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Research Article

HEPATOPROTECTIVE ACTIVITY OF *PATRA SWARASA* OF *GUDUCHI* (TINOSPORA CORDIFOLIA) AGAINST- PARACETAMOL INDUCED

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ABSTRACT

Guduchi (*Tinospora cordifolia* (Willd.) Miers], Menispermaceae family, it is important medicinal plant is widely distributed through India. It is categorized as "*Rasayana*" and the whole plant is used medicinally. The **aim** of the present study is to evaluate the protective effect of *Guduchi patra swaras* against paracetamol induced liver damage in Wister albino rats. **Material & Methods:** Wistar Albino rats (140-250g) will be divided into 6 group and each group 6 rats, into control group, paracetamol control, standard group, test group. The test drug *Guduchi patra swarasa* was administered orally for 10 consecutive days and two dose of the toxicant (paracetamol) were administered orally to each group, on two alternate days i.e., 7th and 9th day, 1hr after test drug administration. After 48 hours of toxicant paracetamol, and standard group. The blood was collected in EDTA tube and sent for biochemistry laboratory for biochemical investigations. **Result:** The analysis of serum biochemical parameters shows that administration of paracetamol leads to significant change in majority of the parameters. The overall activity profile indicated reversal of important parameters like SGOT, SGPT, total bilirubin, total protein, blood sugar level, serum globulin, serum albumin, serum total cholesterol and serum triglycerides. All three test groups of *Guduchi patra Swarasa* administered are found to be effective heapto-protective especially based on histo-pathological study. Among the three groups, the double dose group and therapeutic group has shown good results in comparison with the other two groups**. Conclusion:** In the present study, the overall activity profile of *Guduchi patra Swarasa* administered are found to be effective hepatoprotective among the three groups therapeutic double dose shows good results in comparison with the other group.

INTRODUCTION

Guduchi patra [*Tinospora cordifolia* (Willd.) Miers Ex Hooks F. & Thoms] of Menispermaceae Family,[1] is found throughout country[2], easily available cost effective and have been described in various *Nighantus*. Different pharmacological actions like *Kamalahara, Rasayana, Pramehaghana, Kasahara, Swasahara, Kusthaghana, Jwarahara, Krimighana, Mutrakrichahara* and *Hridya etc.* are mentioned in *Bhavaprakash Nighantu,[3] Kaiyadeva Nighantu,[4] Madanapala Nighantu,[5] Priya Nighantu,[6] Shaligram Nighantu,[7] Shodhala Nighantu,[8] Gunratnamala,[9]* thus indicating its hepatoprotective property.

In *Charaka samhita chikitsa sthana Pandu roga adhyaya Guduchi patra* is indicated in *Kamala[10]* and same references are mentioned in *Bhavaprakash Madhyama khanda,*[11] *Bhavaprakash Nighantu shaka varga,[12] Gunaratnamala Guduchyadi varga*[13] *and Haritkyadi Nighantu*[14] thus supports its hepato-protective property. The liver is the vital organ of the body, its having a pivotal role in regulation of many physiological process such as production of biochemical's necessary for digestion, glycogen storage, decomposition of red blood cells, plasma protein synthesis, hormones production etc.

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furthermore, detoxification of a variety of drugs and xenobiotics also occurs in liver.[15]

Liver diseases are one of the most common prevailing problems in the health sector. They may be classified as acute or chronic hepatitis (inflammatory liver diseases), hepatosis (non-inflammatory diseases) and cirrhosis (degenerative disorder resulting in fibrosis of liver). Liver diseases are manly caused by toxic chemicals (certain antibiotics, chemotherapeutics, peroxidised oil, aflatoxin, carbon-tetrachloride, chlorinated, etc), excess consumption of alcohol, infections and autoimmune disorders.[16]

AIM

To evaluate the hepato-protective activity of *Guduchi* [*Tinospora cordifolia* (Willd.) Miers Ex Hook. f. and Thoms] *Patra Swarasa* in animal model.

OBJECTIVES

- To evaluate hepatoprotective activity by estimating biochemical parameters.
- To evaluate the hepatoprotective activity by histopathalogical examination of liver tissues.

MATERIAL AND METHOD

1. Test drug

The test drug *Guduchi patra* (Tinospora cordifolia) was collected from its natural habitat at the SDM College, Kuthapady, Udupi. The plant was well identified by dr. Sunil Kumar, Research officer, and was distinguished from other one variety of Tinospora found in the habitat. The other common variety found were, *Tinospora Malabarica*. only mature leaf was collected and used for the study.

2. Preparation of *Swarasa*

Mature fresh *Patra* of *Guduchi* was taken in morning hour were washed thoroughly and made free from all the physical impurities. A virtual *Khalva* was used for pounding the *Patra* thoroughly. The pounded *Patra* were then spread on a cloth and then squeezes through the cloth so that the *Swarasa* was extracted. This is the classical method of extracting *Swarasa*. Around 20ml of *Swarasa* was extracted every day for the experiment. Once the *Swarasa* is extracted then it was subjected to get it filtered through a filter paper. It took 15 - 20ml min for the complete *Swarasa* to get it filtered. Once the filtration was over the residue was omitted and the filtrate was used for the administration of dose.

Animals

Wistar albino rats (140-250g) were used in the study taken randomly from well- established animal house attached to S.D.M.C.R. Ayurveda and Allied Sciences, Kuthpady, Udupi. Karnataka. Were used for this study. The rats were housed individually in poly propylene cages in well ventilated rooms. The rats were kept under observation for 7 days with standard laboratory diet. The experiment was conducted after obtaining the permission from the institutional animal ethics committee for usage of animals in the experimental was obtained. Total no of groups 6 each group consisting of 6 rats.

Experimental Grouping & Treatment Schedule

The animals were randomly divided in to 6 groups each consist of 6 rats. Group I the rats were given normal tap water & Standard laboratory diet. Group II the rats were given with paracetamol 3g/kg body weight of rats, single dose on 9th day. Group III the rats were given with *Silymarin* 100mg/kg body weight of rats single dose day 9th day. Group IV,V,VI (TED, TED x 2, TED x ½) the rats were given with *Guduchi patra swarasa*, (4.32ml/kg body wt) dose 1st to 10th days in addition to paracetamol 7th and 9th days, 3g/kg body weight.

Standard Hepatoprotective

Tab. *Silymarin-70mg* It was purchased from the market (Manufactured by Microlabs Limited, Mamring, Namthanga Road South Sikkim- 737132) was use to make suspension in doses of 100mg/kg body weight

Hepatotoxin

Tab. Paracetamol - Dolo 650, It was purchased from the market (Manufactured by Micro Labs Limited, Mamring, Namthang Road South Sikkim- 737132) was use to make suspention in doses of 3g/kg body weight

Route of Drug Administration

The test drug *Guduchi patra* and toxicants administered by oral route with the help of rat feeding tube fixed to the syringe.

Biochemical evaluation

The Test drug *Guduchi patra swarasa* and reference drugs were administered orally for 10 consecutive days and two dose of the toxicant (paracetamol) were administered orally to each group, except the water control group, on 10th day 1hr after test drug administration. After 48 hours of toxicant Paracetamol, and standard group, the blood was drawn from retro-orbital puncture from each rat of all groups under diethyl ether anesthesia. The blood was collected in EDTA tub and sent for biochemistry laboratory for biochemical investigations. i.e., SGOT, SGPT, ALP, TP, serum sugar level, serum albumin level, globulin, total bilirubin, direct bilirubin, cholesterol, HDL, LDL, triglycerides. Then all the rats were sacrificed and important organs like liver were dissected out, cleaned to remove extraneous tissues, blotted to remove blood stain and weighed. A piece of liver tissue was preserved in 10% formalin for histo pathological processing.

Table 1: Effect of *Guduchi* (*Tinospora cordifolia*) *patra swarasa* on serum biochemical parameters during paracetamol induced acute liver damage in rats

	Group I	Group II	Group III	Group IV	Group V	Group VI
	Normal Control	Paracetamol Control	Standard (Silymarin)	Test I [*Guduchi Patra* TED]	Test II [*Guduchi Patra* TED x2]	Test III [*Guduchi Patra* TED x ½]
SGOT	127±15.26	274.5±62.91*	202.16±28.2	144.83±24.64*	145.66±22.60*	161.6 ± 26.05
SGPT	157.8± 3.69	189.75± 78.64	180 ±55.66	83.12± 18.78	74± 13.48	92.2± 21.308
ALP	379±81.81	381.833±117.17	661.5±105.59	586.66±144	519.166±92.631	581±97.64
Total Protein	7.18±0.13	7.34± 0.09	7.01 ± 0.22	6.68 ± 0.66	6.9 ± 0.21	6.98 ± 0.30
Sugar	129± 6.21	117 ± 9.16	120 ± 9.04	116 ± 9.8	115.8 ± 11.22	124 ± 7.96
Total Bilirubin	0.17±0.015	0.20± 0.03	0.28 ± 0.05	0.26 ± 0.05	0.13 ± 0.02	0.14 ± 0.01
Direct bilirubin	0.12±0.01	0.123±0.01	0.17 ± 0.04	0.153 ± 0.041	0.125 ± 0.026	0.125 ±0.026
Albumin	3.31±0.07	3.71± 0.13	3.65 ± 0.26	3.71 ± 0.32	3.21 ± 0.51	3.68 ± 0.27
Globulin	3.93±0.09	3.51± 0.23	3.36 ± 0.24	2.91 ± 0.61	3.68 ± 0.61	3.3 ± 0.20
Serum Cholesterol	69.5±5.52	64.33± 3.6	75 ± 6.10	61 ± 8.09	49.05 ± 8.0	92 ± 9.1
HDL	50 ± 3.85	40.16±1.99	42.5 ± 5.2	37 ± 5.58	35.16 ± 6.92	51.6 ± 5.33
LDL	11.9±1.55	76 ± 2.81 ***	17.93±4.38***	16.21±4.10 ***	12.68±3.19***	17.1±3.59***
Triglycerides	86± 15.82	125±20.14	87.4 ± 8.65	114 ± 25.64	73 ± 11.909	118.6± 10.02

Histopathological study

Liver sections obtained from different groups were scanned under trinocular microscope and all important changes were photographed. The following are the observations made:

Group- 1: positive control group. In this group marked degenerative changes were observed in the form of cell infiltration, cell depletion, fatty degenerative changes, central vein dilatation, focal necrosis and inflammatory changes around the portal triad. These changes were severe (>3) in three rats and moderate to severe in (2-3) in two rats and mild to moderate in one rat. Plate-1; depicts photomicrographs of representative sections.

Group 2: Reference standard – Silymarin administered group. The observed changes were central vein dilatation, cell infiltration, micro fatty changes and necrosis at some sites. The changes were found to be moderate in three rats, mild in one rat; sections from two rats exhibited only minor changes. Plate-2; depicts photomicrographs of representative sections from this group.

Group 3: Test I [*Guduchi Patra* TED dose administered group]. The observed changes were moderate central vein dilatation, sinusoidal dilatation, microfatty changes. The changes were found to be only mild to moderate in the tested rats. The features indicate good hepatoprotection. Plate-3; depicts photomicrographs of representative sections from this group.

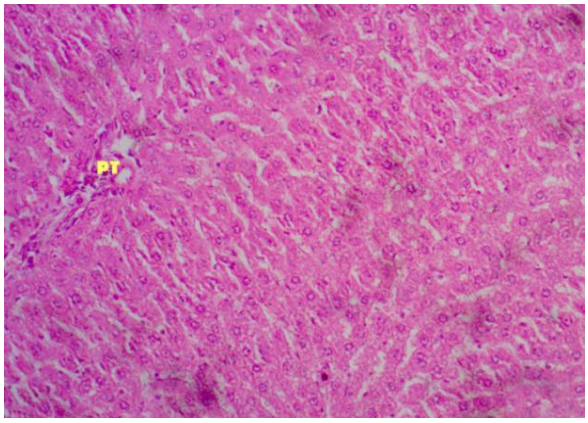
Group 4: Test II [*Guduchi Patra* 2 X TED dose administered group]. The observed changes were moderate central vein dilatation, mild cell infiltration, micro fatty changes and mild necrotic changes. The changes were found to be only mild to moderate in all rats from which the tissue samples were drawn. The features indicate moderate to good hepatoprotection. Plate-4; depicts photomicrographs of representative sections from this group

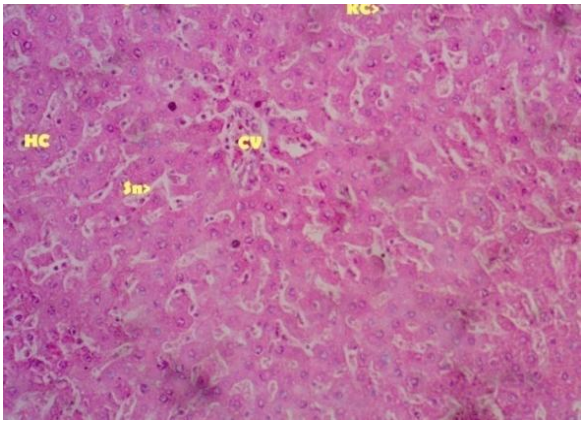
Group 5: Test III [*Guduchi Patra* half TED dose administered group]. The observed changes were central vein dilatation, mild cell infiltration and micro fatty changes. The changes were found to be only mild to moderate in the all the rats from which the tissue samples were drawn. The features indicate moderate hepatoprotection. Plate-4; depicts photomicrographs of representative sections from this group.

Statistical analysis

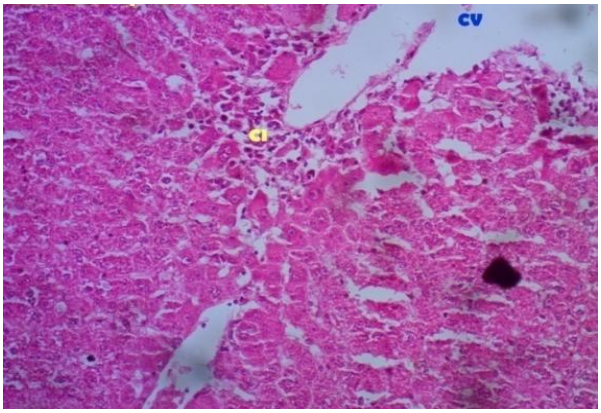
All the values were expressed as mean ± SEM (standard error of mean). The data were analyzed by ANOVA with Dunnet's multiple 't' test. A level of $p< 0.05$ was considered as statistically significant. Level of significance was noted and interpreted accordingly.

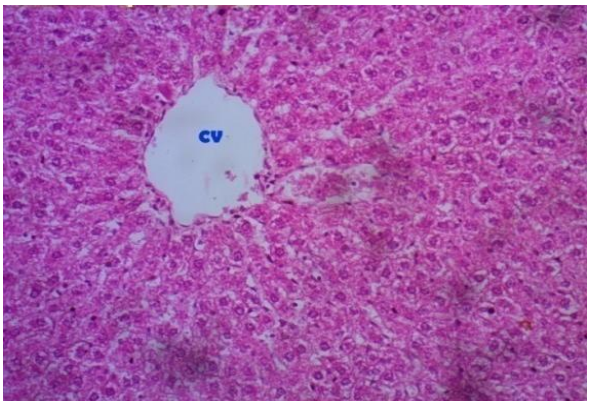
Showing microphotographs of liver of control group animals



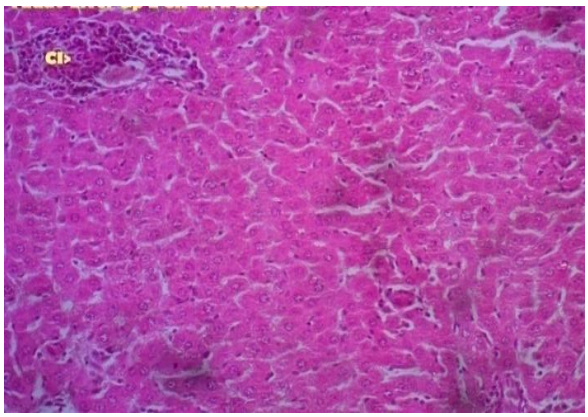


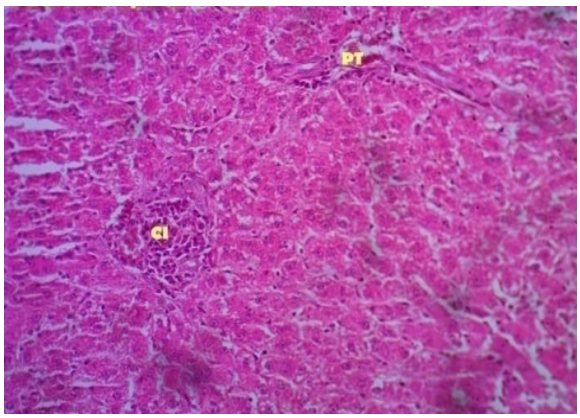
Showing microphotographs of liver of paracetamol group animals





Showing microphotographs of liver of reference standard group animals





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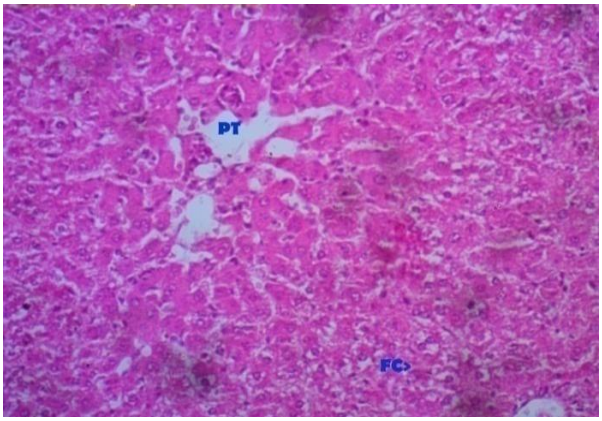
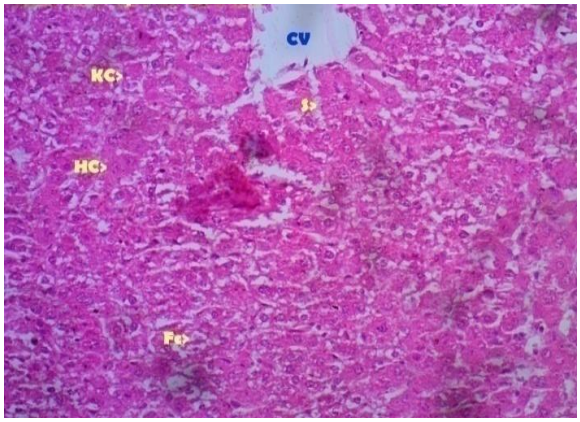
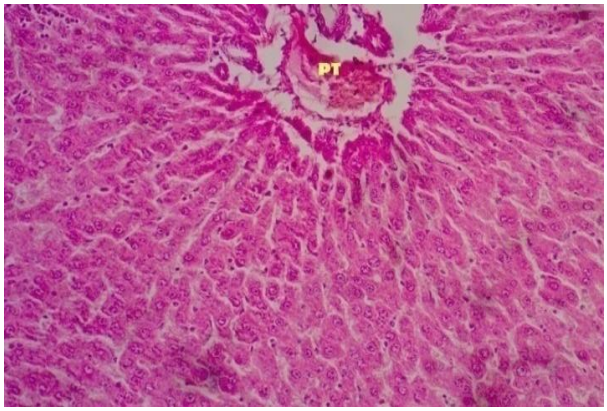
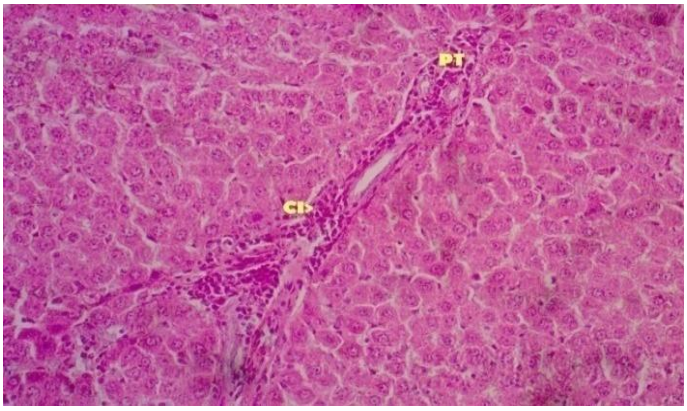


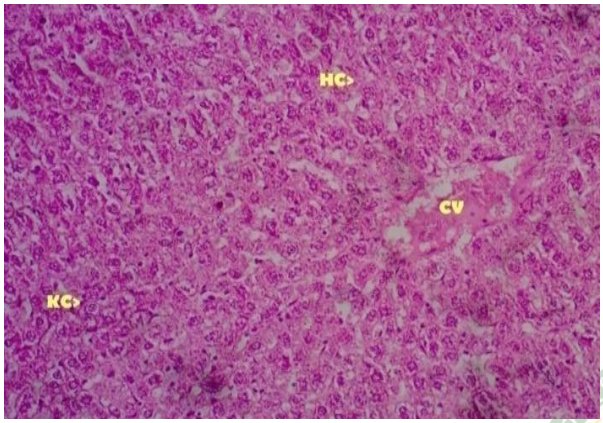


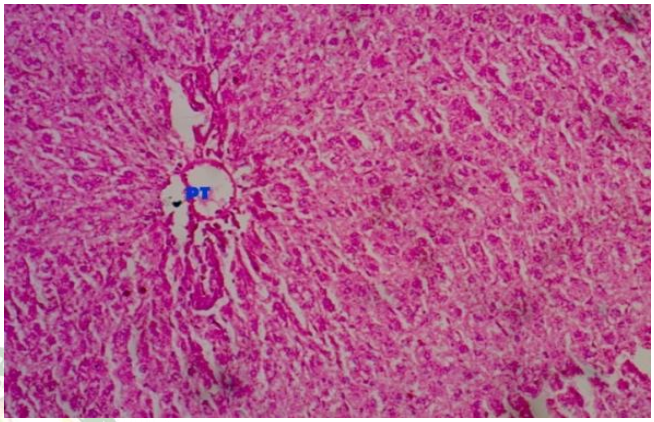
Showing microphotographs of liver of TED2 group animals





Showing microphotographs of liver of TED ½ group animals





DISCUSSION

Serum parameters studied were SGOT, SGPT, ALP, total protein, serum cholesterol, serum triglycerides, blood sugar, total bilirubin, direct bilirubin, serum albumin, serum globulin, HDL serum cholesterol and LDL. SGOT and SGPT get elevated in paracetamol induced hepatotoxicity. In the present study also significant elevation was observed. This elevation of SGOT was significantly decreased by the administration of the test drug at therapeutic dose and Test formulation at Therapeutic dose and Double dose administered groups has shown remarkable decrease in the serum SGOT activity. The elevation of SGPT was extremely significantly decreased by the administration of test drugs. SGPT level was decreased by the administration of the test drug at therapeutic dose, double dose and half dose and reference standard group. The administration of the test drugs elevation of total protein level was observed to moderate extent– this was decreased by the administration of the test drug at therapeutic dose, double dose and half dose. It shows there is moderate increase in serum TB level in paracetamol toxic dose was observed. This parameter was also only moderately elevated. Reference standard and TED dose of test drug showed moderate non-significant elevation where as non-significant decrease was observed with half and 2 TED dose groups- not consistent with histological observation hence not considered for efficacy determination. In this study albumin shows toxicant induced moderate elevation- only marginal effect was observed with reference standard and test drug groups hence no inference was drawn. In this study serum glubin shows toxicant induced moderate decrease- only marginal effect was observed with reference standard and test drug groups hence no inference was drawn. In this study, it shows there is a only a margin decrease in serum cholesterol level in paracetamol toxic dose the observed change where statistically non-significant. Standard drug that is silymarin administered groups has shown slight Increase in the serum cholesterol level and test formulation at therapeutic dose and double dose administered groups has shown slight decrease in the serum cholesterol level therapeutic half dose administered groups has shown slight Increase in the serum cholesterol level. In this study HDL shows toxicant induced moderate decrease- only marginal to moderate effect was observed with reference standard and test drug groups hence no inference was drawn. In this study, it shows there is remarkable increase in LDL cholesterol level in paracetamol toxic dose The observed changes were statistically highly significant in comparison to normal control. The marked toxicant induced elevation in LDL-cholesterol was found to be significantly reversed in reference standard and all the three test formulation receiving groups. The decrease level of serum can be considered as indicative of significant hepatoprotective at double dose, moderate

protection in the remaining doses. The present study, it shows there is moderate increase in serun triglycerides level in paracetamol toxic dose, however the observed changes were statistically non-significant. Reference drug that is silymarin administered groups has shown moderate decrease in the serum triglycerides level, therapeutic dose, double dose and half dose administered groups has shown moderate to near significant decrease in the serum triglycerides. The observed effect can be considered as indicative of significant hepatoprotective at double dose, moderate protection in the remaining doses.

Thus the analysis of serum biochemical parameters shows that administration of paracetamol leads to significant change in majority of the parameters. The overall activity profile indicated in all three test groups of *Guduchi patra swarasa* administered are found to be effective hepato-protective especially based on histopathological study, among the three groups the double dose group and therapeutic group has shown good results in comparison with the other two groups.

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CONCLUSION

Guduchi (*Tinospora cordifolia*) commonly known as *'Amrita'* is one of the best *Rasayana* (rejuvenator) drug and useful in rejuvenating various organs of human body. Stem as well as *Patra*. Remarkable decrease in serum parameters like SGOT, SGPT, albumin, cholesterol and triglycerides level with therapeutic dose and double dose of *Guduchi* [*Tinospora cordifolia* (Willd.) Mier] *Patra swarasa* is an indication of its hepatoprotective activity. The marked toxicant induced elevation in LDL-cholesterol was found to be significantly reversed in reference standard and all the three test groups receiving *Guduchi* [*Tinospora cordifolia* (Willd.) Mier] *Patra Swarasa.* The observed changes with histopathology like moderate central vein dilatation, sinusoidal dilatation, micro fatty acids changes were mild to moderate indicating good heparoprotection.

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