Int J Ayu Pharm Chem
ISSN 2350-0204
www.ijapc.com

ORIGINAL RESEARCH ARTICLE

Hepatoprotective Activity of *Shrikhand Asava* – A Preclinical Study

Author: Ruchi Sharma[1]

Co Authors: Mangala Jadhav[2]

[1,2]Dept. of Rasa Shastra evum Bhaishajya Kalpana, R.A. Podar Medical (Ayu.) College, Worli, Mumbai, MS, India

ABSTRACT

Background: Liver plays important roles in activities such as – metabolism, excretion, etc. Due to these reasons it is more prone to toxicities. Among this alcohol toxicity is most common which causes alcohol liver disease (ALD). ALD is the common reason of morbidity and mortality worldwide. Along with this there is no reliable and complete effective drug for liver protection.

Objectives: To evaluate the *ayurvedic* formulation *Shrikhand asava* of *Bhaisajaya Ratnavali* which is indicated in *madatya roga* for it's hepatoprotective activity.

Methods: *Shrikhand asava* is prepared according to the *Bhaisajaya Ratnavali* reference and then this formulation is evaluated for it's hepatoprotective action by inducing hepatotoxicity with ethanol in Wistar rats. Standard group used for comparison is of Silymarin drug**.** Parameters used in study are- Body weight, Total Bilirubin, SGOT, SGPT, Serum Alkaline Phosphate, Total cholesterol, Total protein, globulin, albumin and histopathology.

Results: Reduction in raised serum liver enzymes and improved histopathological changes of both liver and kidney in *Shrikhand asava* group.

Conclusion: *Shrikhand asava* shows hepatoprotective action comparable to that of Silymarin.

Keywords *Shrikhand asava*, hepatoprotective, Wistar rats, alcohol induced hepatotoxicity, *ayurvedic formulation*

Received 05th September 21 Accepted 07th September 21 Published 10th September 2021

INTRODUCTION

Liver is a vital organ which play a role in metabolizing, detoxifying various metabolites, synthesizes proteins and also produces necessary biochemical for digestion and growth[1,2]. It is located in right upper quadrant of the abdomen, below the diaphragm. It also play a role in the regulation of glycogen storage, decomposition of red blood cells, and the production of hormones. Because of it's function it is also prone to many diseases[3] Liver diseases are diagnosed by liver function tests–blood tests that can identify various markers. If the pathology is not cleared or for more diagnosis ultrasonography is done.

Centers for Disease Control and Prevention (CDC), had declared that in 2014 the number of deaths from alcoholic liver disease in the United States was 19,388, while all causes of chronic liver

ORIGINAL RESEARCH ARTICLE

disease and cirrhosis are estimated to cause 12 fatalities per 100,000 people per year. And in 2015, United States had nearly 20% of all liver transplants occurred as a result of alcoholic liver disease, which makes it the third most common reason for transplant[4]. Also according to World Health Organization, alcohol is said to be a risk factor in more than 200 health disorders which includes high blood pressure, stroke, coronary heart disease, liver cirrhosis and various cancers. Worldwide, 3.3 million people used to die every year due to the alcohol abuse; this represents 5.9% of total of all deaths[5] .

Excessive alcohol consumption caused liver toxicities which damages the liver, leading to a build up of fats, inflammation, and scarring. These are grouped under alcoholic liver diseases which includes- alcoholic hepatitis, fatty liver, and cirrhosis. Factors which contributes to the development of alcoholic liver diseases are not only the quantity and frequency of the alcohol consumption, but it also include gender, genetics, and liver insult. Liver used to metabolize alcohol into the highly toxic acetaldehyde by the enzyme alcohol dehydrogenase. Acetaldehyde oxidase or xanthine oxidase then oxidizes acetaldehyde to acetate by giving rise to Reactive oxygen species(ROS) via cytochrome P 450 2E1. Prolonged consumption of alcohol also increases the nitric oxide (NO) level which leads to formation of the toxic oxidant peroxynitrite. Low capacity of the antioxidants leads to damage of cells of the hepatic cells and the cell organelles with the release of the reactive aldehydes and ROS[6].

In addition to this, there is no such effective medicine in modern science for hepato-protective action. So formulations suggested in *ayurvedic* sciences many years back should be tried and tested for their action. So, a formulation mentioned in *Bhaisajya Ratnavali* for the treatment of *madatya roga* i.e. *shrikhand asava* is taken in this study[7].

One that produces *Mada* is known as *Madya*[8] in *ayurveda* and the disease which is produced due to improper use of *Madya* is known as *Madatyaya*[9]. *Madatyaya* is produced when person used to consume the *Madya* without considering *Prakriti*, *Satmya*, *Agni*, etc[10]. As per *Ayurvedic* concepts *Madatyaya* is a *Tridoshaja Vyadhi* mainly involving *Kapha Sthana* which is vitiated along with *Agni*. The 10 *Gunas* of *Madya* are stated as- *Laghu, Ushna, Teekshna, Sukshma, Vishada, Amla, Vyavayi, Aashu, Vikashi and Ruksha*[11]. All the *Madatyaya* are known to be *Tridoshaja Vyadhi* so the drug of *Tridosha shamak* effects should be used. In this present study the *Tridoshaja shamak* formulation "*shrikhand asava*" is used. *Bhasajya Ratnavali* has stated to use it in *sannipataj madatya* i.e. *madatya* involving three of *doshas*. Also *Acharya Charaka* has mentioned the use of *Madya* in the treatment of *madatayaya Roga* by giving the reason that *Madya* which is *Teekshana, Ushna, Amla* and *Vidahi*, if taken in excessive amount makes the *Annaras Utkleda* and will be digested improperly that is ultimately it gets turns into *Kshara* and

ORIGINAL RESEARCH ARTICLE

causes *Antardaha*, *Jwara*, *Trishna*, *Pramoha*, *Vibhrama* and *Mada*. In this situation if same or other type of *Madya* which is *Amala* in taste is administered in appropriate manner and quantity it gets mixed with *Kshara Rasa* produced during pathology of *madatayaya roga* and convert it into sweet taste i.e *kshara* is neutralized by *amala* rasa. *Madya* is the best among the *Dravya* having *Amla* Rasa. So if this principle is taken into consideration, use of *Arishta* which is also a kind of *madya Kalpana* and has *amala rasa* also act in the same manner and must show effect in the *madatayaya roga.*

OBJECTIVES

- To prepare *Shrikhand asava* in accordance with *Bhaisajya* Ratnavali.
- To study the development of the hepatotoxicity model by inducing alcohol in the Wistar rats.
- To compare the hepatoprotective activity of *Shrikhand asava* with the Silymarin in the Wistar rats.

METHODOLOGY

MATERIALS

For Drug preparation:

Weighing machine, Raw drugs, *Ulukhalyantra*, Porsalin jar, Mulsulin Clot, Sieve no 85 etc.

For Experimental study:

Shrikhand Aasava, Glass beakers, 18G needle, Disposable Syringes, Ethanol Feeding needles, Hand gloves, Glass rod, Biochemical test kit, Standard (Silymarin), Distilled water, Male Wistar rats weighing 180-200gm, Weighing machine

METHODS

The study will be carried out in the following two steps:

A. Pharmaceutical study

B. Experimental study

A. Pharmaceutical Study

Prepration of *Shrikhand Asava* as per reference of *Bhaisajya Ratnavali*[7]. B.R. 22/ 29-33 and A.F.I part 1[12]

श्रीखण्डं मरिचं मांसी रजन्यौ चित्रकं घनम्।

उशीरं तगरं द्राक्षां चन्दनं नागकेशरम्॥

पाठां धात्रीं कणां चव्यं लवङ्गञ्चैलबालुकम्।

लोध्रञ्चार्द्धपलोन्मानं जलद्रोणद्वये क्षिपेत्॥

द्राक्षां षष्टिपलां तत्र गुडस्य च तुलात्रयम्।

धातकीं द्वादशपलाञ्चैकत्र परियोजयेत्।

मासं संस्थाप्य मृद्भाण्डे वस्त्रपूतं रसं नयेत्।

पाययेन्मात्रया वैधौ वयोवह्न्याधपेक्षया॥

पानात्ययं परमदं पानाजीर्णञ्च नाशयेत्।

पानविभ्रममत्युग्रं क्षीखण्डासव आशु च॥(भै. र. २२/२९-३३)

Ingredients of Shrikhand [7,12]:

1. *Shweta Chandana* -24gm
2. *Kali Maricha* -24gm
3. *Jatamansi* – 24gm
4. *Haridra* -24gm
5. *Daruharidra* -24gm
6. *Chitraka mula* -24gm
7. *Nagarmotha* -24gm
8. *Khasa* -24gm

ORIGINAL RESEARCH ARTICLE

9. *Tagara* -24gm
10. *Munakka* -24gm
11. *Rakta Chandana* -24gm
12. *Nagakesar* -24gm
13. *Patha* -24gm
14. *Amala* -24gm
15. *Pippali* -24gm
16. *Chavya* -24gm
17. *Lavanga* -24gm
18. *Elvaluka* -24gm
19. *Lodhra* -24gm
20. Water – 24.576*l*
21. *Draksha* – 2.880kg
22. *Guda*(Jaggery) – 14.400kg
23. *Dhatki pusha* - 576gm

Method of Preparation[7,12]

1. The required quantity of water, to which jaggery as prescribed in the formula was added, boiled and cooled.
2. Then was poured into the fermentation vessel.
3. Now fine powders of the drugs mentioned in the ingredients from 1-19 and crushed draksha were added.
4. At the end, *Dhatki pushpa*, should be properly cleaned and added.
5. Then container was covered with a lid and the edges were sealed with clay-smeared cloth wound in seven consecutive layers.
6. This container is kept in a heap of paddy, so as to ensure that for the duration of fermentation, as far as possible, a constant temperature may impede or accelerate the fermentation.
7. After the specified period, the lid is removed, and the contents examined to ascertain whether the process of fermentation (*Sandhana*) has been completed.
8. After confirmation of the *sandhana lakshana*, the fluid was first decanted and then strained after two or three days. When the fine suspended particles settle down, it is strained again and bottled.

Uses of *Shreekhand* [7,12]

Panatyaya (Acute alcoholism), *Mada* (Intoxication), *Panavibhrama* (Delirium due to alcohol intoxication), *Panajerna* (Alcoholic intoxication)

Human Dose[7,12]**-**

12-24ml

B. Experimental Study

Male Wistar rats of either sex weighing 180-200 gm were used for the induction of hepatotoxicity by giving ethanol. The hepatotoxicity was induced by administration of ethanol orally once in a day for 28 days. The dose of ethanol was increased slowly, as tolerance developed, to maintain blood alcohol levels in the range of 150–300 mg/dl. The starting dose was 8g/kgd; the final dose was 16 g/kgd.

After 28 days, six rats in total were randomly selected and blood is collected from their retro orbital space of the eye and assessed for the induction of hepatotoxicity by sending their blood to the laboratory for analysis of SGOT, SGPT, serum Alkaline Phosphate, Total Bilirubin, Cholesterol, Triglyceride, Total protein, Albumin and Globulin levels. After confirming that hepatotoxicity was induced, the animals were

ORIGINAL RESEARCH ARTICLE

divided into three groups (each group containing 6 rats).

All the selected rats were weighed and randomly divided into 3 groups, containing 6 rats each group as shown below and were given following treatment for 28 days.

Groups	No of animals	Treatment
Control group	6	Ethanol
Test Drug group	6	Shreekhand Aasava(400mg/kg) + Ethanol
Standard group	6	Silymarin 100mg/kg + Ethanol

Parameter

Body weights were measured before and after treatment. Blood investigation was done for both confirming the induction of hepatotoxicity and at the end of the experiment. After collection of blood rats were sacrificed, then their liver and kidney was sent for histopathological studies.

Biochemical and histological analysis

The serum transaminase and lipid profile were analyzed by an automated analyzer using diagnostic kits (ERBA, Czech Republic). The histology was performed using standard protocol and the tissues were stained using hematoxylin and eosin (H&E).

Statistical analysis

Values are expressed as a mean ± standard error of the mean (SEM). Data were analyzed using ANOVA and multiple comparisons were carried out using the Tukey's Honest Significance test. Statistical significance was considered for p values < 0.05.

RESULTS

Values of Weight changes, SGOT, SGPT, Total Bilirubin, Alkaline Phosphate, Total Cholesterol, Triglyceride, Total Protein, Albumin and Globulin are expressed as mean ± SEM, N = 6 animals of each group in Table1.a, 2.a, 3.a, 4.a, 5.a, 6.a, 7.a, 8.a, 9.a and 10.a and in fig 1-10 respectively.

Table1.a Treatment on change in the body weight of rats in the form of Mean ± SEM

	Control	Test drug	Standard
Weight	277.67±12.32	325.67±7.75	312.50±11.90

Table2.a Treatment on SGOT value in the form of Mean ± SEM

	Control	Test drug	Standard
SGOT value	119.67± 7.96	58.63±4.55	63.95±13.57

Table3.a Treatment on SGPT value in the form of Mean ± SEM

	Control	Test drug	Standard
SGPT value	62.17±8.03	31.42±4.62	31.12±2.70

Table4.a Treatment on Total Bilirubin value in the form of Mean ± SEM

	Control	Test drug	Standard
Total Bilirubin value	0.76±0.04	0.40±0.07	0.33±0.03

Table5.a Treatment Alkaline Phosphate value in the form of Mean ± SEM

	Control	Test drug	Standard
Alkaline Phosphate value	225.33±12.06	159.33±9.07	144.83±7.86

Table6.a Treatment on Cholesterol value in the form of Mean ± SEM

	Control	Test drug	Standard
Cholesterol value	263.17±13.22	175.67±13.88	161.67±9. 47

Table7.a Treatment on Triglyceride value in the form of Mean ± SEM

	Control	Test drug	Standard
Triglyceride value	165.83±6.90	129±5.81	111.83±6.02

Table8.a Treatment on Total Protein value in the form of Mean ± SEM

	Control	Test drug	Standard

ORIGINAL RESEARCH ARTICLE

Total Protein value	6.90±0.17	4.85±0.55	4.68±0.43

Table9.a Treatment on Albumin value in the form of Mean ± SEM

	Control	**Test drug**	**Standard**
Albumin value	4.32±0.24	2.96±0.17	2.81±0.48

Table10.a Treatment on Globulin value in the form of Mean ± SEM

	Control	**Test drug**	**Standard**
Globulin value	3.32±0.27	2.44±0.28	2.53±0.13

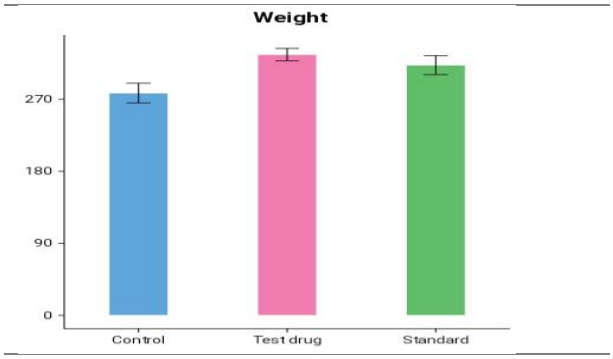

Figure 1 Graphical Representation of showing effect of different treatment on weight of rats

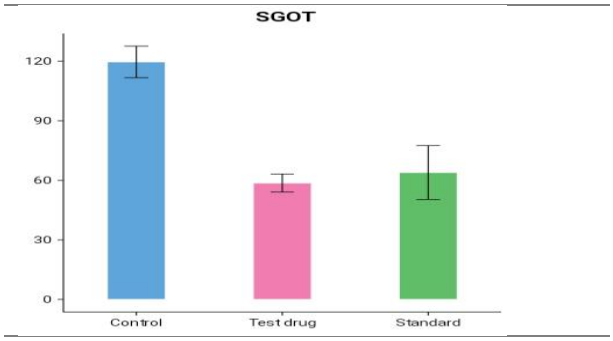

Figure 2 Graphical Representation of showing effect of different treatment on SGOT of rats

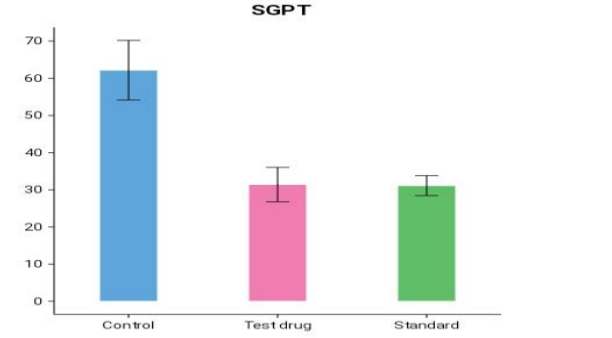

Figure 3 Graphical Representation of showing effect of different treatment on SGPT of rats

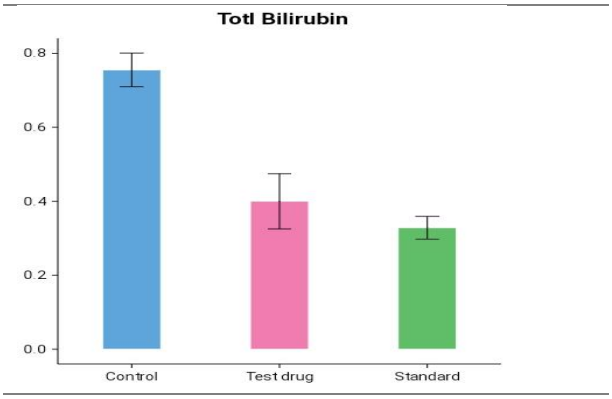

Figure 4: Graphical Representation of showing effect of different treatment on Total Bilirubin of rats

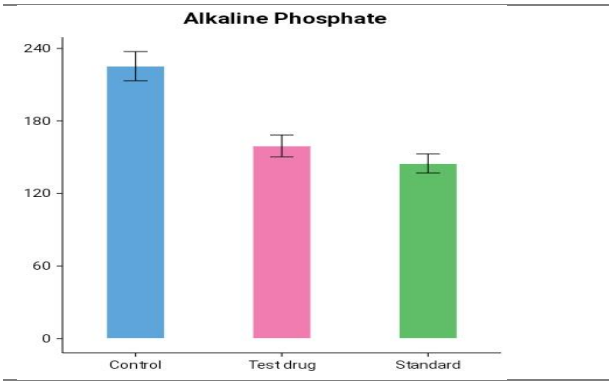

Figure 5 Graphical Representation of showing effect of different treatment on Alkaline Phosphate of rats

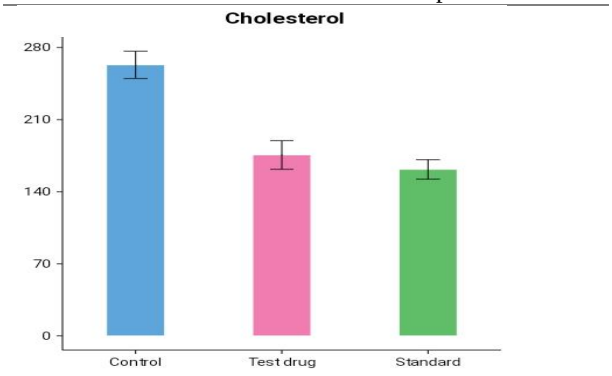

Figure 6 Graphical Representation of showing effect of different treatment on Cholesterol of rats

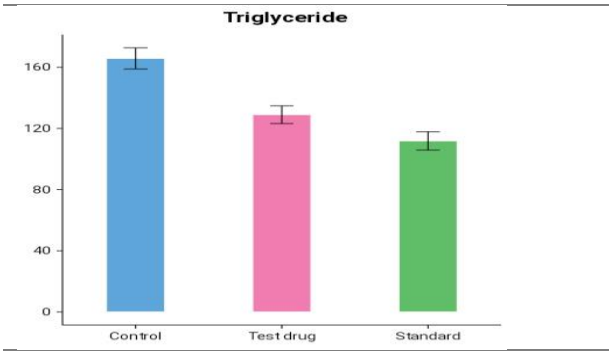

Figure 7 Graphical Representation of showing effect of different treatment on Triglyceride of rats

Int J Ayu Pharm Chem
ISSN 2350-0204
www.ijapc.com

ORIGINAL RESEARCH ARTICLE

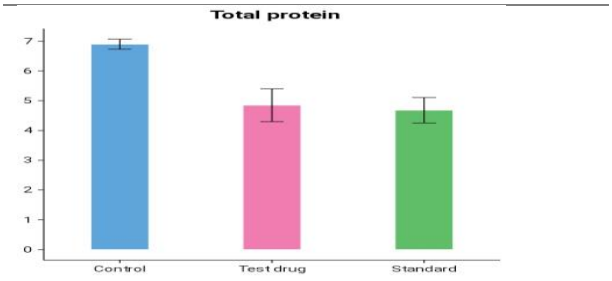

Figure 8 Graphical Representation of showing effect of different treatment on Total Protein of rats

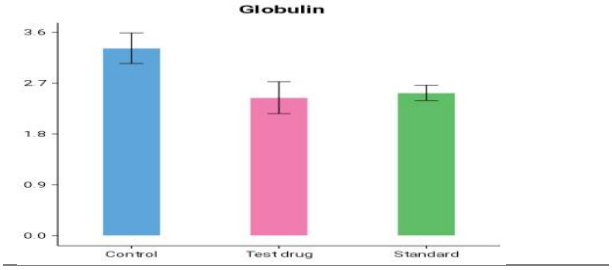

Figure 9 Graphical Representation of showing effect of different treatment on Globulin of rats

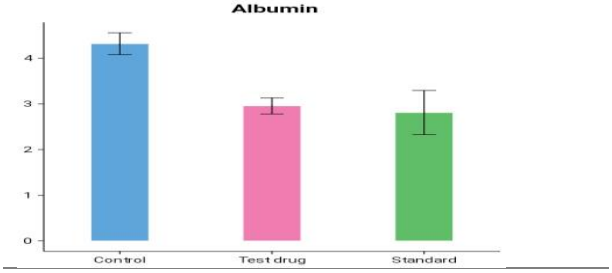

Figure 10 Graphical Representation of showing effect of different treatment on Albumin of rats

Difference between the groups was statistically determined by one way analysis of variance (ANOVA) and P value of each parameters such as- Weight changes, SGOT, SGPT, Total Bilirubin, Alkaline Phosphate, Total Cholesterol, Triglyceride, Total Protein, albumin and globulin found to be:<0.05, <0.001, <0.01, <0.0001, <0.0001, <0.0001, <0.0001, <0.01, <0.01 and >0.05 respectively. The results of all the parameters found to be significant. Then Tukey's Honesty Significance Test was done to compare between the groups and results of all parameters were represented in Table 1.b, 2.b, 3.b, 4.b, 5.b, 6.b, 7.b, 8.b, 9.b and 10.b.

Table1.b Comparative results after applying Tukey's Honest Significance test between groups in relation to SGOT value

Groups	P value	Impression
Control vs TD	<0.05	Significant
Control vs Standard	<0.05	Significant
TD vs Standard	>0.05	Non Significant

Table2.b Comparative results after applying Tukey's Honest Significance test between groups in relation to SGOT value

Groups	P value	Impression
Control vs TD	<0.01	Very Significant
Control vs Standard	<0.01	Very Significant
TD vs Standard	>0.05	Non Significant

Table3.b: Comparative results after applying Tukey's Honest Significance test between groups in relation to SGPT value

Groups	P value	Impression
Control vs TD	<0.01	Very Significant
Control vs Standard	<0.01	Very Significant
TD vs Standard	>0.05	Non Significant

Table 4.b Comparative results after applying Tukey's Honest Significance test between groups in relation to Total Bilirubin value

Groups	P value	Impression
Control vs TD	<0.001	Highly Significant
Control vs Standard	<0.001	Highly Significant
TD vs Standard	>0.05	Non Significant

Table 5.b Comparative results after applying Tukey's Honest Significance test between groups in relation to Alkaline Phosphate value

Groups	P value	Impression
Control vs TD	<0.001	Highly Significant
Control vs Standard	<0.0001	Highly Significant
TD vs Standard	>0.05	Non Significant

Table 6.b Comparative results after applying Tukey's Honest Significance test between groups in relation to Cholesterol value

Groups	P value	Impression
Control vs TD	<0.001	Highly Significant
Control vs Standard	<0.0001	Highly Significant
TD vs Standard	>0.05	Non Significant

Table 7.b Comparative results after applying Tukey's Honest Significance test between groups in relation to Triglyceride value

Groups	P value	Impression
Control vs TD	<0.01	Very Significant
Control vs Standard	<0.0001	Highly Significant

ORIGINAL RESEARCH ARTICLE

TD vs Standard	>0.05	Non Significant

Table 8.b Comparative results after applying Tukey's Honest Significance test between groups in relation to Total Protein value

Groups	P value	Impression
Control vs TD	<0.01	Very Significant
Control vs Standard	<0.01	Very Significant
TD vs Standard	>0.05	Non Significant

Table 9.b Comparative results after applying Tukey's Honest Significance test between groups in relation to Albumin value

Groups	P value	Impression
Control vs TD	<0.05	Significant
Control vs Standard	<0.05	Significant
TD vs Standard	>0.05	Non Significant

Table 10.b Comparative results after applying Tukey's Honest Significance test between groups in relation to Globulin value

Groups	P value	Impression
Control vs TD	>0.05	Non Significant
Control vs Standard	>0.05	Non Significant
TD vs Standard	>0.05	Non Significant

Comparative histopathological reports of liver and kidney of different treatments are mentioned in the Table 11.a and 11.b and Fig 11.a and 11.b respectively.

DISCUSSION

Induction of hepatotoxicity by administration of ethanol is confirmed by the raised parameters of SGOT, SGPT, Total Bilirubin and Alkaline Phosphate. Raised cholesterol and Triglyceride values evident are indication of fatty liver. After confirmation of hepatotoxicity, treatment are given for 28 days whose results are compared by using ANOVA test which shows significant p value for every parameter which depicts that there is significant difference between the treatment of all the three groups. As ANOVA results are found significant, so further post hoc test- Tukey's Honest Significance test was applied to compare results of treatment between each groups. The result depict that both Silymarin and the *Shreekhand Aasava* had showed significant effect in reducing all the raised parameters as compared to control group. And when the treatment of Silymarin and *Shrekhand Asava* are compared, it shows that both had equal potential in reducing the raised levels of all these parameters. Generally, in hepatotoxicity, levels of Total Protein, Albumin and Globulin decreased, but in this study these levels increased after hepatotoxicity induction and both the treatment helps in decreasing these levels that can be a indication of kidney protection activity.

Table 11.a Histopathology report of Liver of different treatment groups

HISTOPATHOLOGICAL ANALYSIS REPORT		
Group	**Histological observations – Liver**	**Overall Pathological lesion score**
Control	The histopathological observations of tissue sections of liver from this group showed Moderate pathological changes. The vascular tissue showed focal congestion of central vein. The hepatocytes showed cellular swelling and granular cytoplasm and presence of fatty accumulation in the hepatocytes. Mild degenerative changes and focal necrotic changes of hepatocytes with loss of cellular border were noted. Focal necrotic changes were also evident with loss of nucleus from hepatocytes. Focal inflammatory cellular infiltration of mononuclear cells was also observed in the liver tissue section.	**Moderate (+3)**
Test Drug	The histopathological observations of tissue sections of liver from Group 4 (**DP III-S 1 to S 3**) showed **focal** and **very minimal pathological** changes in the hepatocytes.	**Minimal (+1)**

	There was absence of any inflammatory cellular changes in the hepatic tissue.	
Standard	The histopathological observations of tissue sections of liver showed **minimal pathological** changes in the hepatocytes. The hepatocytes showed focal and minimal degenerative changes with granular cytoplasmicchanges with occasional presence of fatty changes in the hepatocytes. There was absence of any inflammatory cellular changes in the hepatic tissue.	**Minimal (+1)**

Table 11.b Histopathology report of Kidney of different treatment groups

HISTOPATHOLOGICAL ANALYSIS REPORT		
Groups	**Histological observations –Kidney**	**Overall Pathological lesion score**
Control	The microscopic histopathological observations of tissue sections showed mild pathological changes of renal parenchyma. The vascular tissue showed focal congestion in medulla and cortex. Focal interstitial hemorrhages were also noted in renal parenchyma in the renal parenchyma. Cellular swelling and tubular degeneration with focal necrosis of tubular epithelium were also noted in the medullary region. The renal tubules showed mild to moderate degenerative changes with granular cytoplasm and enlarged tubules. Focal hypertrophic features of glomeruli were also noted in the cortical region.	**Mild (+2)**
Test Drug	The histopathological observations of tissue sections of kidney from Group 4 (**DP III- S1 to S3**) showed **minimal and focal pathological** changes of renal parenchyma. Focal and minimal changes with cellular swelling of renal tubules were noted only	**Minimal (+1)**
Standard	The histopathological observations of tissue sections of kidney showed **minimal and focal pathological** changes of renal parenchyma. Focal and minimal changes with cellular swelling of renal tubules were noted only	**Minimal (+1)**
Note : Overall Grade score as- NAD =No Abnormality Detected, Minimal changes (+1), Mild changes (+2), Moderate changes (+3), Severe changes (+4).		

Also when histopathology reports are studied it shows that, the overall pathological lesion score of liver that is +3 in case of control group is reduced to +1 in case of both treatments and also lesion score of kidney which is +2 in case of control group is reduced to +1 in case of both treatments. Thus it is depicted from histopathology reports that both Silymarin and *Shrekhand Asava* had helped in improving the condition of liver and kidney that are injured due to toxicity of alcohol.

CONCLUSION

Thus from the results of study it's concluded that *Shreekhand Aasava* has hepatoprotective action and ability to reduce the Cholesterol and Triglyceride levels in alcoholic liver disease comparable to that of standard drug. From histopathology reports of kidney it is evident that both Silymarin and *Shrekhand Asava* also had protective action for kidney.

SCOPE FOR STUDY

- *Shreekhand Aasava* can be evaluated for it's hepatoprotective action in other toxicity models.
- Different doses of *Shreekhand Aasava* can be evaluated
- Silymarin and *Shrekhand Asava* can be evaluated for it's renal protective actions more evidently.

REFERENCES

1. Elias, H.; Bengelsdorf, H. (1 July 1952). "The Structure of the Liver in Vertebrates". Cells Tissues Organs. 14 (4): 297–337. doi:10.1159/000140715. PMID 14943381.

2. Abdel-Misih, Sherif R.Z.; Bloomston, Mark (2010). "Liver Anatomy". Surgical Clinics of North America. 90 (4): 643–653. doi:10.1016/j.suc.2010.04.017. PMC 4038911. PMID 20637938.

3. Cirrhosis Overview Archived 2011-10-30 at the Wayback Machine National Digestive Diseases Information Clearinghouse. Retrieved 2010-01-22

4. https://www.medicalnewstoday.com/articles/215638#prevention

5. http://www.who.int/mediacentre/factsheets/fs349/en/ cited date: 14/01/2017

6. Saaluetal. The hepato-protective potentials of Moringa oleifera leaf extract on alcohol – induced hepato-toxicity in wistar rats. American journal . American Journal Biotechnology and Molecular Sciences, 2 (1): 6-14. DOI: 10.5251/ajbms.2012.2.1.6.14

7. Govind das. Bhaisajyaratnavali.vidyotini, 18th edition, 20019, Varanasi; Chaukhambha Prakashan, madatyay, shloka no , p.no- 29-33, p.no- 504

8. Bhavmishra. Bhavprakasha. Brahmshankar mishra, 11th edition, 2004, Varanasi; Chaukhambha Sanskrit sansthan; Sandhanvarga, Shloka no.18. p.785.

9. Vachaspatyam, Dictionary, Tarkavachaspati; Varanasi: Chaukhamba Sanskrit Series; p.-4728

10. Agnivesha. Charaka Smahita, revised by Charaka and Dridhabala, with Ayurveda Dipika commentary of Chakrapanidatta, edited by: Vaidya Jadavaji Trikamji Acharya. Reprint:2009 Subharti Prakashan, Chikitsa Sthan, 24/109, p.-588. Edited by , Pt. AA Sanakrit Comprehensive by: Compiled Sri Taranatha Varanasi: Chaukhmba

11. Vagbhata. Astangahridayam. Edited by: Pt. Hari Sadasiva Shashtri Paradakara. Reprint: 2002, Varanasi: Chaukhamba Subharati Prakashan, Nidan Sthan, 6/1, p.-485.

12. Anonymous, the Ayurvedic Formulary of India. Part I, Second edition, Govt. of India, M.H. & F.W., Dept. of AYUSH, 2003, pg no- 95-97.