity and its protection by methionine in rats. Indian Journal of Experimental Biology, 38(11): 1138-1142.

Ferguson HC. 1962. Dilution of dose and acute oral toxicity. Toxicology and applied pharmacology, 4(6): 759-762.

Humason GL. Animal Tissue Techniques, 4th Ed. 1979, W.H. Freeman, San Francisco.

Kamat JP, Narurkar LM, Narurkar MV. 1989. Induction of hepatic drug metabolizing enzymes by DL-methionine in rats. Journal of Biosciences, 14 (3): 233-241.

Mato JM, Lu SC. 2007. Role of S-adenosyl-L-methionine in

liver health and injury. Hepatology, 45: 1306–1312.

Moss DW, Henderson AR. Clinical enzymology. In: Burtis CA, Ashwood ER, editors. Tietz Textbook of Clinical Chemistry. 3rd Ed. Philadelphia: W.B Saunders Company; 1999. page 617-721.

Pearlman FC, Lee RT. 1974. Detection and measurement of total bilirubin in serum, with use of surfactants as solubilizing agents. Clinical chemistry, 20(4): 447-453.

Persijn JP, Van der Slik W. 1976. A new method for the determination of γ-glutamyltransferase in serum. Clinical Chemistry and Laboratory Medicine, 14(1-12): 421-428.

Quentin M. Anstee, Christopher P. Day, 2012. S-adenosylmethionine (SAMe) therapy in liver disease: A review of current evidence and clinical utility. Journal of Hepatology, 57: 1097–1109.

Schumann, Gerhard, Rainer Klauke, Francesca Canalias, Steffen Bossert-Reuther, Paul FH Franck, F-Javier Gella, Poul J. Jørgensen. 2011. IFCC primary reference procedures for the measurement of catalytic activity concentrations of enzymes at 37 C. Part 9: Reference procedure for the measurement of catalytic concentration of alkaline phosphatase. Clinical Chemistry and Laboratory Medicine, 49(9): 1439-1446.

Thomas L. Alanine aminotransferase (ALT), Aspartate aminotransferase (AST). In: Thomas L, editor. Clinical Laboratory Diagnostics. 1st Ed. Frankfurt: TH-Books Verlagsgesell Schaft; 1998.