



Toxicological & Liver Function Assay Study of Poly-Herbal Drugs on Inflammatory Bowel Disease

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ABSTRACT

Objective: The aim of the current investigation is to evaluate or discover the right combination with less or no side effect to treat the inflammatory bowel disease.

Method: Male Sprague Dawley (S.D) rats (250gm) was randomly allocated to 17 groups (n=6): Group I received water throughout the 18 day study period, groups II and III only were received 0.1 mL 3% N-ethylmaleimide (NEM) and 0.1mL of 1% methyl cellulose intracolonicly at 11 days of the study, respectively. Group-IV to XVII were received selected drug and drug combination throughout 18 days as well as at 11th day also receive 0.1mL 3% N-ethylmaleimide (prepared in 1% methylcellulose). The histopathological features of NEM model control animal included surface epithelial damage, mucosal crypt drop out, edema, desquamated areas and diffuse inflammatory cell infiltration in the mucosa.

Result: Pretreatments of selected drug/s significantly attenuated the extent and severity of the histological signs of cell damage also produce a good effect on serum glutamate pyruvate transaminase (SGPT), serum glutamate oxaloacetate transaminase (SGOT), ALP, LDH, total bilirubin (TB) and direct bilirubin (DB) in the liver function assay.

Conclusion: The drug combination therapy gives better effects than the single drug, but the liver histopathology and serum enzyme level studies shows multiple drug/s combination produce at some extent liver damaging effects.

Keywords: Inflammatory Bowel Disease (IBD), N-ethylmaleimide

(NEM), Toxicity.

INTRODUCTION

Inflammatory bowel disease (IBD) refers to a group of poorly understood condition of chronic inflammation in the digestive tract, most often the small or large bowel (although these diseases can sometimes manifest themselves anywhere along the digestive tract or even outside of it in other organ systems such as joints, skin or eye). Inflammatory bowel disease including ulcerative colitis and Crohn's diseases are among the most challenging human illness¹ and it is the second most common chronic inflammatory disorder after rheumatoid arthritis. Both types of IBD may increase risk of cancer. The risk of colorectal cancer in patients with IBD is increased 4 to 20-fold compared to the general population, and some malignancies can develop in apparently uninvolved sites.² Patients with UC and CD have been reported to develop leukemia, suggesting a potential relationship between IBD and leukemia.³ Most of the current therapies for inflammatory bowel diseases involve treatment with Glucocorticosteroids and 5-Aminosalicylic acid.^{4,5} Immunosuppressive drugs have also been used to control severe illness, regardless of the more serious complications and toxic side effects associated with them.⁶ 5-Aminosalicylic acid, are potent ROS scavengers.⁷ In many studies, it has been reported that antioxidants show beneficial effects on experimental colitis. Therapeutic efficacy of platelet activating factor (PAF) receptor antagonists were reported in animal models of IBD.⁸ The choice of treatment for IBD depends on the severity of the disease. Many people with IBD commonly turn to complementary and alternative remedies. Many researchers have shown that herbal drugs *Gingko biloba*,⁹ *Aegle marmelos* (the

pulp of fresh bael fruit), *Holarrhena antidysenterica*, *Bombax malabaricum*, *Cyperus rotundus*, *Woodfordia floribunda* have good result in treatment of IBD or IBD related symptoms. These drugs give beneficial effect after long term use or taken in combination. Thus, the aim of the current investigation is to evaluate or discover the right combination with less or no side effect to treat the inflammatory bowel disease.

MATERIALS AND METHODS

Male Sprague Dawley (S.D) rats (250gm) were obtained from the animal house facility of the National Institute of Nutrition, (Hyderabad, India). Animals were housed at room temperature (22-25°C) and 50±10% humidity with 12:12-h light-dark cycles. The animals were fed on standard pellets and *water ad libitum*. All animals were acclimated a week before the experiment and were randomized 4 days before the start of the study. The study was approved by the institutional animal ethical committee (IAEC/09/2013-14) which follows the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA, Reg. No. 651/02/C/CPCSEA).

Induction of colitis by N-ethylmaleimide (NEM)¹⁰

24 hours Fasted rats were lightly anesthetized with ether and a flexible plastic catheter with an outer diameter of 2mm was inserted intracolonicly into the colon with the aim to place the catheter tip 8cm proximal to the anus. IBD was induced by intracolonicly instillation of 0.1mL 3% NEM (prepared in 1% methylcellulose) as described by Satoh *et al.*, 1997. Animals

were kept for 5 min in a trendelenburg position to avoid reflux. The rats were inspected for the presence of diarrhea.

A drug treatment protocol for NEM induced model

Male Sprague Dawley (S.D) rats (250gm) were randomly allocated to 17 groups containing six animals each animal in all groups were fasted for 24hr prior to study, given access to water *ad libitum*.

The group I received water throughout the 18 day study period, groups II and III only were received 0.1 mL 3% NEM, and 0.1mL of 1% methylcellulose (MC) intracolonicly on 11 days of the study, respectively.

While group-IV to XVII were received below given drug treatment throughout 18 days as well as at 11th day also receive 0.1mL 3% NEM (prepared in 1% methylcellulose).

PARAMETERS

Toxicological study^{10,11}

Acute toxicity

The treated group of rat was observed continuously for 1 h and then half hour for 4 hours. For any gross behavioral change and general motor activities like writhing, convulsion, response to tail pinching, gnawing, pupil size, fecal output, feeding behavior, etc., and further up to 72 hours. for any mortality.

Sub-acute toxicity

Measure the signs of behavioral, Neurological toxicity and mortality during the 7 days.

Chronic toxicity

After the 4th week animals were dissected out and remove various organs and carry out the histological study and compare it with the normal animal.

Histopathological studies

The rats were sacrificed and liver was rapidly excised followed by fixing it for 48 hours. in 10% formalin, and was dehydrated by passing successively in different mixtures of ethyl alcohol-water (50%, 80%, and 95%) and finally in absolute alcohol, cleared in xylene and embedded in paraffin. Thick sections (4-5 mm) were prepared and then stained with hematoxylin and eosin dye for microscopic observation of cell necrosis, fatty change.

Assessment of liver function assay¹²

Rats of all groups were anesthetized with ether. Then blood was withdrawn from all groups of rats by puncturing retro-orbital plexus and allowed to coagulate for 45 min at room temperature. Serum was separated by centrifugation (Remi centrifuge) at 2500 rpm at 30°C for 15 min. Serum samples were immediately subjected to biochemical estimation of serum glutamate pyruvate transaminase (SGPT), serum glutamate oxaloacetate transaminase (SGOT), ALP, LDH, total bilirubin (TB), and direct bilirubin (DB) by a microplate reader (Power wave XS), according to the colorimetric methods.

Statistical analysis

Results were presented as mean \pm SEM. The statistical difference between the means of the various groups were analyzed using one-way analysis of variance. Data was considered statistically significant at $P \leq 0.05$ and highly significant at $P \leq 0.001$. Statistical analysis was performed using Sigma state statistical software.

RESULTS

Toxicological study

Acute toxicity

All the treated groups did not cause any significant behavioral changes and no mortality was observed.

Sub-Acute toxicity

No any mortality rate was observed in all treated groups of animals.

Chronic toxicity

a). Histopathological studies

Drug treatment with single drug therapy of groups IV to VII shows no necrosis of the centrilobular hepatocytes cells. While combination or multidrug drug therapy of groups VIII to XVII shows minute extend of necrosis of the centrilobular hepatocytes characterized by lymphocytic infiltration and portal trides respectively (Figure 1).

b). Assessment of liver function assay¹¹

Drug treatment with single drug therapy of groups IV to VII *shows* the normal level of LDH, and TB and DB compare to a normal control group and combination of drug therapy in groups VIII to XVII shows some extend the elevation in level of LDH, and TB and DB compare to a normal control group (Figure 2 and Table 2).

DISCUSSION

Over, all inflammatory bowel disease (IBD) affects approximately 1 million people in the United States; it is believed that 15,000 to 30,000 new cases developed each year. The use of natural anti-inflammatory products provides an attractive and relatively nontoxic alternative to modulate inflammatory disorders.¹³ Pharmacotherapy of ulcerative colitis is principally aimed at inhibiting the production of inflammatory mediators and at modulating the immune system. The multitude of reactions in which ROS participate provides a new area of research in intestinal inflammation. The current study tried to reduce pharmacologically the excessive ROS production and/or action on

the inflamed colonic mucosa. Using NEM induced colitis model, the present work supports a possible role for inflammation and antioxidant therapy in inflammatory bowel disease patients. This appears to be a promising approach that may be considered as a complementary treatment of ulcerative colitis.

In our study N-ethylmaleimide (NEM) a sulfhydryl blocker¹⁴ was used to produce IBD which is similar to human. NEM offers the advantage by including IBD in lesser quantity required as compared to other inducements and just within 3 days, NEM model helps evaluate the fine histological and cellular changes present at earlier stages of IBD. Several major causative factors involved in the initiation of human IBD, such as oxidative stress, enhanced vasopermeability, neutrophil infiltration and increase production of inflammatory mediators are all observed in this animal model.

The present study demonstrated that 0.1mL 3% NEM cause a substantial degree of inflammation and tissue injury in the rat colon, which is associated with an infiltration of the colon with polymorphonuclear cells (histology and myeloperoxidase activity) as well as lipid peroxidation. The inflammation induced by NEM involved the mucosa and submucosa and rarely extended into the muscularis propria.

Hepatic cells participate in metabolic activities and contain host of enzymes. In tissue, asparate aminotransferase (AST) and alkaline aminotransferase (ALT) were found to be in higher concentrations in cytoplasm, and AST exists in mitochondria. In liver injury, transport function of the hepatocytes gets disturbed, resulting in the leakage of plasma membrane and thereby causing an increased enzyme level in serum. The elevated activities of these enzymes are indicative of cellular leakage and the functional integrity of the cell membranes of

the liver. ALP is excreted by the liver via bile in the liver injury due to hepatotoxins, which results in a defective excretion of bile from the liver and is reflected in their increased levels in serum. Drug treated groups IV to VII shows the normal level of LDH, and TB and DB compare to a normal control group. While Drug treated groups VIII to XVII shows some extend the elevation in level of LDH, and TB and DB compare to a normal control group.

CONCLUSION

The drug combination therapy gives better effects than the single drug, but the liver histopathology & serum enzyme level studies shows multiple drug/s combination produces at some extent liver damaging effects.

REFERENCES

- Hanan H. Hagar, Azza El Medany, Eman El Eter, Maha Arafa, Ameliorative effect of pyrrolidinedithiocarbamate on acetic acid-induced colitis in rats, *European Journal of Pharmacology*. (2007) 554 69–77.
- Mahmud N., Molloy A., Mc Partlin J., Corbally R., Whitehead A. S., Scott J. M., Weir D. G., Increased prevalence of methylenetetrahydrofolate reductase C677T variant in, patients with inflammatory bowel disease, and its clinical implications. *Gut* (1999)45:389–394.
- Korzenik J.R., Podolsky D.K. Evolving knowledge and therapy of inflammatory bowel disease. *Nat Rev Drug Discov*. (2006) 5: 197–209. PMID: 16518373
- Podolsky D.K., Inflammatory bowel disease. *N. Engl. J. Med.* (1991) 325, 1008–1016.
- Strober, W., Ludviksson, B.R., Fuss, I.J., The pathogenesis of mucosal inflammation in murine models of inflammatory bowel disease and Crohn's disease. *Ann. Intern. Med.* (1998) 128, 848–856. <http://www.annals.org/content/128/10/848.full.pdf+html>
- Shanahan, F., Inflammatory bowel disease: immunodiagnostics, immunotherapeutics, and ecotherapeutics. *Gastroenterology* (2001); 120, 622–635. PMID: 11179240
- Miles A. M, Grisham M. B. Antioxidant properties of aminosalicylates. *Methods Enzymol.* (1994); 234: 555-72.
- Fiocchi C. Inflammatory bowel disease: etiology and pathogenesis. *Gastroenterology* (1998); 115:182-205. [http://dx.doi.org/10.1016/S0016-5085\(98\)70381-6](http://dx.doi.org/10.1016/S0016-5085(98)70381-6)
- Murat M. M. harputluglu, ulvi demrel, neslihan yucel, nefle karada, smail temel, serpilfirat, cengizara, murat alada, melih karincaglu, fatih hilmiglu, The effects of Gingko biloba extract on acetic acidinduced colitis in rats. *Turk J Gastroenterol* (2006); 17 (3): 177-182. <http://www.turkgastro.org/pdf/509.pdf> <http://www.nejm.org/doi/pdf/10.1056/N EJM199110033251406>
- Ghosh M.N. Fundamentals of experimental Pharmacology. 3 rd ed. Kolkata: Hilton and Company; (2005). p. 190-197.
- KumarM. D., AmitaJ., SangeetaS., Hepatoprotective effects of *Polygonum bistorta* and active principle on albino rats intoxicated with carbon tetrachloride, *Indian Journal of Pharmacology*; 2008; 40 (Suppl 2): S160–S171, IP: 164.100.31.85. <http://medind.nic.in/ibi/t08/s2/ibit08s2p160.pdf>
- Reitman S, Frankel S. Colourimetric method for the determination of serum oxaloacetic and glutamic pyruvic trasaminase. *Am J Clin Pathol* (1957) 28:56-63.

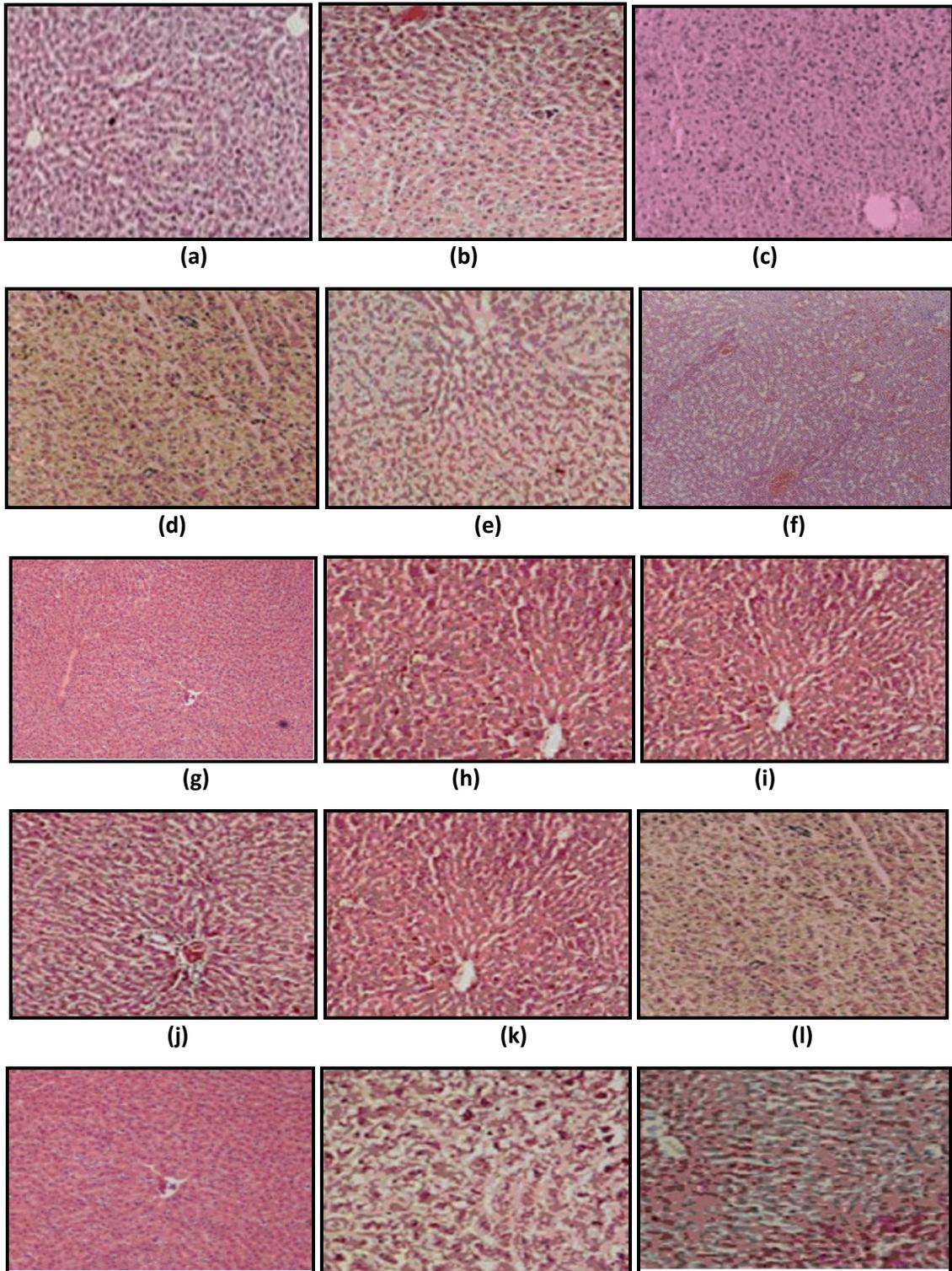
13. Ukil A., Maity S., Karmakar S., Datta N., Vedasiromoni J.R. & Das P. K., Curcumin, the major component of food flavour turmeric, reduces mucosal injury in trinitrobenzene sulphonic acid-induced colitis, *British Journal of Pharmacology* (2003) 139, 209–218. <http://repository.ias.ac.in/30450/1/459.pdf>.
14. Satoh Y, Hirashima N., Tokumaru H, Kirino Y., Activation of adenosine A1 and A2 receptors differentially affects acetylcholine release from electric organ synap 29:325–333.

Table 1. Drug treatment protocol

Group	Receive	Days
I	Water	1 TO 18
II - Vehicle	0.1mL 1% Methyl cellulose	11th
III-Model	0.1mL 3% NEM	11 th
IV- Standard	5-ASA 100mg/kg	1 to 18 (11th day 0.1mL 3% NEM)
V	Prednisone	Same as group IV
VI	Dicyclomine	Same as group IV
VII	Asparagus Racemosus	Same as group IV
VIII	5-ASA 100mg/kg + Prednisone	Same as group IV
IX	5-ASA 100mg/kg + Dicyclomine	Same as group IV
X	5-ASA 100mg/kg + Asparagus Racemosus	Same as group IV
XI	Prednisone + Dicycolime	Same as group IV
XII	Prednisone + Asparagus Racemosus	Same as group IV
XIII	Dicyclomine + Asparagus Racemosus	Same as group IV
XIV	5-ASA 100mg/kg + Prednisone + Dicyclomine	Same as group IV
XV	5-ASA 100mg/kg + Prednisone + Asparagus Racemosus	Same as group IV
XVI	5-ASA 100mg/kg + Dicyclomine + Asparagus Racemosus	Same as group IV
XVII	Prednisone + Dicyclomine + Asparagus Racemosus	Same as group IV

Table 2. Enzyme level in the serum

Groups	SGPT (I.U/L)	SGOT (I.U/L)	ALP (I.U/L)	LDH (I.U/L)	TB (%mg)	Db (%mg)
I.	46	54.6	202	133.5	0.125	0.134
II.	54	57.3	236	145.4	0.09	0.123
III.	48	49.7	247.5	140.1	0.08	0.127
IV.	51.7	55.2	242.3	153.3	0.15	0.136
V.	60.2	63.5	290.5	153	0.1	0.17
VI.	58.5	64.3	277.8	164.1	0.161	0.142
VII.	48.5	56.9	251.3	144.8	0.111	0.121
VIII.	62.4	64.6	298.1	156.2	0.211	0.198
IX.	61.5	67.8	295.1	161.2	0.196	0.21
X.	54.1	57.8	253.3	154.7	0.14	0.176
XI.	65.2	69.5	301	177.9	0.213	0.241
XII.	63.2	65.1	298.4	163.7	0.154	0.176
XIII.	66.2	61.9	276.5	171	0.173	0.165
XIV.	71.4	74.9	310.2	189.4	0.278	0.267
XV.	70.8	72.6	312.1	177.6	0.266	0.287
XVI.	69.4	77	299.1	183.5	0.244	0.268
XVII.	68.5	74.9	302	181.3	0.212	0.231



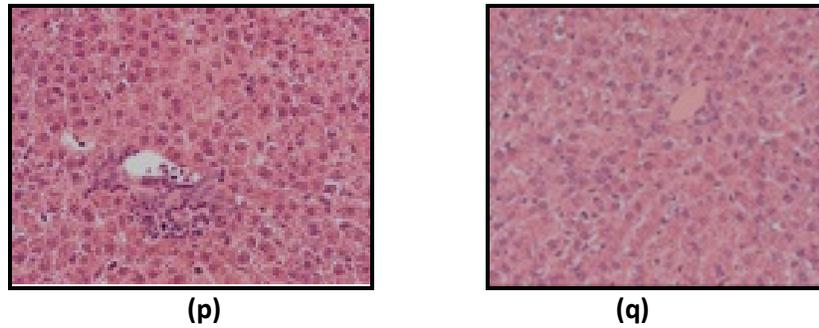


Figure 1. Photographs of hematoxyline and eosin stained paraffin sections of rat liver tissues. a) Normal control b) Vehicle control (1% 0.1 mL methyl cellulose) c) Model control (3% 0.1mL NEM) d)5ASA (100 mg/kg, p.o; for 18 days) e) Prednisone (5mg/kg, p.o. for 18 days) f) Dicyclomine (10mg/kg, p.o. for 18 days) g) Asparagus Racemosus (200mg/kg, p.o. for 18 days) h) 5-ASA + Prednisone (100 mg/kg + 5mg/kg, p.o. for 18 days) i) 5-ASA + Dicyclomine (100 mg/kg + 10mg/kg, p.o. for 18 days) j) 5-ASA + Asparagus Racemosus (100 mg/kg + 200mg/kg, p.o. for 18 days) k) Prednisone + Dicyclomine (5mg/kg + 10mg/kg, p.o. for 18 days) l) Prednisone + Asparagus Racemosus (5mg/kg + 200mg/kg, p.o. for 18 days), m) Dicyclomine + Asparagus Racemosus (10mg/kg + 200mg/kg, p.o. for 18 days) n) 5 - ASA + Prednisone + Dicyclomine (100 mg/kg + 5mg/kg + 10mg/kg, p.o. for 18 days) o) 5 - ASA + Prednisone + Asparagus Racemosus (100 mg/kg + 5mg/kg + 200mg/kg, p.o. for 18 days) p) 5 - ASA + Dicyclomine + Asparagus Racemosus (100 mg/kg + 10mg/kg + 200mg/kg, p.o. for 18 days) q) Prednisone + Dicyclomine + Asparagus Racemosus (5mg/kg + 10mg/kg +200mg/kg, p.o. for 18 days) respectively, attenuated the extent and severity of the histological signs of cell damage. Magnification 100 times

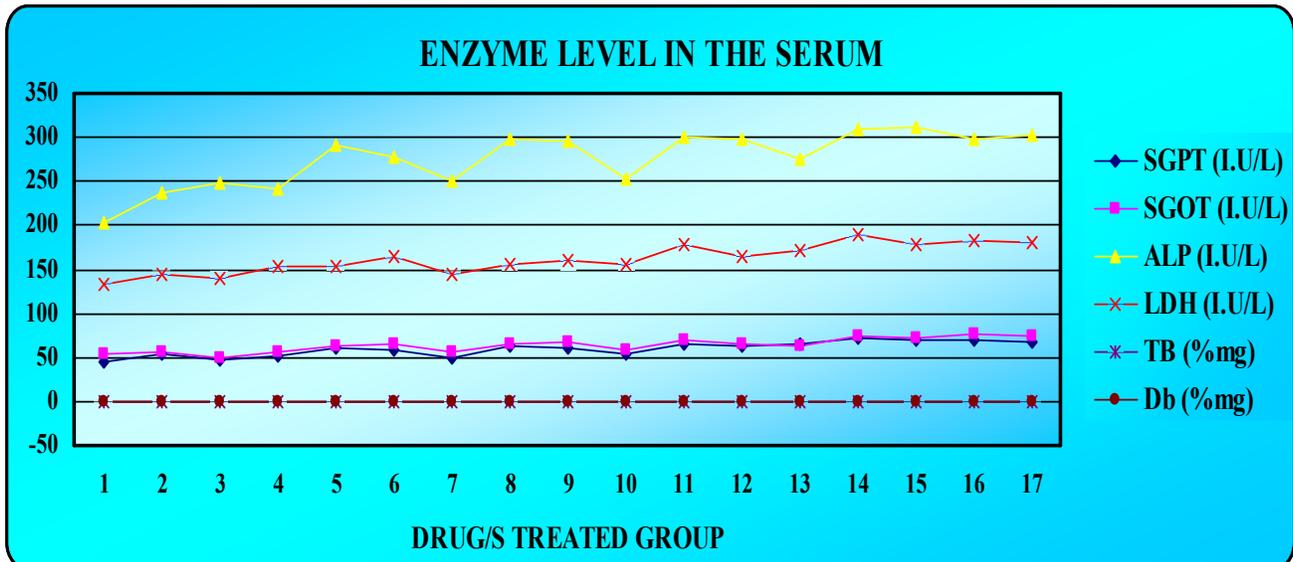


Figure 2. Various enzyme levels in the serum of rat liver