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Research article

Pharmacological Evaluation of Hepatoprotective Activity of Ethanolic extract of *Andrographis lineata Nees* on Hepatotoxicity Induced Rats

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ABSTRACT
Liver diseases are still a worldwide health problem due to drug-induced hepatotoxicity. It may occur as an unexpected idiosyncratic reaction, or nontoxic drug or it may be an expected consequence of the intrinsic toxicity of a drug, taken in a sufficiently large dose to cause liver injury. A highly potential therapeutic agent or a medicinal extract is necessary for the preventive action of the hepatic disorders leading to the inflammation and drug inducing liver injury. The present study proved the medicinal plant with supportive therapeutic efficacy. Albino *wistar* rats of either sex are induced by Rifampicin orally at a dose of 1g/kg and for a period of 14 days, and were treated with ethanolic extract of the stems of *Andrographis lineata Nees* (*EEALN*) orally at a dose of 200 and 400 mg/kg/day. The biochemical parameters such as serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), alkaline phosphatase (ALP), Total protein, and serum bilirubin (Total and Direct) were estimated to assess the liver function. Ethanolic extract of the stems of *Andrographis lineata Nees* (*EEALN*) showed the hepato protective activity by decreasing the levels of serum hepatic marker enzymes. Silymarin is used as a Standard drug. Histopathological studies were performed to confirm the biochemical changes in the hepatocytes. Toxicological studies were carried out with the extract and 2000 mg/kg b.wt. is considered as the safe dose with no mortality and adverse effects.

Keywords: *Andrographis lineata*, Hepatoprotective activity, Rifampicin, SGOT, SGPT, ALP.

INTRODUCTION
Liver diseases remain one of the major threats to public health and is a worldwide problem. Liver disease is one of the major causes of morbidity and mortality in public, affecting humans of all ages. About 20,000 deaths occur every year due to liver disorders. Some of the commonly known disorders are viral hepatitis, alcohol liver disease, non-alcoholic fatty liver disease, autoimmune liver disease, metabolic liver disease, drug induced liver injury, gallstones, etc. Because of the importance of drug-induced hepatotoxicity in clinical medicine, researchers and regulators have vigorously pursued basic knowledge about hepatotoxicity, including types and mechanisms, with the circumstances under which hepatic injury occurs, and measures to reduce the occurrence of this untoward side effect of drugs used for therapeutic purpose[1]. Herbal medicines have stood the test of time for their safety, efficacy, cultural acceptability and minimal side effects. The herbal drug products are prepared from renewable resources of raw materials by eco-friendly processes and will bring economic prosperity to the masses growing these raw materials. In India it is reported that traditional healers use 2500 plant species and 100 species of plants benefit as usual source of medicine.

Andrographis lineata Wall ex. Nees, (Acanthaceae) is a tropical plant which is found in the regional areas of Chithoor district of Andhra Pradesh, and the total plant is used as a medicinal plant. It is

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used as an antipyretic, in snake bite and is considered the most important plant in the traditional system of medicine in the country[2,3]. The ethanolic extract of the stems of *A. lineata* contains higher composition of flavonoids (7.40%)[4] and it is confirmed by the isolation of the phytochemical constituents[5]. Hence the present study was undertaken. And also literature survey revealed that no research work has been carried out on hepatoprotective activity on these models.

MATERIALS AND METHODS

Plant Materials

The stems of the plant *Andrographis lineata Wall ex. Nees* were collected from a botanist Dr. K. Madhava Chetty, Department of Botany, Sri Venkateswara University, Chithoor district, Andhra Pradesh, India in March 2013. And the plant material was authenticated by a botanist. A voucher specimen has been preserved in our College laboratory for future reference.

Preparation of the Extract

Stems of the plant *Andrographis lineata nees* were dried under shade and was powdered with a mechanical grinder. It was then soaked with 70%v/v ethanol at 60-70^{0}C overnight and then loosely packed in a Soxhlet apparatus by a continuous hot extraction process. It was then subjected to estimate the presence of various phytochemical constituents by using different studies. The percentage yield was calculated and then preserved in a sterile bottle under refrigeration conditions.

Experimental Animals

The study was carried out using albino *wistar* rats of either sex weighing about 150-200g. The animals were grouped and housed in a polypropylene cages and feeded with standard pellet diet and water *ad libitum*. Animals were exposed to the alternate cycles of light and darkness of 12h each. And also standard laboratory conditions were maintained at a temperature of (22^{0}C ± 3^{0}C) and relative humidity of 50-70%. The animals were acclimatized for a period of one week before the initiation of the study in order to adjust to the laboratory conditions. The experiments were conducted according to the Institutional Animal Ethics Committee (IAEC no: Reg. No: 51/01/C/CPCSEA/2013/006) at Smt. Sarojini Ramulamma College of Pharmacy, Mahabubnagar and regulations approved by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Acute Oral Toxicity Studies

Acute oral toxicity studies were performed on albino *wistar* rats of either sex with ethanolic extract of *Andrographis lineata Nees* (*EEALN*) as per OECD (Organization for Economic Cooperation and Development) guidelines No.423,(2000)[6]. No behavioral, neurological, and autonomic profiles lethality was evident in any of the test groups upto 2000 mg/kg. Hence one tenth and one-fifth of the doses were selected for evaluation of hepatoprotective activity, i.e, 200 (1/10th) and 400 (1/5th) mg/kg body weight orally.

Rifampicin Induced Hepatotoxicity

Albino *wistar* rats of either sex were randomized and divided into five groups, each group consisting of six animals. The first group received orally with normal saline 10ml/kg body weight daily for 14 days. The second group of treated animals received Rifampicin orally (1g/kg b.wt.). The third group received Rifampicin and Silymarin (25mg/kg body wt.). The fourth group received orally ethanolic extract of *Andrographis lineata Nees* (*EEALN* 200 mg/kg body wt.) and Rifampicin and then the fifth group received orally ethanolic extract of *Andrographis lineata Nees* (*EEALN* 400 mg/kg body wt.) and Rifampicin daily for a period of 14 days respectively. On the 14th day, all the rats in each group were induced with the hepatotoxicant Rifampicin (1g/kg body wt., orally) except group I.

Drugs and Chemicals

Rifampicin-450 mg, Silymarin, Ethyl Alcohol, Normal saline, Formalin, Chloroform, Distilled Water etc.

Biochemical Studies

Animals were fasted for 24 hrs before the estimation of biochemical parameters. The blood was collected by the retro-orbital puncture or tail cutting method. The blood samples were collected and serum was separated by centrifugation at 3000 rpm at 37^{0}C for 15 min. Biochemical parameters such as SGOT[13], SGPT[7], ALP[8], Total protein[9], and Serum bilirubin[10,11] were estimated.

Statistical Analysis

The data were presented as Mean ± SEM using Graph pad Prism version 5.0. The statistical significance between the groups were evaluated by Dunnett's one-way analysis of variance (ANOVA)

followed by Tukey's Multiple Comparisons Test and the value of $p < 0.05$ was considered as test of significant.

RESULTS AND DISCUSSION

The levels of serum hepatic marker enzymes such as SGOT, SGPT, ALP, Total protein, and Serum bilirubin (Total and Direct) is represented in the following Table 1. There was a significant elevation in the levels of serum hepatic marker enzymes and decreased level of proteins on the induction of Rifampicin in treated animals. Administration of *EEALN* (200 and 400 mg/kg body wt.) significantly reduced the elevated levels of serum hepatic marker enzymes to the normal levels on comparing with that of the standard drug Silymarin. Thus, there by showing the significant hepatoprotective activity.

The results of the extract on serum marker enzymes like SGOT, SGPT, ALP, Total Protein, Total and Direct Bilirubin against Rifampicin induced liver damage were represented in the following tables.

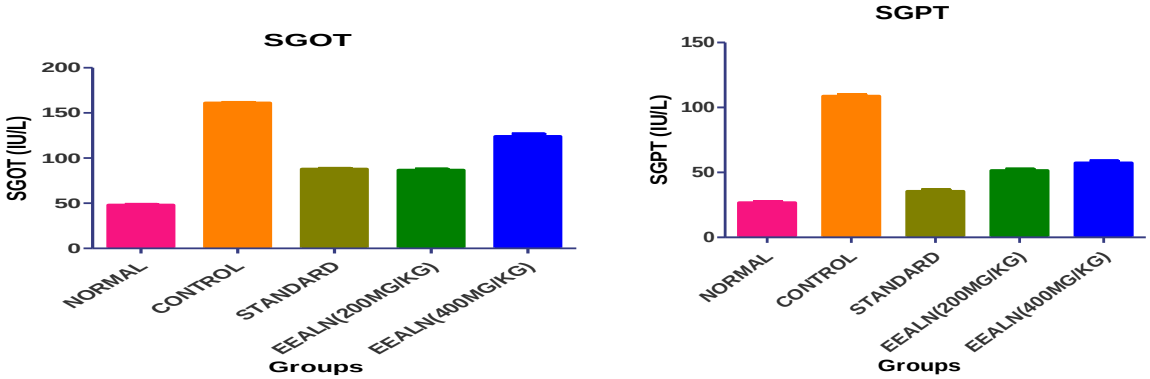
Model I: Rifampicin Induced Hepatotoxicity

Table 1. Mean data of hepatoprotective activity of *EEALN* of different groups against Rifampicin induced rats

Group	Blood Serum Marker Enzymes					
	SGOT(IU/L) Mean ±SEM	SGPT(IU/L) Mean ± SEM	ALP(IU/L) Mean ± SEM	TP(mg/dl) Mean ± SEM	TB(mg/dl) Mean ± SEM	DB(mg/dl) Mean ± SEM
I Normal Control	48.06±0.823	26.69±1.023	81.31±0.593	8.263±0.476	0.156±0.012	0.078±0.016
II Toxicant Control	161.1±0.578**	108.6±1.335***	212.8±2.867***	2.055±0.169**	1.203±0.067***	1.365±0.022***
III Standard	87.87±0.944***	35.41±1.304***	127.9±1.107***	7.042±0.471***	0.218±0.025***	0.280±0.015**
IV Test-I	124.68±1.161***	57.42±1.166***	167.04±1.503**	6.533±0.683***	0.30±0.0006***	0.54±0.078***
V Test II	100.1±2.632***	51.30±1.539***	136.1±0.422***	5.100±0.422**	0.27±0.005***	0.36±0.04**

Values are Mean ± S.E.M., N=6. *P value <0.05, **p <0.01, ***p<0.001, ns = Non Significance Vs. Toxicant Control

1.2 Graphical Representation of Biochemical Parameters of *EEALN* against Rifampicin induced Rats



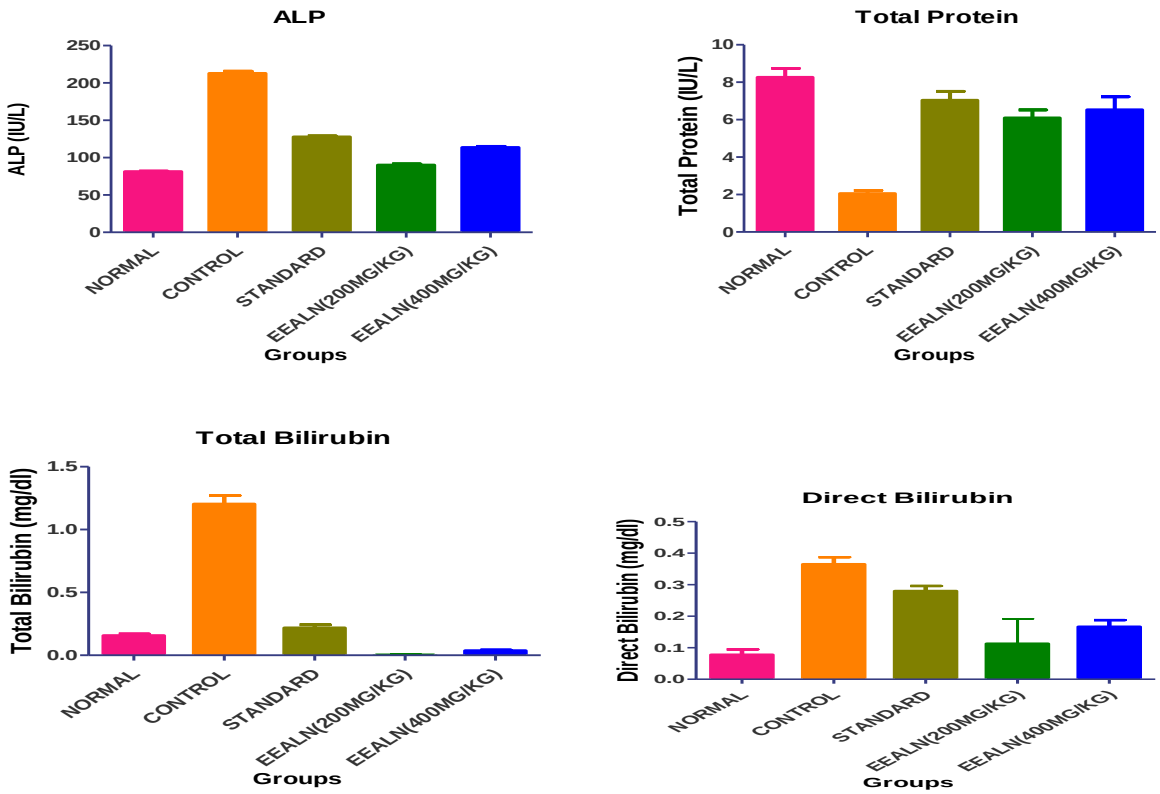
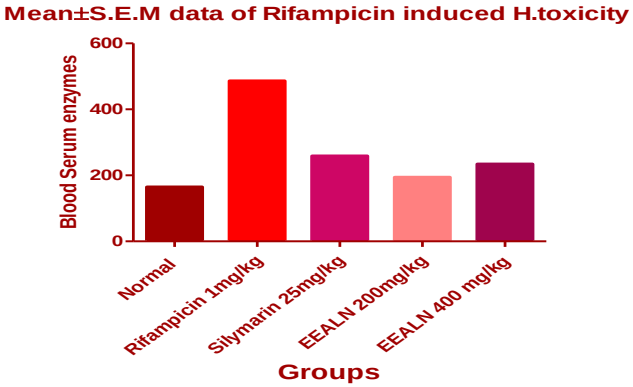


Figure 1.3. Comparison of Hepatoprotective activity of *EEALN* of all groups of Rifampicin induced rats. The values are Mean ± S.E.M., n=6.



Assessment of liver functions can be made by estimating the activities of serum SGOT, SGPT, ALP, Total Protein, Bilirubin etc. An elevation in the levels of the serum marker enzymes is generally regarded as the sensitive index of the hepatic damage[14]. The Rifampicin induced treated liver sections of the liver showed the increased levels of serum marker enzymes leading to the cellular leakage and loss of functional integrity of cell membrane in liver[15]. Therefore, the extract normalizes the elevated levels followed by the stabilization of plasma membrane as well as repair of hepatic tissue damage caused by the Rifampicin. It also leads to the healing of hepatic parenchyma and the regeneration of hepatocytes. The extract stabilizes the biliary dysfunction with concurrent depletion of raised bilirubin level and the normal hepatocytes, prominent central veins.

It is reported to produce intensive inflammatory infiltration followed by the feathery degeneration of hepatocytes, resulting in the lymphocytic infiltration in portal tracts. Protein levels were decreased on Rifampicin and D-Galactosamine treated rats. Oral administration of Rifampicin treated rats increased the levels of protein levels and treatment with ethanolic extract of *Andrographis lineata Nees* (*EEALN*) significantly decreased the protein levels when compared to that

of Silymarin. Treatment with ethanolic extract of *Andrographis lineata Nees* (*EEALN*) significantly p < 0.001 reduced the levels of serum enzymes, produced by Rifampicin caused a subsequent recovery towards normal effect.

Thus, the present studies confirms that the ethanolic extract of stems of *Andrographis lineata Nees* (*EEALN*) showed significant hepatoprotective action against Rifampicin induced liver damage in rats when compared with that of standard drug Silymarin.

Histopathological Studies of Liver

Liver was isolated after killing all the animals and histopathological examination is performed and a small portion was fixed in 10% formalin[12]. It was examined for the changes in the levels of serum marker enzymes along with that of any structural modification of hepatic morphology.

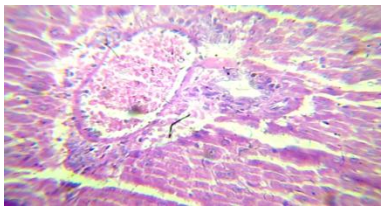


Fig 1. Control Liver (Normal Control)
Shows comparatively normal, no changes in hepatocytes

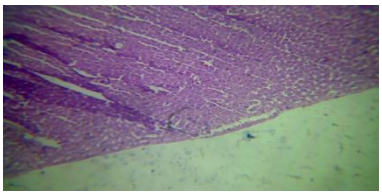


Fig 2. Rifampicin treated Liver (Toxicant Control)
Shows extensive infiltration of lymphocytic portal tracts and feathery degeneration of the hepatocytes

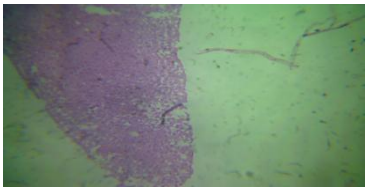


Fig 3. Silymarin treated Liver (Standard)
Shows mild regenerative changes in hepatocytes

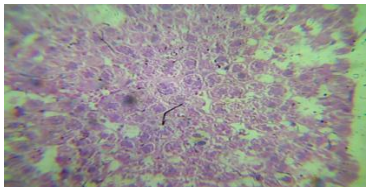


Fig 4.Ethanolic extract of *A.lineata nees (EEALN),* (200 mg/kg) treated Liver
Shows moderate normal hepatocytes and prominent central veins

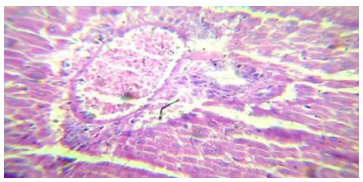


Fig 5. Ethanolic extract of *A. lineata nees (EEALN),* (400 mg/kg) treated Liver
Hepatocytes show normal restoration and prominent central veins

CONCLUSION

The results of the present study shows that the oral administration of *Andrographis lineata Nees* extracts reduced the hepatotoxicity by Rifampicin and D-Galactosamine induction in rats. Moreover, the stem extract of *Andrographis lineata Nees* with a high dose showed good results as compared to a low dose. However, the hepatoprotective activity

shown by the extracts with two different doses is significant and promissory against Rifampicin and D-Galactosamine induced hepatotoxicity in albino *wistar* rats when compared with that of a standard drug Silymarin. Further investigational studies are required to estimate the pharmacological and phytochemical constituents in the plant *Andrographis lineata Nees* that are responsible for its definite pharmacological activities during the mechanism of action of its hepatoprotective activity.

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