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EVALUATION OF HEPATOPROTECTIVE ACTIVITY OF *CANTHIUM DICOCCUM* ON AZITHROMYSIN INDUCED HEPATOTOXICITY

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ABSTRACT

There is a lack of consistent hepatoprotective drugs in contemporary medicine to treat drug-induced liver damage. The aim of the present study was to evaluate hepatoprotective activity of ethanolic extract of *Canthium dicoccum*. Albino rats (150–200 g) were separated into five groups. Groups I, II were normal (2% Tween 80) and toxic controls (Azithromycin 11.2 mg/kg. p.o), respectively. Groups III served as standard control (sylimarin), IV and V received the ethanolic extract of *Canthium dicoccum* 200 mg/kg and 400 mg/kg respectively for 7 days. Hepatotoxicity was induced in Groups II, III, IV and V by Azithromycin (11.2 mg/kg. p.o) twice for 7 days. The hepatoprotective effect was evaluated by physical parameters like wet liver weight and wet liver volume and biochemical parameters like SGOT, SGPT, ALP and bilirubin were estimated by autoanalyser with standard kits. The assay results were presented as mean and standard error of mean (SEM) for each group. In groups III, IV and IV, liver enzymes and albumin globulin ratio were significantly ($P < 0.01$) closer to normal. Histopathological examination revealed reduction in sinusoidal congestion, gloomy swelling and fatty changes and regenerative areas of the liver in groups III, IV and IV. These findings revealed that the *Canthium dicoccum* ethanolic extract has significant hepatoprotective activity.

KEYWORDS: Hepatoprotective, hepatotoxicity, *Canthium dicoccum*, Azithromycin, silymarin

INTRODUCTION

Liver is the heaviest gland of the body weighing about 1.4 kg in an average adult and is inferior to the diaphragm occupying most of the right hypochondriac and a part of the

epigastric region of abdominopelvic cavity. Sodium and potassium salts of bile acids play an important role in emulsification and breakdown of large lipid globules into a suspension of droplets and also in the absorption of lipids following their digestion.[1]

The clinical consequences of liver diseases are hepatic dysfunction in the form of jaundice, hypoalbuminemia, hyperammonemia, hyperglycemia, fector hepatitis, palmar erythema, spider angiomas, hypogonadism, gynecomastia, weight loss, muscle wasting, and portal hypertension from cirrhosis. If these are not treated promptly, they will lead to life threatening complications like hepatic failure in the form of hepatic encephalopathy, hepatorenal-syndrome; or portal hypertension from cirrhosis, malignancy with chronic disease and hepatocellular carcinoma.[2]

A few reports on the hepatoprotective activity are cited here, e.g. *Apium graveolens* Linn. (Umbelliferae), *Boerhaaiadiffusa* Linn. (Nyctagina ceae), *Euphorbia antisyphilitica* (Euphorbiaceae), *Rubia cordifolia* (Rubiaceae), *Solanum lyratum* (Solanaceae), *Tylophora indica* (asclepiadaceae).[3]

MATERIALS AND METHODS

The main objective of the study was to evaluate the hepatoprotective activity of the Ethanolic extract of the Canthium dicoccum in validated experimental animal models. Canthium dicoccum (Gaertn.) belonging to family rubiaceae was collected, authenticated from Chitoor district of Andhra pradesh and the aerial parts of *Canthium dicoccum* used for the present studies. The aerial parts were cut into small pieces and shade dried, pulverized into coarse powder using a mechanichal grinder. The powder obtained was used for extraction. The powdered medicinal plant was packed in Soxhlet apparatus and extraction process was carried out with ethanol for 7 days. Percentage yield obtained was 15 %.

Experimental Animals

Swiss Albino rats adult of any of sexes were used for the study. The rats were randomly grouped into five groups of 6 rats each. Each rat that weighed among 180-200 gm was housed separately. The animals were kept for 48 hrs to acclimatize to the new animal room environment. Animals were maintained at standard laboratory environment of temperature 22±2^{o}C, 70% humidity and 12 hours light and dark cycles. Animals were fed with standard pellet diet and adequate distilled pure water.

Method

The Ethanolic extract of Canthium dicoccum (EECD)was suspended in 2% Tween 80 solution to prepare two dose levels, 200 and 400mg/kg body weight of the animals. The plant was Screened for hepatoperotective activity. 30 healthy male albino rats were randomly divided into Five groups of 6 rats each. Control received(Group I) 2% Tween 80 and served as normal control. Negative control (Group II) received Azithromycin (11.2 mg/kg. p.o) twice for 7 days. Standard (Group III) received drug silymarin (25 mg/kg. p.o.) for 7 days once daily and Azithromycin (11.2 mg/kg. p.o) twice for 7 days. Ethanolic extract of Canthium dicoccum (Group IV and V)group received (200 mg/kg and 400 mg/kg) 7 days once daily and Azithromycin (11.2 mg/kg. p.o) twice for 7 days.

The same animals were then anesthetized using anesthetic ether, blood collected by retro orbital puncture and biochemical parameters like ALT, AST, ALP, Direct Bilirubin, Total Bilirubin, Triglycerides, Cholesterol, Total Proteins and Albumin were estimated.[4] The animals were sacrificed by overdose of ether and autopsied. Livers from all animals were removed, washed with ice-cold saline, weighed and measured the wet liver volume. Small piece of liver tissue was collected and preserved in 10% formalin solution for histopathological studies. Livers of some animals were homogenized with ice-chilled 10% KCl soln and centrifuged at 2000rpm for 10 minutes. Then the supernatant liquid was collected and the antioxidant parameters like Catalase, Super oxide Dismutase and Thiobarbiturate were estimated.

Physical Parameters like wet liver weight, Wet Liver Volume were determined and the biochemical parameters [5,6] like SGPT, SGOT, ALP and bilirubin were estimated by autoanalyser bu using standard kits.

Statistical analysis

The data were expressed as mean ± standard error mean (S.E.M).The Significance of differences among the groups was assessed using one way and multiple way analysis of variance (ANOVA). The test followed by Dunnet's test P values less than 0.05 were considered as significance.

RESULTS

Table 1: Details of qualitative phytochemical tests of Ethanolic extract of *Canthium dicoccum*.

S.NO	TEST	Pet ether Extract	Chloroform Extract	Ethanolic Extract
1	Carbohydrates	_	_	+
2	Proteins and amino acids	_	_	_
3	Alkaloids	_	+	+
4	Fixed oils and fats	+	_	_
5	Glycosides	_	+	+
6	Triterpenoids	+	_	_
7	Phenolics and tannins	_	_	_
8	Saponins	_	+	+
9	Flavones and Flavonoids	_	+	+

$1/10^{th}$ (400mg), $1/20^{th}$ (200mg) of maximum dose tested were selected for the present study.

Hepatoprotective activity

Physical parameters: Wet liver weight wet liver volume

Ethanol treatment in rats resulted in enlargement of liver which was evident by increase in the wet liver weight and volume. The groups were treated with Silymarin and ethanolic extract of *Canthium dicoccum* showed significant restoration of wet liver weight and wet liver volume nearer to normal. The *EECD* at 200mg/kg b.wt and 400mg/kg body weight showed reduction of wet liver weight and wet liver volume significantly at $p<0.05$.The results are shown in table 2, Fig 1 and 2..

Table 2: Effect of ethanolic extract of *Canthium dicoccum* on Wet liver weight and Wet liver volume in Azithromycin induced hepatotoxic rats.

Group	Dose	Wet Liver weight (gm/100)	Liver volumes (ml/100gm)
I	10ml/kg p.o	2.43 ± 0.535	2.535±0.53
II	50mg/kg, p.o	4.24 ± 0.095	4.19±0.04
III	200mg/kg, p.o +50mg/kg	2.49 ± 0.110*	2.78±0.23*
IV	200mg/kg, p.o + 50mg /kg	2.68 ± 0.120*	2.973±0.07*
V	400mg/kg, p.o + 50mg /kg	2.36 ± 0.27*	2.77±0.11*

Values are mean ± SEM (n=6) one way ANOVA. Where, * represents significant at $p<0.05$,** represents highly significant at $p<0.01$, and *** represents very significant at $p<0.001$. All p values are compared with toxicant.

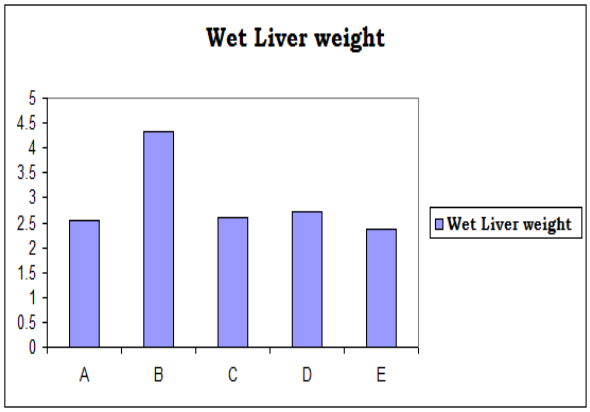


Fig 1: Effect of ethanolic extract of *Canthium dicoccum* on Wet weights in Azithromycin induced hepatotoxic rats.

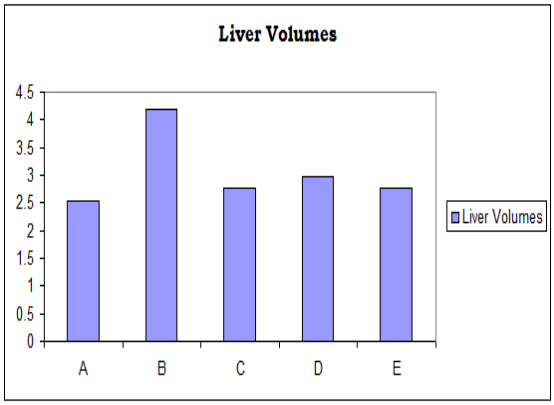


Fig 2: Effect of ethanolic extract of *Canthium dicoccum* on Wet liver volume levels in Azithromycin induced hepatotoxic

Table 3: Effect of ethanolic extract of *Canthium dicoccum* on SGPT, SGOT & ALP levels in Azithromycin induced hepatotoxic rats.

Group	Dose	SGPT levels (U/L)	SGOT levels (U/L)	ALP levels (U/L)
I	10ml/kg p.o	29.35±0.90	34.90±1.50	28.15±1.141
II	50mg/kg, p.o.	123.9±1.50	177.95 ±1.350	81.24±1.388
III	200mg/kg,p.o+50mg/kg	50.57±0.05***	86.86±0.7025***	30.8±2.05***
IV	200mg/kgp.o +50mg/kg	75.6±0.55*	102.56±0.750*	62.0±2.05*
V	400mg/kg,p.o + 50m/kg	72.4 ±0.05**	102.3±0.50*	38.6±0.97***

Values are mean ± SEM (n=6) one way ANOVA. Where, * represents significant at $p<0.05$, ** represents highly significant at $p<0.01$, and *** represents very significant at $p<0.001$. All values are compared with toxicant.

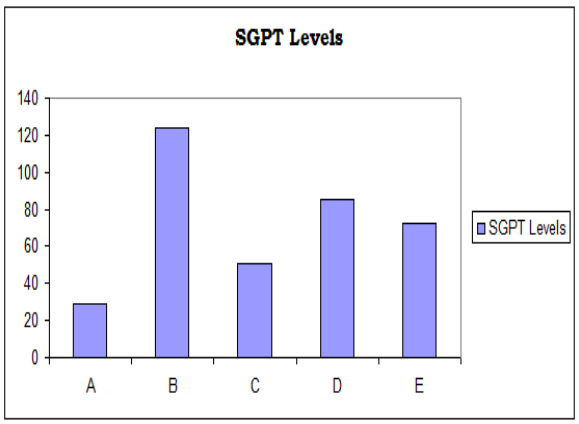


Fig 3: Effect of ethanolic extract of *Canthium dicoccum* on SGPT levels in Azithromycin induced hepatotoxic rats.

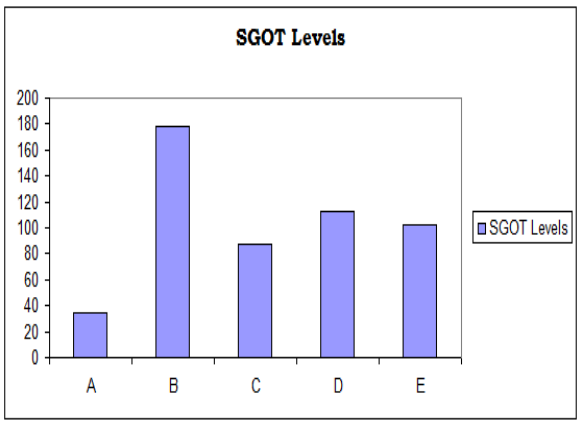


Fig 4: Effect of ethanolic extract of *Canthium dicoccum* on SGOT levels in Azithromycin induced hepatotoxic rats.

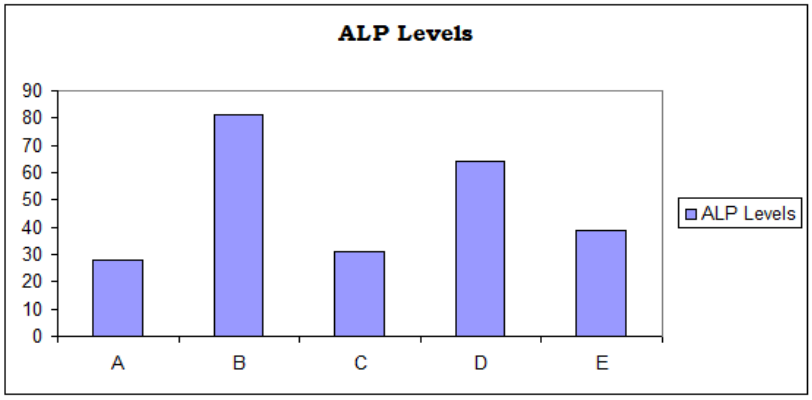


Fig 5: Effect of ethanolic extract of *Canthium dicoccum* on ALP levels in Azithromycin induced hepatotoxic rats.

Table 4: Effect of Ethanolic extract of *Canthium dicoccum* on Direct bilirubin & Total bilirubin levels in Azithromycin induced hepatotoxic rats.

Group	Dose	Direct bilirubin levels(mg/dl)	Total bilirubin levels (mg/dl)
I	10ml/kg p.o	0.188±0.0092	0.20±0.01
II	50mg/kg, p.o.	0.86±0.0301	1.39±0.08
III	200mg/kg,p.o + 50mg/kg	0.33±0.019***	0.45±0.05***
IV	200mg/kgp.o +50mg/kg /kg,p.o+50mg/kg	0.55±0.02*	0.98±0.17
V	400mg/kg,p.o + 50mg /kg	0.43±0.02**	0.71±0.05**

Values are mean ± SEM (n=6) one way ANOVA . Where, * represents significant at $p<0.05$, ** represents highly significantat $p< 0.01$, and *** represents very significant at $p<0.001$. All values are compared with toxicant.

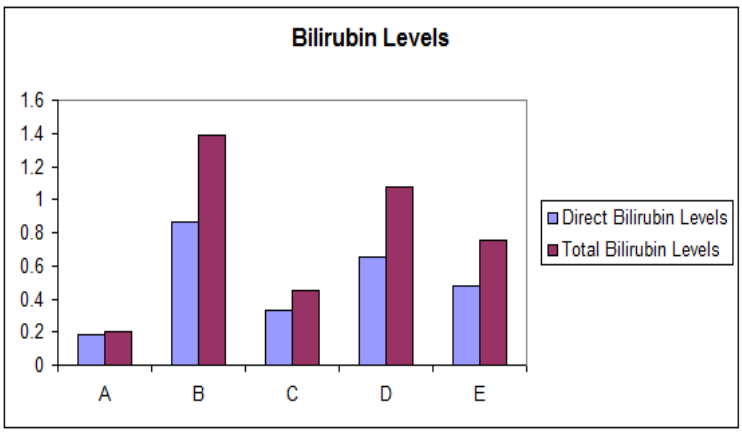
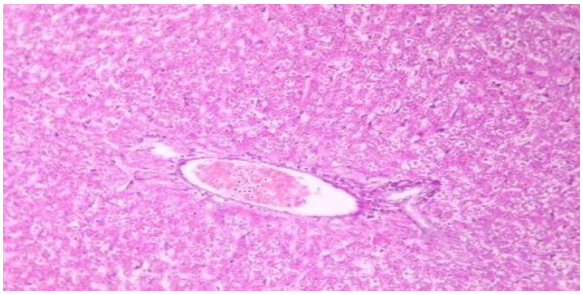
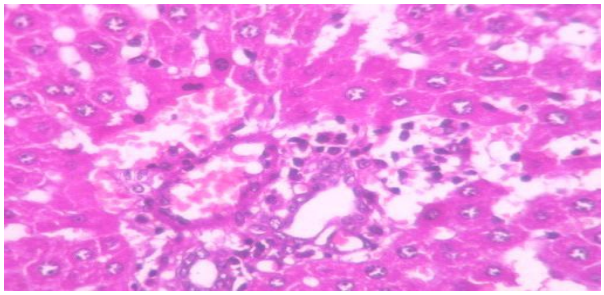


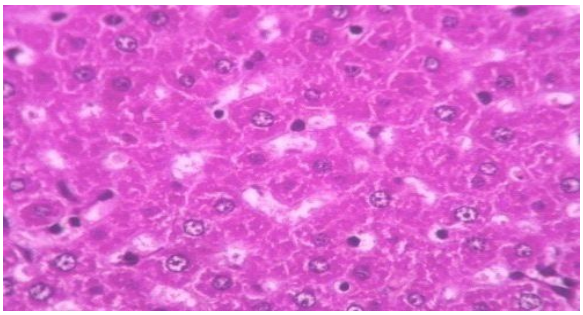
Fig 6: Effect of Ethanolic extract of *Canthium dicoccum* on Direct Bilirubin& Total bilirubin levels in Azithromycin induced hepatotoxic rats.Normal Control (Azithromycin)



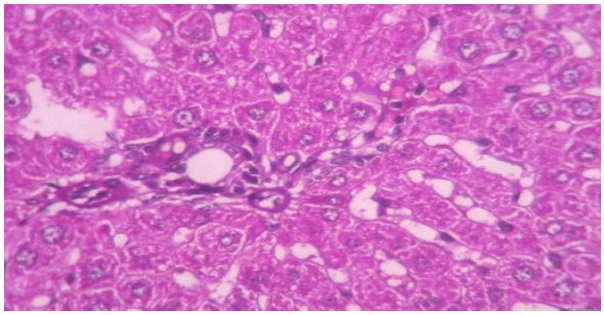
Normal Control



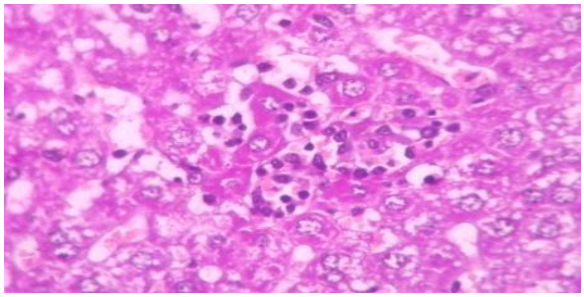
Toxic control (Azithromycin)



Standard (Silymarin 200mg/kg)



EECD (200mg/kg)



EECD (400mg/kg)

Figure no.7: Histopathology of the liver in Azithromycin induced hepatotoxicity studies.

DISCUSSION

Liver participates in a variety of metabolic activities perhaps by virtue of presence of number of enzymes and thus may self-expose too many toxicants, chemicals and drugs which could injure it. In present hepatoprotective study, azithromycin was used as hepatotoxicant to induce liver damage.

In case of toxic liver, Wet liver weight and Wet liver volumes were increased due to water retension in the cytoplasm of hepatocytes resulting in increased total liver mass and volume. It is reported that liver mass and volume are important parameters in ascertaining the hepatoprotective effect of the drugs. So in this study treatment with ethanolic extract of the leaves of *Canthium dicoccum* significantly reduced the wet liver weight and wet liver

volumes of animals and hence it possesses statistically significant($p<0.05$) hepatoprotective activity.

Hepatotoxin gets converted into radicals in liver by action of enzymes & these attacks the unsaturated fatty acids of membranes in presence of oxygen to give lipid peroxides consequently. The functional integrity of hepatic mitochondria is altered, leading to liver damage.[7] During hepatic damage, cellular enzymes like AST, ALT and ALP present in the liver cells leak into the serum, resulting in increased concentrations Azithromycin administration for 7 days significantly increased all these serum enzymes. Serum levels of SGPT can increase due to damage of the tissues producing acute hepatic necrosis, such as viral hepatitis and acute cholestasis.[8] Azithromycin induced liver damage and alcoholic cirrhosis also can associate with mild to moderate elevation of transaminases.[9] In the current study treatment of rats with ethanolic extract of leaves of *Canthium dicoccum* significantly ($p<0.05$ in 200mg/kg b.w. and $p<0.01$ in 400mg/kg b.w.) decreased the levels of SGPT in serum which is an indication of hepatoprotective activity. SGOT is a mitochondrial enzyme released from heart, liver, skeletal muscle and kidney. In the current study treatment of animals with ethanolic extract of leaves of *Canthium dicoccum* significantly ($p<0.05$) decreased the levels of SGOT in serum which is an indicative of hepatoprotective activity. In case of toxic liver, alkaline phosphatase levels are very high, which may be due to defective hepatic excretion or by increased production of ALP by hepatic parenchymal or duct cells.[10] In the current study treatment of animals with ethanolic extract of Canthium dicoccum significantly ($p<0.05$ in 200mg/kg b.w. and $p<0.001$ in 400mg/kg b.w) decreased the levels of ALP in serum as an indication of hepatoprotective activity.

In case of toxic liver, bilirubin levels are elevated. Hyperbilirubinemia can result from impaired hepatic uptake of unconjugated bilirubin. Such a situation can occur in generalized liver cell injury. Certain drugs (e.g., rifampin and probenecid) interfere with the net uptake of bilirubin by the liver cell and may produce a mild unconjugated hyperbilirubinemia. Bilirubin level rises in diseases of hepatocytes, obstruction to biliary excretion into duodenum, in haemolysis and defects of hepatic uptake and conjugation of bilirubin pigment such as in Gilbert's disease. In the current study treatment of animals with ethanolic extract of *Canthium dicoccum* significantly ($p<0.05$ in 200mg/kg b.w. and $p<0.01$ in 400mg/kg b.w) decrease the levels of bilirubin (direct and total) in serum which is an

indication of hepatoprotective activity.

CONCLUSION

The present study was aimed to assess the hepatoprotective activity of ethanolic extract of *Canthium dicoccum* by using azithromycin as toxicant. The Physical parameters such as wet liver weight and wet liver volume, biochemical parameters like serum SGPT, SGOT, ALP, direct and total bilirubin. Azithromycin induced hepatotoxicity was significantly prevented by pretreatment with ethanolic extract of *Canthium dicoccum*. Decrease in wet liver weight and wet liver volumes, reduction in elevated biochemical parameter levels like serum SGPT, SGOT, ALP, direct and total bilirubin, after treatment with ethanolic extract of *Canthium dicoccum* indicates the hepatoprotective effect of extract. In liver injury models in rats restoration of hepatic cells with minor fatty changes and absence of necrosis after treatment with extract was observed, indicating satisfactory hepatoprotection.

Based on improvement in serum marker enzyme levels, physical parameters, functional parameters and histopathological studies concluded that ethanolic extract of *Canthium dicoccum* possesses significant hepatoprotective activity in the doses used.

REFERENCES

1. Tortora G.J, Grabowski S.R. Principles of Anatomy and Physiology. 8thed. Harper Collins College publishers inc, 1996; 824-3.
2. Salman Nusrat, Muhammad S Khan, Javid Fazili, and Mohammad F Madhoun. Cirrhosis and its complications: Evidence based treatment. World J Gastroenterol, May 14, 2014; 20(18): 5442–5460.
3. Vipul Gujrati, Nilesh Patel, Venkat N Rao, K Nandakumar, TS Gouda, Md.Shalam S et al. Hepatoprotective activity of alcoholic and aqueous extracts of leaves of Tylophora indica (Linn.) in rats. Ind J. Pharmacol, 2007; 39(1): 43-47.
4. Yuan SS, Chang HL, Chen HW, Kuo FC, Liaw CC, Su JH, Wu YC. Selective cytotoxicity of squamocin on T24 bladder cancer cells at the S-phase via a Bax, Bad-, and caspase-3-related pathways. Life Sci., 2006; 78(8): 869-74.
5. Bowers G N, Mc Commb R.B. Recommendations of the german society for clinical chemistry. J Clin. Chem Bio., 1972; 10: 182.
6. Jendrassik L, Grof P. Uncertainity and traceability of a calibrator of conjugated bilirubin and problems of an automated Jendrassik- Grof reagent. Biochem, 1938; 2:

281-97.

7. FahadHussain, Arif Malik, Ujala Ayyaz, Hassan Shafique, Zohaib Rana, Zahid Hussain. Efficient hepatoprotective activity of cranberry extract against CCl4-induced hepatotoxicity in Wistar albino rat model: Down-regulation of liver enzymes and strong antioxidant activity Asian Pacific Journal of Tropical Medicine, 2017; 10(11): 1054-1058.
8. Rekha, D.S. Jayaprakash Murthy. Assessment of Serum Enzymes Gamma Glutamyl Trasnferase, Aspartate Transaminase and Alanine Transaminase in Liver Diseases. Journal of Pharmaceutical Sciences and Research, 2011; 3(5): 1221-1226.
9. Melissa A Martinez, Raj Vuppalanchi, Robert J. Fontana, Andrew Stolz, David E. Kleiner, Paul H. Hayashi, Jiezhun Gu, Jay H. Hoofnagle, and Naga Chalasani. Clinical and Histological Features of Azithromycin-Induced Liver Injury. Clinical gastroenterology and hepatology, 2015; 13(2): 369–376.
10. Ian D DAgata, and William F Balistreri. Evaluation of Liver Disease in the Pediatric Patient. Pediatrics in Review, 1999; 20(11): 376-389.