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Rhinacanthusnasutusreverses Paracetamol induced liver damagein rats

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ABSTRACT: Hepatotoxicity by excessive use of medications like paracetamol causes impaired liver function leading to liver failure with serious health complications. Many Herbals, with its biochemical products and their secondary metabolites have been used as medicines for various diseases. This research paper aims to identify various phytochemical constituents present in ethyl acetate extract of Rhinacanthusnasutusleavesand to evaluate its hepatoprotection in paracetamol induced liver damage in Wistar rats.

KEYWORDS: Hepatoprotective, Rhinacanthusnasutus, Phytochemicals

1. INTRODUCTION

Medicinal plants are used as a source of medicine globally in all cultures. Medicinal plants seems to be safer compared with the synthetic medicines which are not safer for human and environment. Many biochemical products are synthesized and preserved by green plants of which, some are extracted and used as raw materials for scientific investigations. Secondary derivatives derived from many plants have been commercially utilized in various pharmaceutical compounds. Recently there is a comeback of medicinal plants and its renaissance in all over the world [1]

Liver, playing a vital role regulates the metabolism, biochemical pathways, metabolic wastes excretion and detoxifications in our body. It is the main organ which receives nutrients from food products, environmental pollutants, drugs and toxicants from the digestive system. Injuries due to exposure to toxins can destructively change the liver functions. The free radicals from toxins can cause many disorders like hepatitis, diabetes and cardiac arrest [2].

Liver toxicity or hepatotoxicity can be caused by consumption of excessive amounts of medications leading to serious health complications. Certain medicationslike antibiotics, paracetamol, antipsychotics when used in high doses will not be able to detoxify completely by the liver, causing hepatotoxicity. This can lead to pathological changes in liver causing oxidative stress and inflammation, impairment in the proper functioning and causing hepatotoxicity. The symptoms of hepatotoxicity include fatigue, abdominal pain, nausea, jaundice leading to liver failure in severe cases. Treatment include discontinuation of the medication, supportive care and liver transplantation in severe cases.

Phytopharmaceuticals from many plants containing diversified bioactive constituents have hepatoprotective activity and have been used for treating liver toxicity. Phytochemicals like flavonoids, saponins and polyphenols reduces inflammation, shows antioxidant and hepatocyte-regenerating capacities, making them novel candidates for reducing liver damage by medications or toxins.Also,In comparison with synthetic drugs, phytopharmaceuticals possess very less side effects which also adds an advantage to liver health [3].

The genus Rhinacanthus, belonging to Acanthaceae family and Justiciinae subtype consists of nearly 25 species which are distributed

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widely in all the tropical regions. Rhinacanthusnasutus which is also known as Snake Jasmine, belonging to this genus is a traditional herb of Asian countries. This plant is a small slender shrub which grows well in rainy seasons and the aerial part dries during summer leaving the root areas intact.

Different parts of this herb are used for treating many disorders like inflammation, ringworm, snake bite, eczema, pulmonary tuberculosis, hepatitis, hypertension, diabetes etc. It is well known for its antioxidant properties [1,4,5,6].

This study aims to evaluate the hepatoprotective effect of "Rhinacanthusnasutus" leaves against paracetamol induced liver damage in Albino wistar rats and its comparison with Silymarin which is a herbal drug used for treating liver diseases.

II. METHODOLOGY

1. Extraction of Rhinacanthusnasutus

Rhinacanthusnasutus was collected from Tirunelveli in September 2023 and authenticated by taxonomist

Fig 1- Rhinacanthus nasutus

The fresh leaves of Rhinacanthusnasutus, was collected, washed, air dried, separated, pulverized by a mechanical grinder and sieved in a 40-mesh sieve. The extraction of the leaves was done by multiple stage maceration method by immersing in ethyl acetate solvent. The filtrate is obtained by filtering with muslin cloth. The crude drug is obtained by simple distillation process and evaporating in a china dish. The extract is stored in the desiccator for further use.

Fig 2-maceration

2. Phytochemical analysis

Quantitative screening

The ash values and the extractive values of the crude extract was determined quantitatively by standard methods.

Qualitative screening

The qualitative analysis for the presence of glycosides, carbohydrates, phytosterols, Flavanoids, Fats, Oils, Tannins, Phenolic acids, Proteins, Aminoacids, Saponins and Alkaloids was done by standard procedures [7] .

3. In vivo preclinical evaluation of hepatoprotective effect of Rhinacanthusnasutus

The experiment was conducted on 30 wistar albino rats (180-200g/ body weight) after obtaining the animal ethical clearance (2009/PO/Re/S/18/CCSEA). Five groups with 6 animals in each group were used for the study. Animals were fed with standard pellet diet. (Sai enterprises pvt. Ltd, Chennai, India) and water. They were well maintained in controlled laboratory conditions of 12 hrs dark/light cycle, temperature at 22 ± 2°C and humidity at 50±5% according to CCSEA guidelines. Group1 was the normal control. All the remaining groups received Paracetamol 500mg/kg for 10 days as hepatotoxin for inducing hepatotoxicity. Group II was considered as disease control without any treatment. Silymarin at a dose of 10mg/kg was given to Group III intraperitoneally from 11th to 21st day. Group IV and V received Rhinacanthusnasutus as treatment at a dose of 200mg and 400mg from 11th to 21st day orally once daily respectively. After 21 days, the rats were sacrificed by using chloroform and cervical decapitation. Blood samples were collected from

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the rats through cardiac puncture and made to clot for 30 mins at room temperature. The serum thus obtained was separated from the blood by centrifugating at 3000rpm for 15 mins and stored at -400C until use for the evaluation of biochemical parameters like Serum glutamic oxaloacetic transaminase (SGOT), Serum glutamic pyruvic transaminase (SGPT), and Total Bilirubin. Liver tissues were excised for histopathology evaluation.

Statistical analysis

The results were tabulated as the Mean ± SD for each group. Statistical difference was evaluated using a one-way analysis of variance (ANOVA) followed by Dunnett's t-test. Results were considered as statistically significant at p<0.

III. RESULTS

1. Phytochemical analysis

Table 1: Physio chemical parameters values

S. No	Parameters	Percentage (%w/w)
1	Extractive value	71.5%
2	Total ash	23%
3	Acid insoluble ash	11.6%
4	Water soluble ash	9.6%
5	Loss on drying	8.93%

Table 2: phytochemical screening of ethyl acetate extracts of leaves of Rhinacanthusnasutus:

S. No	CHEMICAL TEST	INFERENCE
1	**TEST FOR CARBOHYDRATES:**	
a.	Molich's test	- ve
b.	Fehilings test	-ve
c.	Benedicts test	-ve
d.	Barfoed's test	-ve
2	**TEST FOR GLYCOSIDES**	
a.	Legals test	-ve
b.	Baljet's test	-ve
c.	Bontragers test	-ve
d.	Keller killani test	-ve
3.	**TEST FOR PHYTOSTEROLS**	
a.	Libemannburchard test	+ve
b.	Salkowski test	+ve
4.	**TEST FOR FLAVANOIDS**	
a.	Shinoda test	+ve
b.	Alkaline reagent test	+ve
5.	**TEST FOR FIXED OILS & FATS**	
a.	Spot test	-ve
b.	Saponification test	-ve
6	**TEST FOR TANNINS /PHENOLIC COMPOUNDS**	
a.	Ferric chloride test	+ve
b.	Lead acetate test	+ve
c.	Potassium dichromate	+ve
d.	Potassium ferricyanide	+ve
7	**TEST FOR PROTEINS & AMINOACIDS**	
a.	Biuret test	-ve
b.	Ninhydrin test	-ve
c.	Xanthoprotein test	-ve
d.	Millons test	-ve

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8	**TEST FOR SAPONINS**	
a.	Forthing test	+ve
b.	Emulsifying test	+ve
9	**TEST FOR ALKALOIDS:**	
a.	Wagners test	+ve
b.	Dragendroffs test	+ve
c.	Mayers test	+ve
d.	Hagers test	+ve
10	**TEST FOR CUMARINS**	
a.	UV fluorescence test	-ve
b.	Ferric chloride test	-ve

The Phytochemical screening of ethyl acetate extract of Rhinacanthusnasutus leaves showed the presence of phytosterols, flavanoids, tannins /phenolic compounds, saponins and alkaloids.

2. In vivo HEPATOPROTECTIVE ACTIVITY

Table 2: Effect of Rhinacanthusnasutus on liver weight:

Groups	Liver weight (gm)
Group I (Normal control)	7.10 ± 0.3
Group II (Diseases control)	13.5 ± 1.5***
Group III (Standard Drug)	7.10 ± 0.2***
Group IV (200mg of Rhinacanthusnasutus)	7.25 ± 1.0***
Group V (400mg of Rhinacanthusnasutus)	7.10 ± 0.2***
*** P< 0.001	

Values are tabulated as mean ± SEM from all 6 animals in each group and the difference in means were calculated by ANOVA followed by Dunnet's post hoc test. The values of liver weight of group II were significantly increased compared with group I. The values of liver weight of Group III, IV and V were significantly reduced on comparing with Group II.

Table 4: Effect of Rhinacanthusnasutusin liver enzymes and Bilirubin:

Groups	SGOT (U/L)	SGPT (U/L)	Total Bilirubin (mg/100ml)
Group I (Normal control)	52.50±0.325	21.25±0.476	0.85±0.008
Group II (Disease control)	138.03 ±1.268***	61.13±2.267***	2.04±0.025***
Group III (Standard Drug)	54.24±1.254***	21.77±0.547***	0.86 0.37***
Group IV (200mg of Rhinacanthusnasutus)	58.16±1.457***	24.10±0.124***	0.91±0.08***
Group V (400mg of Rhinacanthusnasutus)	56.13±1.254***	23.83±0.547***	0.80±0.37***
*** P< 0.001			

The values of bilirubin and liver enzymes of group II was significantly increased compared with group I and the values of Group III, IV and V were significantly decreased compared with Group II.

Effect of Rhinacanthusnasutusin histopathology of liver tissues:

The tissue sections of liver tissue showed normal lobular architecture with central vein and portal triads along the periphery of the lobules. Many sinusoids were seen passing radially from the central vein with the presence of hepatocytes in the space between the sinusoids. The paracetamol treated Group II tissue sections showed features of hepatic damage like dilated & congested sinusoids, degenerated hepatocytes with centrilobular necrosis, disarrangement of hepatocytes and presence of chronic inflammatory infiltrate around the portal triads. The tissue sections of Silymarin

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treated group III showed apparently normal hepatocytes, sinusoids and central vein showing significant hepatoprotective activity. Rhinacanthusnasutus treated group IV showed reduced vacuolation, reduction in sinusoidal congestion and dilation, less disarrangement and degeneration of hepatocytes compared with Group II indication of good hepatoprotective activity. Rhinacanthusnasutus treated group V showed significant hepatoprotective activity seen by reduction of histological changes compared with Group II. Individual demarcation of the hepatocytes from the canalicular space is also seen suggesting regeneration of hepatocytes.

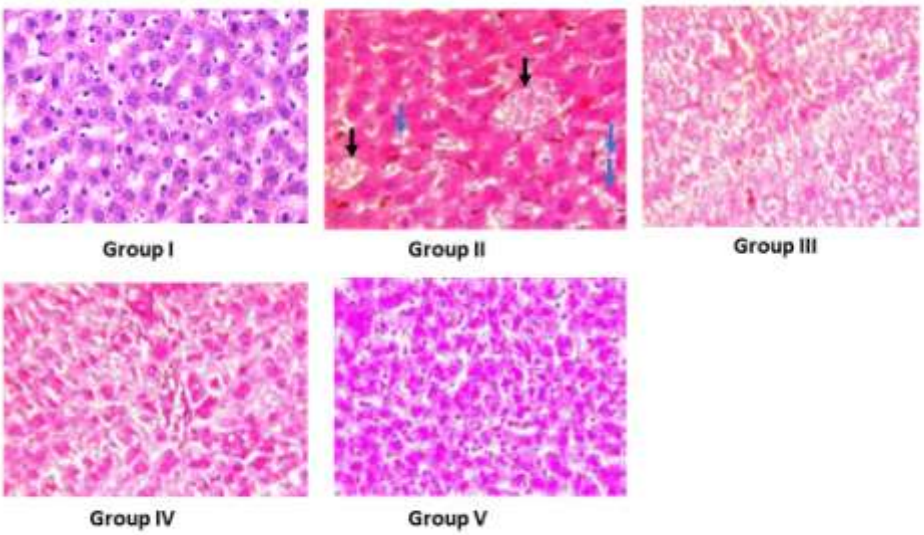

Fig 3: Histology of liver tissues of Group 1-V

Group I showing normal histology of liver tissue with the presence of hepatocytes between the sinusoids.
Group II with Hepatocytes shows ballooning degeneration (blue arrow) and hepatic congestion at sinusoids (black arrow)
Group III showing mild hepatic congestion and degeneration
Group IV showing mild hepatic congestion and degeneration with reduction in histological changes
Group V showing good reduction in histological changes looking like normal

IV. DISCUSSION

Paracetamol is one among the commonly used antipyretics and analgesics. Even though it is considered safe when taken at therapeutic levels, it causes severe liver damage leading to acute liver failure when consumed in doses above therapeutic levels. When consumed at therapeutic levels, the metabolism of paracetamol takes place through the sulfation and glucuronidation pathways and then eliminated from the bodythrough urine. The leftover paracetamol is changed into N-acetyl-p-benzoquinone imine (NAPQI), its hepatotoxic metabolite, through cytochrome P450 enzymes. Thus, formed NAPQI is converted to a soluble, non-toxic mercapturic acid by glutathione which is also eliminated in urine. In Paracetamol overdose, the sulfation and glucuronidation pathways become saturated producing excessive NAPQI through the cytochrome P450 pathway causing the decline of glutathione supplies which causes liver toxicity moderated by the formation of free radicals [8].

Herbal products used by native healers has become a potential source of compounds with therapeutic values. Many pharmaceutical compounds are prepared from the secondary metabolites of plants. Recent trend is people returning back to natural products instead of using synthetic medicines. Recently more research focus is on plants in collecting lots of evidence on the potential of medicinal plants used in different traditional systems. Recently immense developments are seen in the phytomedicine research with more emphasis on scientifically evaluating herbal drugs against synthetic drugs owing to their beneficial properties. The various phytochemical compounds produced by the medicinal plants has made these herbs suitable as

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an alternative source of medicines compared with synthetic drugs [1, 9] This study is a small attempt of analysing the phytochemical screening and the hepatoprotective activity of Rhinacanthusnasutus in comparison with silymarin a strong antioxidant which promotes regeneration of liver cells.

The Phytochemical evaluation of the ethyl acetate extracts of Rhinacanthusnasutus leaves in our study showed the presence of phytosterols, flavanoids, tannins /phenolic compounds, saponins and alkaloids

A study by Nirmala et al on phytochemical evaluation of aqueous extract of Rhinacanthusnasutus revealed the presence of phenols, saponins, steroids, terpenoids, alkaloids, tannins, proteins and carbohydrate [10]. Our study which was done on Ethyl acetate extract did not contain carbohydrates and proteins. The phytochemical evaluation of methanolic extract of the Rhinacanthusnasutus leaves by Sivagnanam has revealed the presence of, alkaloid, glycerides carbohydrates, phenolics, tannins, flavonoids, volatile oils and phenolics [9] . Studies done on Phytochemical analysis of Rhinacanthusnasutus have shown steroids, anthraquinones, terpenoids, lignans and napthaquinones [1]

Our in vivo experiments done to see the efficiency of Rhinacanthusnasutus on paracetamol induced liver damage has shown significant reduction in liver weight, liver enzymes and bilirubin compared with the disease control.

Shyamol et al has shown that Rhinacanthusnasutus root extract has hepatoprotective effect in Aflatoxin B1 treated rats in which Aflatoxin B1 causes hepatotoxicity by creating oxidative stress in liver cells, and further damaging lipids, DNA and proteins [11]. Another study by Shah et al has demonstrated significant reduction of SGOT and SGPT by semi purified Rhinacanthusnasutus extract [12]. The study by Rao et al has significantly reduced the liver enzymes in Streptozotocin induced diabetes in rats [13].

Any stress or abnormality in amino acid or protein metabolism will lead to changes in the tissue and these changes lead to metabolism of catabolic products like ammonia. Metabolism of amino acid is very complex and it involves oxidation and transamination. The enzymes SGOT and SGPT are widely distributed in animal cells and they behave as a link between protein and carbohydrate metabolism. SGOT catalyses the interchanging mechanism of α-ketoglutaric acid and alanine to glutamic and pyruvic acids and the final result is including keto acids into the tricarboxylic acid cycle. As SGOT and SGPT is reduced by Rhinacanthusnasutus, gluconeogenesis can be also further reduced by Rhinacanthusnasutus [6]. So, this indicates that Rhinacanthusnasutus can be a candidate for further investigation for Diabetes mellitus treatment.

Steroids, Flavonoids, terpenoids, lignans, anthraquinones and napthoquinine analogues, isolated from Rhinacanthusnasutus might contribute for its hepatoprotective activity [1]. The hepatoprotective mechanism of Rhinacanthusnasutus might be due to antioxidant mechanism. Antioxidants show three modes of action like prevention, interception and repair and any one of this action might be responsible for the hepatoprotective action of Rhinacanthusnasutus. Free radicals are prevented by enzymes like superoxide dismutase (SOD) which catalysis the conversion of superoxide to H2O2 and in turn breaks into water. Controlling the gene expression of enzymes like cyclooxygenase (COX) and nitric oxide synthase (NOS) regulates the production of Reactive oxygen species. Scavenging of free radicals involves interception of free radicals. Repairing is done by enzymes which restore the damage caused by Reactive oxygen species, many studies has correlated the total phenolic content with the antioxidant activity [4]. The phytochemicals like phenolic compounds, flavonoids, anthraquinone can also enhance the antioxidant activity which in turn can promote hepatoprotective activity of the Rhinacanthusnasutus.

V. CONCLUSION

This study, evaluating the effect of Rhinacanthusnasutus with its significant reduction in liver weight, bilirubin, liver enzymes and histopathological changes has shown that Rhinacanthusnasutus can be a promising drug for hepatoprotective activity. Our findings have shown that Rhinacanthusnasutus can be focused for research as an adjunct drug for liver diseases.

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