Research Article

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HEPATOPROTECTIVE ACTIVITY OF DUSHIVISHARI AGADA IN PARACETAMOL INDUCED HEPATOTOXICITY OF WISTAR RATS

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ABSTRACT

The study was designed to evaluate the hepato-protective activity of Dushivishariagada in acute experimental liver injury induced by paracetamol in wistar albino rats. Five groups of albino wistar rats were selected for the study. Various serum biochemical parameters and histopathological studies were measured to evaluate Dushivishariagada for its anti-hepatotoxic potential in paracetamol induced hepatotoxicity. The histological study exhibited reduced signs of paracetamol (acetaminophen) induced hepatotoxicity. The hepato-protective activity of Dushivishariagada was also substantiated by significant decrease in levels of the bio-chemical parameters. By experimental research, the overall activity profile of serum biochemical parameter indicated reversal of important parameters like SGOT, SGPT, ALP and Total bilirubin in both test drug and reference standard. Analysis of the histo-pathological study data generated during the study clearly indicates both reference standard and test drugs indicating presence of good cyto-protective effect that reversed the injurious effects produced by the toxicant, paracetamol. Hence, the trial drug Dushivishariagada can be used as an anti-hepatotoxic drug.

Keywords: Hepatotoxicity, Paracetamol, DushivishariAgada, Garavisha, Silymarin, Acetaminophen

INTRODUCTION

Paracetamol (Acetaminophen) is such a drug, which is dispensing without any prescriptions. With more than 89 million prescriptions, acetaminophen was the most common dispensed medication worldwide in 2003[1]. Paracetamol, a mild painkiller sold over the counter in India, is being increasingly consumed in higher doses to bring down fever in both adults and children. According to pharma industry estimate, India consumes nearly 1500 tons of paracetamol a month. There are as many as 50 single ingredients and another 24 combination brands in the market. Paracetamol, which causes hepato-nephro toxicity with excess consumption, is selling without any proper dosage on the strips and bottles[2]. Acetaminophen is the leading cause of acute liver failure in the United States[3]. Liver toxicity may result from an acute overdose as well as from chronic excessive ingestion. In 2009, the American Association of Poison Control Centers' national poison data system reported 401 deaths caused by acetaminophen or an acetaminophen combined product.

In the present scenario, all these types of low potent poisons fall under the category of GaraVisha concept of Ayurveda. Gara Visha is the artificial type of poison prepared by the combination of different non-poisonous substances[4] according to Ayurveda. In olden days these types of poisons were given by women for yielding love from their husband, wealth from king and to take revenge on enemies[5]. Gara is described as "Krutrimam Garasamjnam Tu Kriyatevividhoushadai:"[6], i.e. artificially prepared by the combination of different medicaments. The effect of this poison entirely depends on the potency of the combination. The symptoms of Garavisha include diseases like pandu (anemia), agnimandya (indigestion), jwara (fever), mahodara (ascites), Yakrit-pleehavikaras (liver-spleen disorders) etc.[7,8]

Liver has a great capacity of detoxifying toxic substances and to synthesize useful principles. It is involved with almost all the biochemical pathways to grow, fight against disease, nutrient supply and energy provision[9]. Liver diseases are the most serious ailments and are mainly caused by toxic chemicals (excess consumption of alcohol, high doses of paracetamol, anti-tubercular drugs, chemotherapeutic agents, etc.)[10]. Unfortunately, conventional or synthetic drugs used in the treatment of liver diseases are inadequate or can have serious side effects[11]. Paracetamol induced hepatotoxicity is caused by the reaction metabolite N-acetyl-p-benzoquinoneimine (NAPQI), which causes oxidative stress and glutathione depletion[12].

Silymarin has been used for 20 years in clinical practice for the treatment of toxic liver diseases[13]. Silymarin is a flavonolignan and extracted from the seed and fruit of the plant *Silybum marianum*, also called "milk thistle"[14,15]. It can cause allergic skin reactions, bloating, blood clots, decreased platelets, diarrhea, eczema, high bilirubin in blood, impotence, tremor etc. in some people. In the absence of reliable liver protective drugs in allopathic medical practices, Ayurvedic drugs play a role in the management of various toxic liver disorders. In this study, Silymarin was used as a positive control against PCM-

induced acute hepatic damage in rats. The ingredients of Dushivishari Agada have anti-toxic[16] property and are found to be anti-hepatotoxic also. So here an effort is taken to evaluate the effect of Dushivishari Agada in paracetamol induced hepatotoxicity in Wistar rats.

MATERIALS AND METHODS

The materials and techniques used in the presented work are described in the following pages. Drugs used for the evaluation are as follows:

Test Drug
The test drug Dushivishari Agada – After collection of ingredients from local market and its authentication from Dravyaguna department of SDMCAH Hassan, Dushivishari Agada was prepared as per the classical references at Bhaishajya kalpana Department of SDMCAH, Hassan using standard reference procedure.

Standard Hepatoprotective Drug
The reference standard drug Silymarin was purchased from the market with the trade name Silybon -70 mg, Batch no- SIAD0033, manufacturing date- Jun 2012, Exp- May2015, Manufactured by micro labs limited, HB – 211, village katha, P.O. Boddi, tehsil, Nalagarh dist, Solan – 173205 (H.P.)
Toxicants: The toxicant used to induce hepatic injury as per the protocol is as follows:
Paracetamol - Paracetamol injection. (Brand name: FEVASTIN.), BatchNo. TAB 2029, MFG.-OCT.2012, EXP.-SEP.2016, Manufactured in India by TABLETS (INDIA) LIMITED, 179, T.H Road, Chennai.

Animals
Wistar strain albino rats of either sex of body weight ranging from 160 - 270 g were obtained from animal house attached to S.D.M Centre for Research in Ayurveda and Allied Sciences, Udupi, Karnataka. They were maintained on "Amrut" brand animal pellet feed of "Pranav agro industries" and tap water was given ad-libitum. The temperature and humidity were kept at optimum and animals were exposed to natural day night cycles. The experiments were carried out in conformity with the Institutional Animal Ethics Committee (IAEC) and after obtaining its permission SDMCAH/IEC/44/11-12.

Dose: The dose of the drug, vehicle and toxicants was calculated by extrapolating the therapeutic dose of human to rat dose based on surface area ratio by referring to the table of Paget and Barnes (1969).

(a) Dusivishari Agada
Suggested human dose = 12 g
Rat dose = 12× 0.018 (Conversion factor for 200g rat)
= 0.216 g /200g rat or 1.08g/kg

Two dose levels were studied:
TED (rat dose equivalent to human therapeutic dose) and TED x 02

(b) Reference Standard
Silymarin - Rat dose = 50 mg / kg

(c) Toxicants
Paracetamol- Rat dose = 1 g / kg

Route of administration
The test drug Dushivishari Agada and silymarin were administered orally by using rubber catheter No. 3 sleeved to the syringe. Toxicant was administered by injecting intramuscularly.

Drug dosing schedule
In Paracetamol induced hepatotoxicity model the vehicle, test drug and reference standard were administered between 8 to 10 am and toxicant was administered 1 hour after drug administration.

Evaluation of anti-hepatotoxic activity
A number of experimental models are employed to assess anti-hepatotoxic activity. Paracetamol induced liver injury in rodents continues to be one among the most widely used models. Wagner demonstrated the anti-hepatotoxic effect of a compound, derived from extract of the white flowered varieties of Silybum marianum – Silymarin that is generally taken as reference standard drug.
When the rats are exposed to single or multiple doses of paracetamol, drug metabolites produce liver damage and this leads to changes in different types of parameters like ponderal, biochemical and histological. Administration of drug prior to toxicants administration will inhibit liver damage whereas administration of the anti-hepatotoxic drugs after toxicant helps in quick regeneration of hepatocytes. This fact has been used to design experimental models for assessing anti-hepatotoxic activity.
In the present study anti-hepatotoxic activity evaluation of dushivishari agada was carried out using Paracetamol induced hepatotoxicity models in rats.

Treatment protocol

- **Group I Vehicle treated:** animals received tap water.
- **Group II Paracetamol treated:** animals received distilled water.
- **Group III Standard drug treated:** animals received Silymarin (50 mg/Kg, p.o.) in addition to paracetamol.
- **Group IV Test drug:** animals received dushivishari agada,
  TED - (1.08 g / kg, p.o.) in addition to paracetamol
- **Group V Test drug:** animals received dushivishari agada,

TED × 02- (2.16 g / kg, p.o.) in addition to paracetamol
The test drug Dushivishariagada, vehicle and reference drugs were administered orally for 9 consecutive days and one dose of the toxicant (paracetamol) were administered intra-muscular to each group, except the water control group, on 7th day 1h after test drug administration. After 48 hours of toxicant Paracetamol IM injection, the blood was collected in the tubes and sent for biochemistry laboratory for biochemical investigations after being sedated. Then all the animals were sacrificed by cervical

dislocation. Important organs like liver and kidney were dissected out, cleaned to remove extraneous tissues, blotted to remove blood stain and weighed. Liver is cut into two pieces. A piece of liver tissue and kidney were preserved in 10% formalin for histo-pathological processing.

Parameters employed for assessing the extent of liver injury

Ponderal Changes

(i) Body weight - The change in the body weight was calculated. For this purpose the body weight was taken before and after the test drug administration.

(ii) Organ weight – Liver and kidney. The organ weight was presented in terms of both absolute and relative weight.

Serum Biochemical Parameters

The blood was collected and sent to biochemistry laboratory for biochemical investigations involving serum parameters. Serum biochemical parameters were estimated with the help of clinical auto analyzer (Transasia, India) by feeding serum sample to the concerned port as per the directions of the manufacturer for the estimation of various biochemical parameters namely SGOT, SGPT, ALP, Total Protein, Serum Urea, Serum Creatinine, Serum Cholesterol, Serum Triglyceride, Blood Sugar, Total Bilirubin and Direct Bilirubin.

Histopathological study

Procedure followed to prepare histopathological Slides

Fixation: The tissues were excised out immediately after sacrificing the animals, cleaned of extraneous tissue, cut into pieces of appropriate thickness and were transferred into 10% formalin solution. The tissues were allowed to remain in it till they are taken up for processing.

Tissue processing: Tissue processing involves dehydration, clearing and infiltration of the tissue with paraffin. Tissue were thoroughly washed by placing them under running tap water and then passed through a series of solvents as per schedule for dehydration, clearing and paraffin infiltration. Next the tissues were embedded in paraffin wax to prepare tissue blocks. Tissue blocks were fixed to metal object holder after trimming them to suitable size.

Section cutting: The tissue sections of the 5-6 µm thickness were cut with the help of Spencer type rotating microtone and floated in a water bath between 50-55 ^{0}C for 30 minutes. Then they were mounted on clear glass slides with a drop of Mayer's egg albumin dried on hot plate at about 50 ^{0}C for 30 minutes.

Staining: After fixing the section on slide, the sections were stained by serially placing them in the reagents.

After passing through the reagents and stains, the slides were covered with D.P.X. (Diphenylphthalein Xylene) and cover slips were placed. Care was taken to avoid the air bubble formation during mounting the slide.The slides were viewed under binocular research Carl-Zeiss's microscope (Germany) at various magnifications to note down the changes in the microscopic features of the tissues studied and their photomicrographs were taken for the evaluation of histo-pathological changes.

RESULTS

To evaluate dushivishari agada for its anti-hepatotoxic potential in paracetamol induced hepatotoxicity, Effect of 1.08 and 2.16 g/kg dusivishariagada treatment on ponderal changes and various biochemical parameters in paracetamol induced hepatotoxic animals are summarized as follows.

Statistical Tests

The data obtained was analyzed by using analysis of variance (ANOVA) followed by Dunnett's 't' test for determining the level of significance of the observed effects. All the values were expressed as mean ± SEM (Standard Error of Mean). A level of $P<0.05$ was considered as statistically significant and the value of $P<0.01$ or $P<0.001$ was considered statistically highly significant. Level of significance was noted and interpreted accordingly.

Table 1: Grouping of experimental animal

S. No.	Groups	Abbreviation	Dose
1	Water control	WC	-----
2	Paracetamol control	PC	1.0 g/kg
3	Reference Silymarin + Paracetamol	RS	50mg/kg
4	Dushivishari Agada (Therapeutic dose) + Paracetamol	TED	1.08 g/kg
5	Dusivishari Agada (Double dose) + Paracetamol	TEDX2	2.16 g/kg

Biochemical Study

The effect of Dushivishariagada on amount of body and organ weights, SGOT,SGPT, alkaline phosphatase, serum urea, creatinine, cholesterol, triglyceride, blood glucose, bilirubin and total protein in paracetamol induced hepatotoxicity in rats are given in the tables below. Administration of paracetamol intoxication resulted in a significant elevation of hepato-specific serum markers. On administration of Dushivishariagada and Silymarin, the level of these enzymes was found reverting towards normal. The effect of the test drug in both the doses was comparable to that of standard reference drug, Silymarin.

Histopathological Study

The hepatoprotective effect of Dushivishariagada was confirmed by histo-pathological examination of the liver and kidney tissues of control and the treated groups. The liver section of rats intoxicated with paracetamol showed disarrangement and degeneration of hepatic cells with severe necrosis and disappearance of nuclei. The animals treated with Dushivishariagada and intoxicated with paracetamol showed no visible changes confirming the safety of the formulation and a good hepato-protective activity. Rats treated with Silymarin and intoxicated with paracetamol showed less disarrangement and degeneration of hepatocytes, indicating marked regeneration activity.

Table 2: Effect of test drug on ponderal changes

Parameters	Paracetamol Control	Reference + PC (silymarin)	TED+PC	TED*2+PC
Before treatment body weight	232.33 ± 16.50	200 ± 13.90	241.14 ± 6.38	315.8 ± 7.552
After treatment body weight	222.83 ± 16.56	197.83 ± 15.96	242.14 ± 5.83	317.6 ± 5.555
Relative liver weight	7.44 ± 0.32	7.49 ± 0.35	8.79 ± 0.39	10.61 ± 0.78**
Relative kidney weight	1.68 ± 0.13	1.59 ± 0.12	1.56 ± 0.09	2.15 ± 0.16

**P<0.01

Table 3: Effect of test drug on serum bio-chemical parameters

Parameters	Paracetamol control	Reference + PC (silymarin)	TED+PC	TED*2+PC
SGOT	955.08 ± 87.43**	452.53 ± 93.18**	384.16 ± 21.19**	317.86 ± 10.08**
SGPT	505.18 ± 65.93**	125.83 ± 27.28**	93.12 ± 13.53**	95.12 ± 30.71**
Alkaline phosphatase	734.83 ± 297	625.57 ± 127	147.50 ± 17.00**	126.12 ± 12.60**
Total protein	5.47 ± 0.25**	6.07 ± 0.15	6.61 ± 0.16**	6.51 ± 0.43**
Serum urea	215.14 ± 74*	44.16 ± 9.06*	18.4 ± 3.73*	96.43 ± 61
Serum creatinine	1.90 ± 0.57	0.97 ± 0.24	0.74 ± 0.09	3.46 ± 1.50
Serum cholesterol	81.66 ± 6.67**	57.33 ± 8.50*	83.14 ± 4.83	99.80 ± 8.34
Serum triglyceride	134.66 ± 21.66	101.50 ± 20.41	126.17 ± 20.94	179.16 ± 39.40
Serum glucose	112.16 ± 8.5	126.15 ± 8.82	156.66 ± 19.17	182.28 ± 14.92
Total bilirubin	0.52 ± 0.09**	0.19 ± 0.01**	0.30 ± 0.07	0.27 ± 0.06*
Direct bilirubin	0.10 ± 0.00	0.10 ± 0.001	0.11 ± 0.012	0.17 ± 0.07

**P<0.01,*P<0.05

Histopathological examinations

Microscopical examination of liver sections

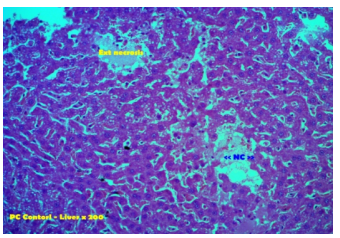

Figure 1: Paracetamol treated

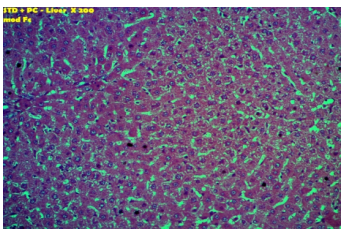

Figure 2: Silymarin and paracetamol

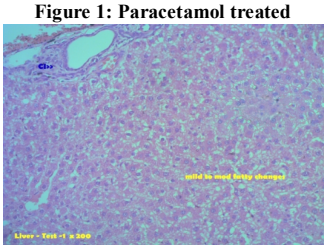

Figure 3: TED and paracetamol

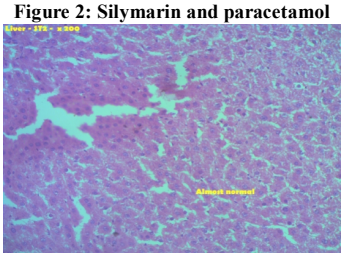

Figure 4: TED *2 and paracetamol

Microscopical examination of Kidney sections

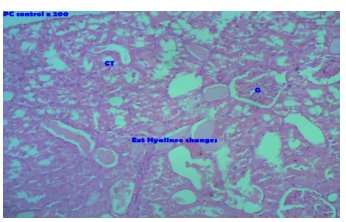

Figure 5: Paracetamol treated

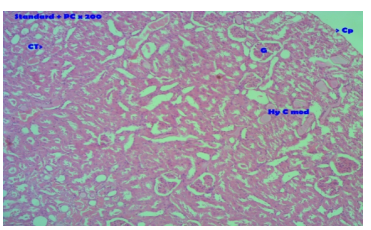

Figure 6: Silymarin and paracetamol

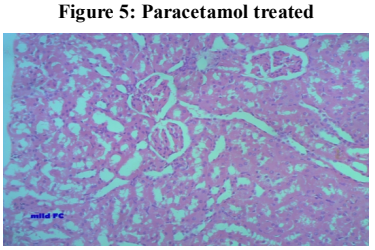

Figure 7: TED and paracetamol

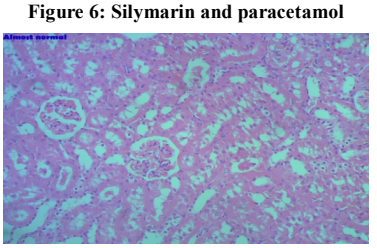

Figure 8: TED *2 and paracetamol

DISCUSSION

Paracetamol is a safe drug at therapeutic doses. It is likely to produce fatal reactions in overdoses both in human and in experimental animals. Paracetamol is metabolized by a cytochrome P-450 dependant pathway to an electrophile metabolite (intermediate reactive) N-acetyl-p-benzoquinoneimine (NAPQI). Therapeutic dose of this drug are safely bio transformed and eliminated as non-toxic conjugates of sulphate and glucuoronic acid and only a small fraction is converted to NAPQI, which is detoxified by glutathione (GSH) and eventually eliminated in the urine/bile. During overdose of paracetamol, the glucouronidation and sulfation routes become saturated and more extensive metabolism (of paracetamol), leads to rapid depletion of hepatic GSH levels. Unconjugated NAPQI can exert its cytotoxic effect by covalent binding to cellular macromolecules, membrane lipid peroxidation and alteration of calcium homeostasis.

In the present study, a therapeutic, and double dose of the Dushivishari Agada, an anti-toxic formulation were screened for Anti-hepatotoxic activity and the effects obtained were compared with a paracetamol control group and with that of reference standard silymarin, a standard anti-hepatotoxic agent for paracetamol intoxicated rats. The ingredients of Dushivishariagada are Pippali, Dhyamaka, Jatamansi, Lodhra, Ela, Gokshura, Syonaka, Tagara, Kushta, Yashti Madhu, Chandana and Gairika. The individual drug profile shows that the general properties are predominant of Madhura Thiktha Kashaya Rasa, Laghu Rooksha Guna, Sheetha Veerya and Katu Vipaka. The formulation is anti-toxic in action and hence will be having anti-hepatotoxic property also.

In the present study, it was observed that the animals treated with the paracetamol resulted in significant hepatic damage as shown by the elevated levels of serum markers. The rise in the SGOT is usually accompanied by an elevation in the levels of SGPT, which plays important role in the conversion of amino acids to keto acids[17].When the hepatic cell membrane is damaged, the enzymes SGOT, SGPT and SALP which are normally located in the cytosol, leak into circulation from hepatocytes leading to increased serum level of SGOT, SGPT and SALP. The pretreatment with formulation at both the dose levels significantly attenuated the elevated level of the serum markers. The normalization of serum markers by formulation suggests that the formulation protect the membrane integrity against paracetamol induced leakage of marker enzymes into the circulation. The above changes can be considered as an expression of the functional improvement of the hepatocytes, which may be caused by the accelerated regeneration of parenchyma cells.

In paracetamol control, decreased body weight was observed. Here the loss in body weight was found to be reversed in Dushivishari Agada at both the doses. It is an indication of the anti-hepatotoxic action of the test drug. The second parameter was liver weight a slight non-significant increase in liver weight of rats treated with paracetamol. The liver weight was found to be further increased in all the 3 test groups. Rats treated with Dushivishari Agada in double dose (42.60%) shown maximum increase followed by Dushivishari Agada in therapeutic dose (18.14%) and silymarin (0.67%). The third parameter was kidney weight. In this parameter, administration of paracetamol leads to a non-significant increase in weight. But an increase was observed in double the therapeutic dose group.

Administration of paracetamol lead to marked elevation in SGOT, SGPT, ALP activities and in the level of total protein, serum urea, serum creatinine, serum cholesterol, serum triglycerides, blood sugar, total bilirubin, and direct bilirubin. It was found that all the 3 test groups had shown extremely significant reversal of increased SGOT level, where the maximum extent was found in Dushivishari Agada at double dose (66.71%) treated rats. The reversal percentage of Dushivishari Agada in single dose (59.22%) was found to be more than that of silymarin (52.62%). It was found that all the 3 test groups had shown highly significant reversal of SGPT activity elevation induced by the toxicant. In TED single dose group significant decrease of SGPT was observed, whereas double dose of TED group SGPT level was more significantly reduced. In silymarin group, a non-significant decrease of ALP was observed compared to paracetamol control group. Both groups of Dushivishari Agada have shown a significant decrease in serum ALP level in comparison to paracetamol control group. It might be due the cytoprotective activity of Dushivishari Agada and shows its effect as an anti-hepatotoxic drug. The elevated activities of AST and ALT in serum are indicative of cellular leakage and loss of the functional integrity of cell membranes in liver. Administration of Dushivishari Agada and silymarin causes decrease in the activity of the above enzymes, which may be a consequence of the stabilization of plasma membrane as well as repair of hepatic tissue damage caused by paracetamol. In this study, administration of paracetamol lead to highly significant decrease in serum total protein level. In reference standard non-significant increase was observed. In test drug at both doses a highly significant increase of serum total protein in comparison to the paracetamol control group was observed. The reversal of elevated Total Protein level by Dushivishari Agada might be due to the inhibition of acute stress of paracetamol metabolite on liver cells and the protein synthesis mechanism. In this study, the serum urea was found to be significantly increased in paracetamol induced rats. All the 3 groups had shown reversal of serum urea level. Both Dushivishari Agada at therapeutic dose and silymarin had shown significant decrease while Dushivishari Agada at double dose shown no significant effect. The possible mode of action might be due to the decreased catabolism of proteins and liver enzyme and hence decreased levels of serum urea was observed. In the present study, a non-significant increase was observed in Serum Creatinine level in Paracetamol induced rats. The elevation of serum creatinine was antagonized in both silymarin and Dushivishari Agada at therapeutic dose, but not to a significant level. But the serum creatinine was further increased in double dose of Dushivishari Agada. In the present study, serum cholesterol was found to be significantly increased in paracetamol- induced rats. The

level was found to be reduced in silymarin treated rats only. The Dushivishari groups had shown mild increase compared with that of paracetamol control group. In the study serum triglyceride level was observed to be non-significantly increased in paracetamol treated rats in comparison to normal control group. In both reference standard and TED group, a non-significant decrease was observed as compared to paracetamol control group. A non-significant increase of serum triglyceride was observed in TEDx2 group in comparison to paracetamol group. In the present study, it is observed that serum Glucose level was decreased to a non-significant extent in paracetamol administered rats. All the 3 test groups have shown reversal in decreased level of serum Sugar, but not to any significant extent. In the present study, the observed value of total bilirubin was found to be increased in highly significant manner in paracetamol treated group in comparison to normal control group. In reference standard group, the level was found to be decreased in highly significant manner as compared with paracetamol treated group. The total bilirubin was observed to be significantly decreased in TED group and non-significantly decreased in TED×2 groups in comparison with the paracetamol control group. The observed value of bilirubin direct shown a non-significant increase in paracetamol group compared with normal control group. Reference standard group was found to have no change in comparison with the paracetamol control group. A further non-significant increase was observed in Dushivishari Agada at both doses was observed in comparison to the paracetamol control group.

Microscopic examination of liver sections showed significant damage in the cell cyto architecture of paracetamol treated rats, i.e. presence of necrosis, appearance of balloon cells, leukocyte infiltration, micro and macro fatty changes, sinusoidal dilatation and areas of regenerations. Liver sections of all the three test groups have shown only mild damage. This may be due to the cytoprotective action of these drugs. This shows the anti-hepatotoxic effect of Dushivishari Agada.

Microscopic examination of kidney sections showed only mild damage of cell structures. This proves the preventive action of the test drug to the damage of kidney tissue caused by paracetamol. This proves the anti-toxic effect of Dushivishari Agada.

Probable mode of action of drug

Dushivishari Agada is mentioned in all types of Visha. Hence Dusivishari Agada is effective in sthavara (inanimate), jangama (animate) and also Gara Visha. The properties of Dusivishari Agada are Raktashodhaka (blood purification) and Vishaghna (anti toxic)[16]. Rakta shodhaka property corrects the vitiated Raktadhatu and helps in maintaining normal functions of Rakta Dhatu. Vishaghna property helps in detoxifying the Gara Visha, which is having Alpa virya and is deep seated in Rakta dhatu. The drug profile shows that the general properties are predominant of Madhura Thiktha Kashaya Rasa, Laghu Rooksha Guna, Sheetha Veerya and Katu Vipaka. Being a combination of drugs bearing similar qualities, a synergistic drug action can be assumed. Due to the above-mentioned properties, Dushivishari Agada is Tridoshahara, Twak doshahara and Raktha sodhaka. Almost all the drugs of this yoga are Sheetha Veerya (cold potency) in nature capable of antagonizing the adverse effect of Visha. Drugs of this yoga are fairly met within many Agada preparations, some of these drugs are individually Vishagna and again certain, combination of drugs would bring about miraculous effects apart from their total effects (Yoga Samyogajam Phalam).

CONCLUSION

By experimental research, the overall activity profile of serum biochemical parameter indicated reversal of important parameters like SGOT, SGPT, ALP and Total bilirubin in both test drug and reference standard. It shows presence of good anti-hepatotoxic effect. Analysis of the histo-pathological study data generated during the study clearly indicates in both reference standard and test drugs indicating presence of good cytoprotective effect that reversed the injurious effects produced by toxicant paracetamol. Hence, the trial drug Dushivishariagada can be used as an anti-hepatotoxic drug.

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