IJBPAS, September, 2021, 10(9): 3192-3203

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

HEPATOPROTECTIVE EFFECT OF A INDEGENOUS POLYHERBAL FORMULATION SUMUKTI SYRUP AGAINST ETHANOL AND PARACETAMOL INDUCED LIVER DAMAGE IN SWISS ALBINO RATS

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Received 26th Oct. 2020; Revised 27th Nov. 2020; Accepted 12th Dec. 2020; Available online 1st Sept. 2021

https://doi.org/10.31032/IJBPAS/2021/10.9.5634

ABSTRACT

A polyherbal formulation was designed for alcohol use disorders and an experimental validation of the same was carried out for its hepatoprotective activity against ethanol and paracetamol induced liver damage in swiss albino rats. The rats were divided into four groups of ten animals each with two study groups, one toxic control group and one normal control group. The polyherbal formulation was coded as Sumukti and was prepared in syrup form by combination of 11 drugs. The study groups were fed with Sumukti syrup for 21 days and 31 days respectively along with hepaotoxic drugs like ethanol and paracetamol. Biochemical parameters like Liver function test Histopathological studies of liver cells were carried out. Results were suggestive of hepatoprotective activity of study formulation.

Keywords: Sumukti syrup, polyherbal, Hepatoprotective, indigenous

INTRODUCTION

In India alcoholism is prevalent for generations and problems due to alcoholism are increasing in all age groups. According to WHO survey report

as on September 2018, 3 million deaths every year result from harmful use of alcohol worldwide, which amounts to 5.3 % of all deaths. Apart from death, Alcohol is key factor in more than 200 disease conditions and injury. Total alcohol per capita consumption in India over 15 years of age rose from 2.4, litres of pure alcohol in 2005 to 4.3 litres in 2010 and 5.7 litres in 2016 **[1]**. More than 30 conditions listed in the WHO's International Classification of Diseases, 10th Edition (ICD–10) (WHO2007) include the term "alcohol" in their name or definition, indicating that alcohol consumption is a necessary cause underlying these conditions. The most important disease conditions in this group are alcohol use disorders (AUD), which include alcohol dependence and harmful use or alcohol abuse. Even though AUD in themselves do not rank high as a cause of death globally, they are the fourth-most disabling disease category in low- to middle-income countries and the third-most disabling disease category in high-income countries **[2]**. WHO have developed a system called AUDIT which is a simple method of screening and assessing excessive alcoholism **[3]**.

It is a known fact that alcohol affects all organs, the most predominant being liver. The damage is mostly due to Acetaldehyde, Lipid per oxidation and glutathione depletion resulting in cirrhosis of liver. The multi-organ damages by alcohol are also linked to its addiction, tolerance and dependence liability. In chronic alcoholism the treatment is aimed at preventing addiction, protecting damaged organs from further damage and controlling withdrawal syndrome. Various drugs are available for above purposes however none of the above are found reliable in all patients **[4]**.

In Ayurveda, alcohol related disorders are dealt under the name Madatyaya **[5]**. In this disease, there will be vitiation of body humors and faculties of mind (Shareerika and Manasika Dosha), which ruins the physical, psychological, social, economical and occupational wellbeing. In Ayurvedic texts, treatment of Madatyaya is built around holistic approach of correcting the vitiation of the Doshas in all systems. Number of Ayurveda formulations like Ashtangalavana, Guduchyadi yoga, Ashwagandharishta, Shreekhandasava has been proved to be effective against the symptoms of alcohol use disorder **[6-9]**. Even though the above clinical trials have shown a promising result, the treatment protocols are not applicable much, as it requires in patient care to carry out the procedures **[10]**. Further, few medications aim only at mental

faculties whereas few formulations aim at hepatic damage. In some cases, the formulations are designed to curb the craving. Hence there was need for generating a formulation with holistic approach of correcting the vitiation of the Dosha in all systems. So an attempt was made to formulate a polyherbal formulation which was coded as Sumukti syrup.

MATERIALS AND METHODS

Sumukti syrup preparation:

The study formulation was prepared at Teaching pharmacy of Sri Dharmasthala Manjunatheshwara College of Ayurveda & Hospital Hassan using authenticated ingredients such as Guduchi (*Tinospora cordifolia),* Bringaraja (*Eclipta alba),* Kiratatikta (S*wertia chirayita*), Amalaki (*Emblica officinalis*), Hareetaki (*Terminalia chembula*), Vibhitaki (*Terminalia bellerica*), Draksha (*Vitisvinifera*), Mandukaparni (*Centellaa sciatica*), Ashwagandha (*Withenia somnifera*), Yashtimadhu *Glycirizha glabra*), Katuki (*Picririz hakurroa*), Sugar and Sodium benzoate. Dose of the animal was calculated on the basis of body surface area ratio as per Paget and Barnes- 1964. The test formulation was administered in syrup form with a dose of 0.5 ml per 100g body weight once daily orally which was calculated based on body surface ratio **[11]**.

Experimental animals:

Wistar albino rats of either sexweighing 200 ± 50 g were obtained from animal house attached to department of Pharmacology, SDM Centrefor Research in Ayurveda and Allied Sciences, Udupi.The experimental protocol was approved by the Institutional Animal Ethics Committee under the reference no. SDMCRA/ IAEC-2012-13DGM 02ab. The animals were fed with normal rat diet and water *ad libitum* throughout the study period. The animals were acclimatized fortwo weeks at the laboratory condition prior to the experimentation. The animals were maintained at controlled lighting of 12:12 hours light and dark cycle, temperature of 25ºC and relative humidity of approximately 50 ± 5%.

Evaluation of hepatoprotective activity:

Control group rats were administered with normal tap water at a dose of 5ml/kg in 0.5% gum acacia for 30 consecutive days. Group II, the toxicant control group was administered with 1mL of 20% ethanol for 21 consecutive days and paracetamol, 2g per kg body weight on 21st day and sacrificed on 22nd day. In Group III Sumukti syrup 1 ml/200 g body weight was given along with toxicants for 21 days and the animals were sacrificed

on 22 day. Group IV was administered with 1 ml of 20% ethanol and 2g per kg paracetamol for 21 days. Sumukti syrup 1 ml/200g body weight was coadministered and was continued till 30th day and the animals were sacrificed on 31day. The hepatoprotective effect of Sumukti syrup was evaluated by the assay of serum biochemical markers such as SGOT, SGPT and ALP according to standard methods.

Histopathological studies:

The animals were sacrificed and the abdomen was cut open to isolate the liver. Theliver was washed with normal saline and for histopathological study biopsy specimen was fixed in 10% formalin solution and then the liver tissue was embedded in paraffin. The section was cut into 5 μm thickness and stained with hematoxyline – eosin stain and mounted in diphenylxyline. The histopathological changes of liver tissue were observed under compound microscope and their microphotographs

Statistical analysis:

The data was represented as Mean ± SEM and was analyzed by employing one way ANOVA followed by Dunnet's multiple't' test using Graph pad prism version 3.5. The values were considered significant at the levels of $P<0.05$.

RESULTS

Repeated administration of 20% ethanol and single dose paracetamol 2g/kg body weight significantly increased serum SGPT and ALP as compared to the control group. Co-administration of Sumukti syrup has significantly reduced the elevated serum SGPT and ALP as compared to the toxic control group rats **(Table 1)**.

Repeated administration of 20% ethanol and single dose paracetamol 2g/kg body weight significantly increased serum total protein as compared to the control group. Co-administration of Sumukti syrup has markedly reduced the elevated serum total protein as compared to the toxic control group rats **(Table 2)**.

Repeated administration of 20% ethanol and single dose paracetamol 2g/kg body weight significantly increased serum triglyceride and significantly reduced HDL level as compared to the control group. Co-administration of Sumukti syrup has significantly reduced the elevated serum triglyceride and considerable increase in the HDL level as compared to the toxic control group rats **(Table 3)**.

There were no significant changes observed in the serum total bilirubin, direct bilirubin, urea and creatinine as compared to the control group rats. However there was significant increase in the serum total bilirubin in the treatment

II group as compared toxic control group **(Table 4)**.

Histopathological results:

The histopathological studies of liver sections from normal control rats showed normal cytoarchitecture of liver **(Figure 1 a & b)**. Liver sections from alcohol and paracetamol administered rats **(Figure 1 c & d)** showed hepatocyte necrosis, appearance of balloon cells, leukocyte infiltration, micro and macro fatty changes in the hepatocytes, sinusoidal dilatation and appearance of areas of regenerations. Sumukthi co-administration has shown mild to moderate protection in alcohol and paracetamol induced liver toxicity by maintaining normal cytoarchitecture of liver tissue in both treatment I groups. Mild degenerative changes in the form of necrosis, macro fatty changes and mild cell infiltration **(Figure 1 e, f, g & h)**.

Table 1

Group	SGOT	SGPOT	ALP
Normal control	145± 17.16	105.3± 13.28	334.37± 38.66
Toxic control group (Alcohol+ paracetamol)	218.14±24.32	342.42±88.10$^{@@}$	786.25±92.80 $^{@@}$
Treatment -1	123.37±7.72	80.28± 4.59$^{\#\#}$	783.87± 93.03
Treatment -II	627.75±162.35 $^{\#\#}$	78.75± 7.49 $^{\#\#}$	454±62.18$^{\#}$

Data expressed in Mean ± SEM, @- In comparison to normal control, # - In comparison to toxic control

Table 2

Group	TP	ALBUMIN	GLOBULIN
Normal control	6.59±0.12	3.46±0.099	3.18±0.09
Toxic control group (Alcohol+ paracetamol)	7.32±0.14$^{@@}$	4.22±0.116	3.16±0.14
Treatment -1	6.97±0.13	4.13±0.08	2.83±0.07
Treatment -II	6.9±0.125	3.64±0.16	3.21±0.085

Data expressed in Mean ± SEM, @- In comparison to normal control, # - In comparison to toxic control

Table 3

Group	CHOLESTEROL	TG	LDL	HDL	VLDL
Normal control	56.14± 3.5	65.5± 5.67	8.98±0.60	53.44± 3.18	15.88± 2.14
Toxic control group (Alcohol+ paracetamol)	51.25±2.9	98.85± 8.52$^{@}$	12.14± 1.80	27.75±1.82 $^{@@}$	18.15±2.09
Treatment -1	56.87±2.98	89.83± 13.36	7.8±1.48 $^{\#}$	35.5±2.78	20.67±2.67
Treatment -II	51.12±3.27	85.71± 8.21	11.85±0.38	31.37± 0.65	18.45± 1.19

Data expressed in Mean ± SEM, @- In comparison to normal control, # - In comparison to toxic control

Table 4

Group	Total bilirubin	Direct bilirubin	Urea	Creatinine
Normal control	0.191 ± 0.023	0.07 ± 0.009	36.8± 2.58	0.53±0.055
Toxic control group (Alcohol+ paracetamol)	0.122 ± 0.017	0.11 ± 0.02	30.62±1.25	0.56±0.046
Treatment -1	0.163 ± 0.023	0.16±0.023	37.25±1.46	0.625± 0.055
Treatment -II	0.218 ± 0.022 #	0.13± 0.02	33.5±1.32	0.45±0.032

Data expressed in Mean ± SEM, @- In comparison to normal control, # - In comparison to toxic control

Table 5

Group	Na^+	K^+	Cl^-
Normal control	139.11± 0.48	4.75±0.23	98.9± 0.69
Toxic control group (Alcohol+ paracetamol)	136.5±0.65 $^{@@}$	4.56±0.04	97.14±1.29
Treatment -1	137.87± 0.44	4.65±0.08	97.5± 0.70
Treatment -II	136.75±O.52	4,5±0.03	96.14± 1.079

Data expressed in Mean ± SEM, @- In comparison to normal control, # - In comparison to toxic control

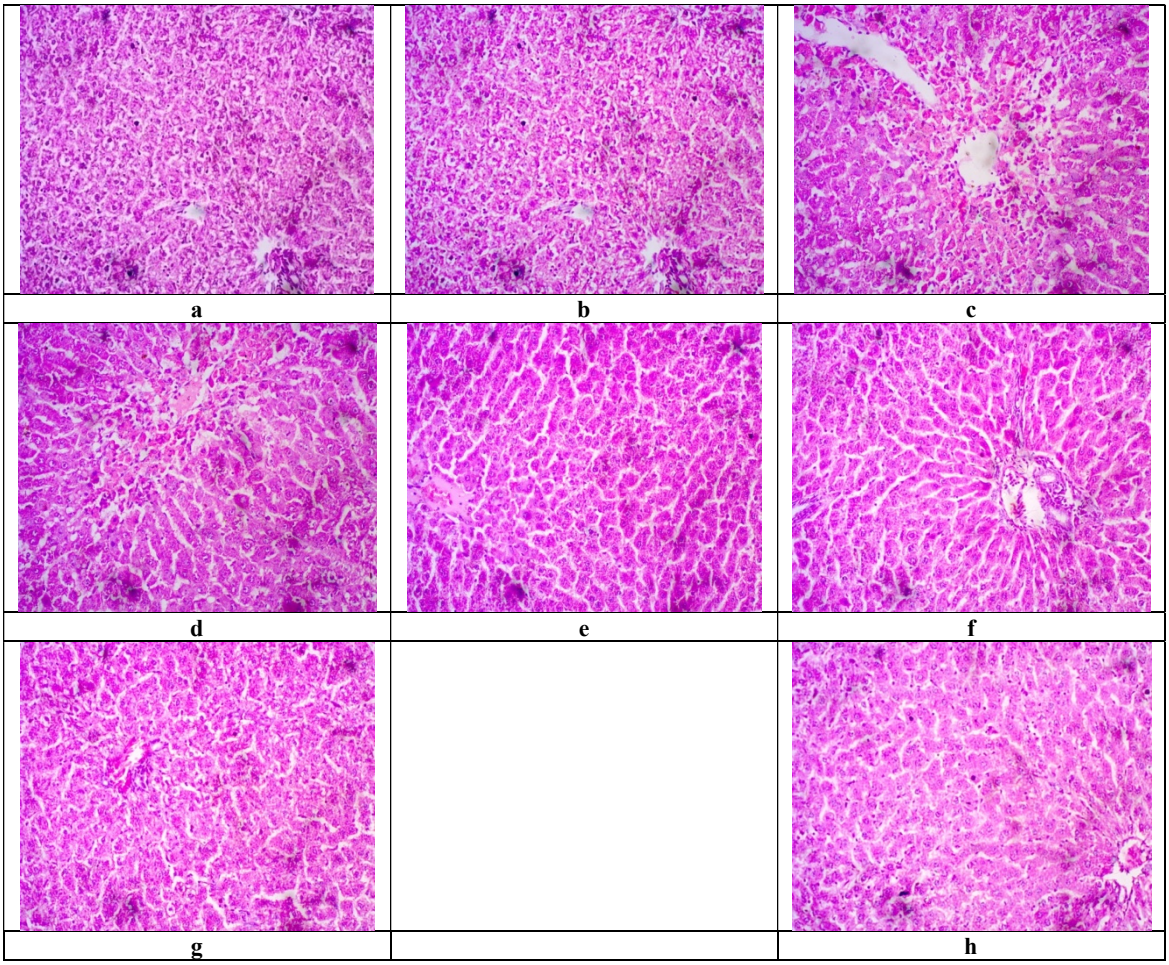

Figure 1: Effect of Sumukti on histopathology of liver in alcohol and paracetamol induced hepatotoxicity

DISCUSSION

The present work was aimed at preclinical evaluation of a new polyherbal syrup. The syrup was developed with an aim of alcoholic deaddiction activity. It is observed that the liver is one of the major organs that is affected due to alcoholism. Hence in this work an attempt was made to assess hepatoprotective activity of the study drug sumukti syrup.

The liver plays a very important role in the maintenance of vital functions and performance and regulating homeostasis of the body. Liver is considered to be of the most vital organs that functions as a centre of metabolism for nutrients such as carbohydrate, proteins and lipids and excretions of waste metabolites. Additionally, it has a role in the body's fight against disease, energy production and detoxification of foreign substances by detoxifying and eliminating them **[12]**. Because of its location and role in body's metabolic functions it is constantly exposed to factors which may inure it and making it one of the frequently affected organs.

There are numerous plants and traditional formulations available for the treatment of liver disorders **[13]**. Medicinal plants are significant source of

hepatoprotective drugs. Mono and poly-herbal preparations have been used in various liver disorders since ages. The Indian traditional medicine like *Ayurvedic, Siddha* and *Unani* are predominantly based on the use of plant materials. Silymarin is being used for this purpose in an extensive manner. However in *Ayurveda* many indigenous plants have been used as hepatoprotective agents. Herbal drugs have gained importance and popularity in recent years because of their safety, efficacy and cost effectiveness. Hence, there is an ever-increasing need for safe hepatoprotective agent **[14]**.

Several models are available for screening potential hepatoprotective drugs. Alcohol and Paracetamol induced liver injury model was selected for present study. Paracetamol induced hepatotoxicity is thought to be caused by N-acetyl-pbenzoquinoneimine (NAPQI), a cytochrome P450 mediated intermediate metabolite. NAPQI can react with sulphydryl groups such as glutathione and protein thiols. The covalent binding of NAPQI to cell proteins is considered the initial step in a chain eventually leading to cell necrosis. It has been established that a hepatotoxic dose of paracetamol depletes the endogenous glutathione level to below a threshold value <20% of control), therefore permitting interaction of NAPQI with cell macromolecule **[15]**.

Pondoral changes:

Three parameters namely body weight, weight of liver and kidney were studied under this. In normal control and therapeutic dose group, body weight gain was observed where as in toxic group, moderate decrease was observed. Due to short duration of the study there were no remarkable changes in the body weight pattern hence it is not considered important for assessment of the hepatoprotective potential of the testdrug. Moreover, the observed variation was not statistically significant.

Liver weight was found to be increased in toxicant group as well as study groups. This may be possible because of the changes in the hepatic structure that occurs due to hepatotoxicity. It shall be considered as a positive and intended change in the liver tissues. However the increase was marginal and does not provide statistical significance.

The third parameter was kidney weight. In this parameter Toxic control did not show much changes in the weight. There was further increase in the kidney weight in treatment groups. This observation could not be related to hepatoprotective activity of drugs.

Biochemical parameters:

Serum parameters studied were SGOT, SGPT, ALP, total protein, serum urea, serum creatinine, serum cholesterol,

serum triglycerides, blood sugar, total bilirubin, and direct bilirubin.

SGOT is a mitochondrial enzyme released from heart, liver, skeletal muscle and kidney. SGOT level may rise in acute necrosis or ischemia of other organs such as the myocardium, besides liver cell injury. It is well established that level of serum enzymes such as SGOT and SGPT gets elevated in ethanol and paracetamol induced hepatotoxicity. In the present study also, moderate elevation was observed. It is natural phenomenon and hence the elevation of SGOT in toxic control group is acceptable. This elevation of SGOT should be reversed with medications to establish the efficacy of study drug. Decrease of SGOT was seen in treatment group 1 where the concomitant use of medicine was there with drugs used to induce hepatotoxicity. But there was significant surge in the levels of SGOT in treatment II group where the medicine was given after inducing hepatotoxicity. This may be due to elevated levels contributed by injury to different organs. Hence it can be interpreted that the test drug might not have protective effect on all the organs. Further it can be interpreted as a preventive mode of the study drug than curative mode. However, such conclusion cannot be drawn only with one parameter.

SGPT is a cytosolic enzyme primarily present in the liver. Thus, serum estimation of SGPT which is fairly specific for liver tissue is of great value in liver cell injury. In the present study significant elevation of SGPT was observed in toxic control group. This elevation of SGPT was significantly antagonized in both the treatment groups. This can be considered as indicative of significant hepatoprotection along with histopathological examination.

Serum alkaline phosphatase (ALP) is produced by many tissues, especially bone, liver, intestine and placenta and is excreted in the bile. Elevation in activity of the enzyme can thus be found in diseases of bone, liver and in pregnancy. In the absence of bone disease and pregnancy, an elevated serum alkaline phosphatase levels generally reflect hepatobiliary disease. The greater elevation occurs in biliary tract obstruction. Slight to moderate increase is seen in parenchymal liver disease such as in hepatitis and cirrhosis and in metastatic liver disease **[16]**. In the present study significant increase of alkaline phosphatase activity was observed after ethanol & paracetamol injection. This increase was significantly reversed in treatment group II. This reversal was not seen in Treatment group I. Reversal of ALP decrease can be considered as an indicator of hepatoprotection.

Total serum Protein values decrease below normal range in different clinical conditions associated with nephrotic

syndrome, malnutrition, cirrhosis of liver and in other liver disease in which liver cells are severely damaged. Increased total protein value may be found in multiple myeloma and conditions associated with high globulin concentration **[17]**. In the present study significant increase in total protein was observed by toxic control group. Elevated protein levels were reduced in both treatment groups. However the reduction was not statistically significant. The mean value of total protein in normal control group was 6.59 which was elevated to 7.32 in toxic control group and was reduced to 6.97 and 6.90 respectively in treatment groups. This is an indicator that the medicines given along with and after inducing toxicity are effective in reversing the condition.

Bilirubin level rises in disease of hepatocytes, obstruction to biliary excretion into the duodenum, in hemolysis and defects of hepatic uptake and conjugation of bilirubin pigment such as Gilbert's disease. Bilirubin, a breakdown product of the porphyrin ring of heme- containing proteins, is found in the blood in two fractions- conjugated and unconjugated. The unconjugated fraction (indirect), is insoluble in water and is bound to albumin in blood. Elevation of the unconjugated fraction of bilirubin is rarely due to liver disease. An isolated elevation of albumin is seen primarily in hemolytic disorders and in a number of genetic conditions. In the present study there was no change that is seen direct bilirubin, indirect bilirubin, Albumin and Globulin parameters in toxic as well as treatment groups. Hence its estimation did not contribute to the determination of the hepatoprotective activity of the test drug.

A mild decrease in serum cholesterol and significant elevation in serum triglyceride level was observed after inducing hepatotoxicity in toxic control group when compared to normal control group. Lipid synthesized in the liver includes cholesterol and cholesterol esters, phospholipids and triglycerides. These lipids are insoluble in water and are carried in circulation with three major types of lipoproteins. They are high density lipoprotein (HDL), low density lipoprotein (LDL) and very low-density lipoprotein (VLDL). Estimation of total serum cholesterol, triglyceride, and lipoprotein fraction are frequently done in liver disease. Serum triglyceride is also elevated in cholestasis. These values are lowered in acute and chronic diffuse liver disease and in malnutrition **[18]**. There was no change in toxic control and both treatment groups on serum cholesterol. Even the other parameters like triglycerides, HDL and VLDL there were no marked changes in treatment groups when compared to normal control group. Significant elevation of

Triglycerides and significant decrease of HDL was seen in toxic control group. This is an expected variation because of hepatotoxicity that was induced. But it is interesting to note that Treatment I was able to show a significant decrease of LDL cholesterol which is only positive change that was seen in cholesterol spectrum.

Elevated levels of urea are observed in pre-renal conditions like diabetes mellitus, dehydration, cardiac failure etc., renal conditions like diseases of kidney and post renal conditions like enlargement of prostate etc. Decreased values have been reported in severe liver disease, protein malnutrition and pregnancy **[19]**. In the present study there was nonsignificant decrease of Urea was seen in toxic control group. The observed value was increase to the level of normal control level in both the treatment groups.

Serum Creatinine is increased in renal failure and also observed in certain other conditions like congestive heart failure, shock and mechanical obstruction of the urinary tract **[20]**. In the present study slight but insignificant elevation in serum Creatinine was observed in toxic group. Moderate non-significant reversal was observed in Treatment II group. Since creatinine is a bio-marker which indicates renal injury-the moderate elevation can be considered as indicative of weak to moderate renal injury and its reversal by test drug indicating presence renal protective effect.

Histopathology:

Liver sections from alcohol and paracetamol administered rats showed hepatocyte necrosis, appearance of balloon cells, leukocyte infiltration, micro and macro fatty changes in the hepatocytes, sinusoidal dilatation and appearance of areas of regenerations. This is an indication that there will be a severe grade of hepatotoxicity induced as expected in the protocol of experimental study. Co-administration of study drug has shown mild to moderate protection in alcohol and paracetamol induced liver toxicity by maintaining normal cytoarchitecture of liver tissue in both treatment group. Severe damage to the cells could be reversed by taking degenerative changes in the form of necrosis, macro fatty changes and mild cell infiltration in to account.

CONCLUSION

Overall analysis of serum biochemical parameters shows that administration of ethanol and paracetamol leads to significant change in majority of the parameters. These altered biochemical parameters were found to be reversed in some of the instances like SGPT, ALP, TP, TG, LDL and HDL. There were exceptions like alteration of SGOT in treatment group and no changes seen in parameters like Serum Cholesterol, direct bilirubin, Serum

Creatinine and Serum Urea. The overall activity profile indicated reversal of important parameters and hence presence of moderate to good hepatoprotection.

The observations of histopathological study can be considered as an important and un-equivocal evidence for the presence of heapatoprotective activity in the test formulation.

Acknowledgements:

Authors would like to acknowledge the research grants provided by Rajiv Gandhi University of Health Sciences, Karnataka Bangalore for present research work

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