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Research Article

AN EXPERIMENTAL STUDY TO EVALUATE THE HEPATOPROTECTIVE ACTIVITY OF NILITANDULIYADI LEHA IN PARACETAMOL INDUCED HEPATOTOXICITY IN WISTAR ALBINO RATS

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ABSTRACT

Background: Paracetamol toxicity is currently the single most important cause for acute liver failure and is associated with significant number of deaths. Nilitanduliyadi leha is one among the formulation explained in the context of Vishahara yogas (anti-poisonous formulations) in the text Vishavaidya Jyotsnika. Objectives: To experimentally evaluate the hepatoprotective activity of nilitanduliyadi leha in paracetamol induced hepatotoxicity in Wistar albino rats. Methods: Albino Wistar rats of either sex weighing 200 – 250, g were selected and divided into four groups of six animals in each group (n = 6). Treatment was given for 7 days. Blood was drawn and sent for tests and important organs like liver and kidney 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 histopathological processing. Results: The formulation has helped in balancing the biochemical parameters studied almost as efficiently as the standard drug. In the antioxidant study also, the drug has given good results and shows even slightly more effective than the standard drug. The histopathology study also reveals mild protection and regeneration of tissues by the effect of test drug. Conclusion: This present study proves that the formulation is having a comparable hepato protective activity with that of silymarin.

Key words: Hepatoprotective, Paracetamol, Nilitanduliyadi leha, Agada, Vishavaidya Jyotsnika.

INTRODUCTION

Paracetamol (acetaminophen) has both analgesic and antipyretic effect[1]. Paracetamol being well tolerated, lacks many of the side-effects of aspirin and is available without prescription, thus it has earned a prominent place as a common household analgesic[2]. This popular analgesic is said to be safe when taken in a dosage of 1.2 g/d for an adult.[3]. However acute over dosage leads to fatal hepatic damage and the number of self-poisoning and suicides with paracetamol have grown alarmingly in recent years[4].

Paracetamol toxicity is currently the single most important cause for acute liver failure in the USA and is associated with significant number of deaths. More than 200 million persons take paracetamol each year. Of these, about 200 persons a year die of fulminate hepatic failure from paracetamol over dosage.

The most serious adverse effect of acute over dosage of paracetamol is a dose- dependent, potentially fatal, hepatic necrosis. Renal tubular necrosis and hypoglycemic coma may also occur. The mechanism by which over dosage with paracetamol leads to hepato-cellular injury and involves its conversion to a toxic reactive metabolite[5] - n-hydroxy n-acetyl p-hydroxy aniline.

In the Malayalam text Vishavaidya Jyotsnika, Nilitanduliyadi leha is one among the formulation explained in the context of Vishahara yogas (anti poisonous drugs). It is indicated in all types of poison (vishas) – Sthavara (plants and animals), Jangama (animal), and Kritrima (artificial). Kritrimavisha (artificial poison) is also termed as Garavisha (compound poison)[6]. The Garavisha causes several diseases like Pandu (anaemia), Agnimandya (impaired digestion), Jwara (fever), Mahodara (ascites), Yakrithpleehavikaras (hepatic and splenic disorders) etc[7,8]. In present scenario all the low potent poisons fall under this category only.

Almost all of the drugs in this Agada (anti-dote) are having Vishaghna Guna (anti-poisonous property) and some of the drugs in this Agada have shown hepatoprotective activity[9,10], so here is an effort to evaluate the effect of nili tanduliyadi leha in paracetamol induced hepatotoxicity in Wistar rats.

Table 1: ingredients of Nilitanduliyadi Leha

DRUG	BOTANICAL NAME	PART USED	QUANTITY USED
Nili	*Indigofera tinctoria*	Patra (Leaves)	1 part
Tanduliya	*Amaranthus spinosus*	Patra (Leaves)	1 part
Tagara	*Valeriana wallichii*	Mula (Root)	1 part
Shunti	*Zingiber officinale*	Kanda (Rhizome)	1/3 part
Maricha	*Piper nigrum*	Phala (Fruit)	1/3 part
Pippali	*Piper longum*	Phala (Fruit)	1/3 part
Saindhava (Salt)			1 part

OBJECTIVES

To experimentally evaluate the hepatoprotective activity of nilitanduliyadi leha in paracetamol induced hepatotoxicity in wistar albino rats.

MATERIALS & METHODS

Source of data: Raw materials of Nilitanduliyadi was procured from Kerala. The raw drugs were authenticated at **Dravyaguna Department** of SDMCA, Hassan. Preparation of *nilitanduliyadi* was done as per classics – Visha Vaidya jyotsnika, 9th chapter 1st sloka at Teaching Pharmacy of Sri Dharmasthala Manjunatheshwara College of Ayurveda and Hospital, Hassan. Animal house facilities in Sri Dharmasthala Manjunatheshwara Centre For Research Ayurveda, Uduppi was used.

Methods of collection of data

Sampling: Wistar rats of either gender with an average of 200 to 250 gm is selected randomly for the study. They were obtained from well-established animal house attached to S.D.M Centre for Research in Ayurveda and Allied Sciences, Udupi, Karnataka. They were maintained on feed of "Sai Durga feed and food, Bangalore" 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 IAEC approval no: CPSEA/IEAC/SPMH-AT-20.

Inclusion criteria: Healthy Wistar albino rats of either sex, weighing about 200-250g was included.

Exclusion criteria: Pregnant and diseased Rats and Rats which were under trial of other experiments were excluded.

Grouping: Albino Wistar rats of either sex weighing 200 – 250, g was selected and divided into four groups of six animals in each group (n = 6).

Group I Vehicle treated: animals received tap water.

Group II Paracetamol treated: animals received paracetamol (3g/kg, p.o.),0.5 % gum acacia and 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 Nilitanduliyadi leha, TED (4.32 gm /kg, p.o.) in addition to paracetamol

Treatment protocol: The Test drug Nilitanduliyadi leha and reference drugs were administered orally for 7 consecutive days and one dose of the toxicant (paracetamol) was administered orally to each group, except the water control group, on 7th day 1h after test drug administration. After 48 hours of toxicant Paracetamol, the blood was collected in the tubes and sent for biochemistry laboratory for biochemical investigations. 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. A piece of liver tissue was preserved in 10% formalin for histopathological processing.

STATISTICAL ANALYSIS

The data were expressed as Mean ± standard error of the mean (SEM) of 6 animals per group. Parametric one-way Analysis of Variance (ANOVA) test was performed using Graph pad prism 5.0. The minimum level of significance was identified at $p<0.05$- with Dunnett's multiple 't' test as post hoc test.

OBSERVATION AND RESULTS

Table 2: Results of biochemical parameters statistical analysis

BIOCHEMICAL PARAMETER	PARACETAMOL	STANDARD	TEST DRUG
SGOT	*75.101↑@	38.98↓#	39.72↓#
SGPT	*318.06↑@	57.87↓#	24.25↓#
Alkaline phosphatase	1.28↑@	8.42↑#	13.82↑#
Total protein	3.02↑@	3.70↑#	4.17↑#
Serum albumin	2.23↑@	**11.16↓#	*7.52↓#
Serum globulin	4.25↓@	29.78↑#	25.25↑#
Total bilirubin	**135↑@	*31.91↓#	**42.55↓#
Direct bilirubin	**257.14↑@	*40↓#	*32↓#
Serum urea	**28.40↑@	8.21↓#	2.98↓#
Serum creatinine	11.76↑@	8.77↓#	10.52↓#
Serum cholesterol	19.81↑@	1.67↓#	10.61↑#
Serum triglycerides	**33.24↓@	51.13↑#	152.17↑#

Table 3: Results of ponderal index statistical analysis

PONDERAL INDEX	PARACETAMOL	STANDARD	TEST DRUG
Body weight	**526.41↓@	**135.25↑#	**233.03↑#
Liver weight	**31.95↑@	6.07↑#	13.83↑#
Heart weight	**26.66↑@	7.89↓#	1.75↓#
Kidney weight	**61.53↑@	4.76↓#	0.43↓#

Table 4: Antioxidant parameter statistical analysis

ANTIOXIDANT PARAMETER	PARACETAMOL	STANDARD	TEST DRUG
Catalase action	*86↓@	2.12↑#	64.2↑#
Protein estimation	*70↑@	98.3↓#	98.7↓#
Glutathione peroxidation	*73.8↓@	30.8↑#	3.75↑#
Lipid peroxidation	90↑@	26.1↓#	52.3↓#

DISCUSSION

Probable mode of action: All the drugs contained in the formulation *nili, tanduliya, tagara* and *trikatu* have been proved to have hepatoprotective activity in various studies conducted. *Saindhava* is *tridoshagna* (antagonise all three doshas), *sookshma* (microscopic), *yogavahi*(synergic) and have *marganusari* (clears passage) effect. This helps in removing visha even from sookshma srothas (minute passages). Nili (*Indigofera tinctoria)* is told to be effective in *udara* (ascites)and is having *vishaghna* karma (anti poisonous). Tanduliya (*Amaranthus spinosus)* was previously studied and the hepatoprotective and antioxidant activity of 50% ethanolic extract of whole plant of *Amaranthus spinosus* was evaluated against carbon tetrachloride induced hepatic damage in rats[9]. The results strongly indicate that it is a potent hepatoprotective and the possible mechanism may be due to the presence of flavonoids and phenolic compounds of the drug.Tagara (*Valeriana wallichii)* is *tridoshahara* (antagonise all three doshas) and is having *vishagna karma*[10]. Hepatic inflammation is a common trigger of liver disease and is considered the main driver of hepatic tissue damage. According to a study it showed anti-inflammatory properties, similar to those observed for non-steroidal anti-inflammatory drugs, such as aspirin. Another study proves that tagara is having hepatoprotective activity which may be due the presence of phytochemicals such as alkaloids, tannins, saponins and hesperidin which have antioxidant properties.Pippali (*Piper longum)* is *tridoshahara, rasayana* (rejuvenating) and *udarahara*(cures ascites). It also increases the bioavailability of the drug. Study conducted exerts a clear hepatoprotective potential of the drug due its antioxidant property of inhibiting lipid peroxidation[11]. Maricha (*Piper nigrum)* is *vishagna*(anti poisonous) and *shophahara* (anti-inflammatory) which helps in reducing the hepatic inflammation. When Anti-hepatotoxic and anti-oxidant effects of extracts from *Piper nigrum* was studied and results show that it possess hepatoprotective activity as well as anti-inflammatory activity[12].Shunti (*Zingiber officinale)* is *shophahara* (anti-inflammatory) and *udarahara*(cures ascites). According to Study of the hepatoprotective effect of ginger aqueous infusion in rats, it was found that zingerone present in ginger inhibits liver lipid peroxidation, making it a potent antioxidant. The hepatoprotective activity may be due to the volatile oils, which showed anti-inflammatory, analgesic and immune modulatory effects[13].

Biochemical parameters

It is well established that level of serum enzymes such as SGOT and SGPT gets elevated in paracetamol induced hepatotoxicity. In the present study also, similar elevation was observed. Elevation of transaminase activity is indicative of tissue destruction and inflammation. It was found that both the test drug group and the standard group shows moderate decrease in the SGOT and SGPT. The reversal percentage nilitanduliyadi leha was even slightly greater than that of silymarin. This proves the efficacy of nilitanduliyadi leha as anti-hepatotoxic drug.

Serum alkaline phosphatase 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. Nilitanduliyadi leha have shown a non-significant decrease in serum ALP level in comparison to paracetamol control group. It might be due the cytoprotective activity of the drug.

In this study, administration of paracetamol lead to highly significant decrease in serum total protein level. In standard group and test drug group non-significant increase was observed in comparison to the paracetamol control group. The reversal of elevated Total Protein level by nilitanduliyadi leha might be due to the inhibition of acute stress of paracetamol metabolite on liver cells and the protein synthesis mechanism.

A decreased serum albumin is usually normal in liver failure. In this study there was a mild decrease in serum albumin level in paracetamol control group when compared to the normal control. In the standard and the test drug group there was a mild increase in the serum albumin level when compared to paracetamol control group, which shows that the test drug is an effective hepatoprotective drug.

Serum globulin is a critical plasma protein produced by the liver. A decreased serum globulin is usually seen in liver failure. Here there was a mild decrease in serum Globulin level in paracetamol control group when compared to the normal control. The standard drug (silymarin) and nilitanduliyadi leha showed a moderate increase in serum Globulin level when compared with paracetamol control group.

Bilirubin, a breakdown product of hemoglobin, is the predominant pigment produced in the liver. Excess bilirubin causes yellowing of body tissues (jaundice). There are two tests for bilirubin direct-reacting (conjugated) and indirect-reacting (unconjugated). Total bilirubin increases in case of obstructive condition of the bile ducts, hepatitis, cirrhosis, in hemolytic disorders. 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 significant manner as compared with paracetamol treated group. The total bilirubin was observed to be very significantly decreased in Test Drug group.

In this study, the serum urea was found to be very significantly increased in paracetamol induced rats. Other two groups had shown reversal of serum urea level. Both the test drug and silymarin had shown non-significant decrease. 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.

Serum creatinine is more specific and sensitive indicator of renal functions. Simultaneous estimation of serum urea and creatinine provides better information. 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 nilitanduliyadi leha groups, but not to a significant level.

CONCLUSION

This present study proves that the formulation is having a comparable hepato protective activity with that of silymarin. The formulation has helped in balancing the biochemical parameters studied almost as efficiently as the standard drug. In the antioxidant study also the drug has given good results and shows even slightly more effective than the standard drug.

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