

EVALUATION OF HEPATOPROTECTIVE (PREVENTIVE & CURATIVE) ACTIVITY OF LEAVES EXTRACT OF *ROSA CENTIFOLIA* ON EXPERIMENTAL ANIMAL MODELS

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ABSTRACT

OBJECTIVE: The experimental study was designed to evaluate the hepatoprotective (preventive and curative) activity of Ethanolic extract of leaves of *ROSA CENTIFOLIA* (RC) in experimental animals (rats). **METHODS:** In both models (preventive and curative) the Liver was damaged by giving Carbon tetra chloride (CCl₄)-1ml/kg-p.o. Increased in the levels of biochemical markers of Hepatic damage like SGPT, SGOT, ALP and BILURUBIN (TB). Treatment with extract of *ROSA CENTIFOLIA* (300mg, 600mg/kg) in preventive model for 7 days and then from 7 to 14 days in curative model. The extract of RC was effectively reduced the high levels of enzymes in the plasma in preventive than curative models in the dose dependent manner. The enzyme level reduction in serum was considered as Hepatoprotective property of RC. Pathological changes like centribular necrosis and vacuolization were observed in CCl₄ treated rats, the same pathological changes were not observed in groups treated with RC and Silymarin (100mg/kg/day). **CONCLUSION:** The Ethanolic extract of RC has shown good hepatoprotective activity in preventive than curative research models against CCl₄ induced hepatotoxicity in rats.

Keywords: Carbon tetrachloride, Silymarin, *ROSA CENTIFOLIA*, Hepatoprotection

INTRODUCTION

Liver is the largest organ in the body and is main site for metabolism. It regulates the body homeostasis. It involves in almost all the biochemical pathways to growth, fight against disease, nutrient supply, energy provision and reproduction [1]. The important functions of the Liver are carbohydrate, protein, and fat metabolism, detoxification, secretion of bile and storage of vitamin. So it was considered as chief organ in the body to maintain perfect health. But when it exposed to any air pollutants, toxins and by continues alcohol can leads to liver diseases like hepatitis, cirrhosis, and alcohol liver disease, fatty liver, jaundice. In liver damage there is also increased serum enzyme levels like SGPT, SGOT, ALP and Bilirubin and also cellular necrosis is present[2]. In the traditional system of medicine, there are many medicinal plants to treat the hepatic diseases by antioxidant and hepatoprotective properties. In the present study *ROSA CENTIFOLIA* was selected to evaluate its hepatoprotective property due to its antioxidant action in CCl₄ induced Liver damage on animal models-Rats. The plant *ROSA CENTIFOLIA* belongs to family rosaceae, contain many chemical components like Phenyl ethanol (43%), Geranyl acetate (15.6%), Geraniol (10.5%), Benzaldehyde (1.5%), and also contains tannins, saccharine matter, mineral salts, salt of mallic acid, pectin, riboflavin, sugars, purgative glycosides (multiflorin A& B). Traditionally these chemical compounds are reported to use in Hepatopathy, inflammation, intestinal ulcers, asthma, cough, diarrhoea, bacterial infection, fever, insomnia, and headache. And also used as cardio protective, local aesthetic, anti depressant, antioxidant, vasoconstricting agent.[3,4] The antioxidant property is due to presence of tannins, and other chemical agents in RC. Since, antioxidants are known to prevent the chemically induced hepatic damage, the effect of ethanolic extract of leaves of the plant *ROSA CENTIFOLIA* has been evaluated for its hepatoprotective activity in Preventive and Curative animal models.

MATERIALS AND METHODS

Collection of plant material: Fresh leaves of *ROSA CENTIFOLIA* were collected during June-July from Thirupathi surrounding areas in Andhra Pradesh. The plant medicinal properties were identified and authenticated by Dr. Shekhar, Head of the department of pharmacognosy and phytochemistry, Sai College of

pharmacy, Kurnool, AP. The literature was collected from the book-Indian Medicinal Plants by Vaidyaratnam P.S. Varier vol-5(8).

Preparation of alcohol extract: The dried fine powder of leaves of the *ROSA CENTIFOLIA* was weighed on balance 30g, and taken into the sac like cloth material and placed in the Soxhlet basket. 300 ml of ethyl alcohol was taken as solvent into the Soxhlet flask. Cold tap water must flow through the inlet and outlet of the condenser. The Soxhlet apparatus kept running for 24hours for proper extraction. The extract laden solvent falling from the Soxhlet basket is dark in color and it becomes clearer, that indicates the extraction process is finished. At the end of the extraction process the solvent is then evaporated and the remaining mass is measured. [5]

Table 1: the percentage yields are calculated as Mg per Grm dried powder.

S. No.	Solvent	Weight in gm. Dry powder of Rosa Centifolia	Extract	Percentage Yields
1.	Ethyl Alcohol-300ml	30gm.	6gm.	20%

The yield of the ethyl alcohol extract is 20%. The extract was suspended in 2ml of 2% Gum acacia and used for the oral administration.

Toxicity study: the acute oral toxicity study was conducted according to the OPPTS (office of prevention, pesticide and toxic substances) Up and Down procedure. [6].

Chemicals: all the drugs and materials used for this study are of pharmacopeia grade. CCl₄ (E.Merck), Silymarin (sigma) and gum acacia-2%, olive oil, were purchased from the local supplier.

Experimental animals: Swiss albino Rats weighing 150-200g male and female rats were supplied from Srinath Agencies, Chennai, India. They were randomly distributed into groups and housed in cages (6/cage) and kept under standard conditions at 26±2°C and relative humidity 44-56% and 10 hours light: 14 hrs dark cycles each day for 1 week before and during the experiments. All animals were fed the standard rodent pellet diet and libitum. This study was cleared by

institutional animal ethical committee according to CPCSEA guidelines.

Experimental design: The animals used for the experiment were divided into 4 groups for preventive model and 5 groups for curative model, 6 rats for each group. [7].

Food was withdrawn 12 hr before CCl₄ administration to enhance the acute liver toxicity in all test groups of preventive and curative models.

Preventive model: four groups of rats were pre-treated with test drugs, Group-I (control) received 2% gumacacia-2ml/100g, Group-II (standard) received Silymarin (100mg/kg) and group-III, IV were treated with Ethanolic extract of RC- 300mg/kg, 600mg/kg respectively orally 1 hr prior to CCl₄ administration. After 1 hr of giving test drugs to all group of animal, Hepatic injury was induced by intraperitoneal injection of 1:1 v/v Carbon tetrachloride (CCl₄) in olive oil (1ml/kg) daily for 7 days. On 8th day, all the animals were anesthetized and blood was collected from the carotid artery at the neck for the determination of enzyme levels in serum, then all animals were sacrificed and dissected for Liver and preserved in 10% formalin for the histological study of each lobe of the liver. [8].

Curative model: the animals were divided into 5 groups, and all groups (I to V) were treated with CCl₄ (1:1 v/v)-1ml/kg in olive oil intraperitoneally daily once for 7 days. On 8th day, group-I

anesthetized for blood collection to determine enzyme level in serum, and then dissected for liver for the histological examination. From 8 to 14 days, group II-V were treated with test drugs. Group-II (control) received 2% gumacacia-2ml/100g, Group-III (standard) received Silymarin (100mg/kg) and group-IV,V were treated with Ethanolic extract of RC- 300mg/kg, 600mg/kg respectively orally up to 14 days. On 15th day, from all groups II-V blood was collected for biochemical determination of serum enzymes and dissected for liver for the histological examination.

Statistical analysis: The results obtained were expressed as Mean±SEM and were analyzed by the application of One-way Analysis of Variance (ANOVA), and P<0.05 was considered significant.

RESULTS

In preventive model: three groups (II-IV) of animals were treated with ethanolic extract of *ROSA CENTIFOLIA* (300, 600mg/kg) and Silymarin (100mg/kg) 1 hr before giving CCl₄ respectively. The extract and Silymarin treated animals were shown significant reduction in Serum marker enzymes ($p<0.001$). High dose of extract (600mg/kg) and Silymarin (100mg/kg) greatly reduce enzyme levels but low dose of extract (300mg/kg) has shown less effect than high dose (600mg/kg) but shown better effect than CCl₄ induced hepatotoxicity in control group rats. Liver weight almost comes to normal. All the results were depicted in table no: 1.

Table 2: Effect of ROSA CENTIFOLIA on preventive model (n=6, Mean±SEM)

GROUPS (I-IV)	SGOT (IU/ml)	SGPT (IU/ml)	ALP (IU/ml)	TOTEL BILIRUBIN (Mg/dl)	LIVER WEIGHT-g/100gm bw
Control (CCl ₄ 1ml/kg)	266.8±0.50	251.65±1.25	211.2±0.47	2.07±0.01	3.64±0.05
Standard (Silymarin-100mg/kg)	167.2±0.21***	151.29±0.13***	166.4±0.21***	0.89±0.06***	2.69±0.02***
Test-1 (Extract-300mg/kg)	199.7±0.10*	178.45±1.15*	201.9±0.17	1.80±2.21*	3.34±0.01*
Test-2 (Extract-600mg/kg)	175.4±0.09**	91.65±2.33**	159.2±0.15***	1.36±0.01**	2.73±0.30*

*P<0.05, **P<0.01, ***P<0.001 compared to Control.

Histological examination of the hepatic tissue in CCl₄ treated rats shown that CCl₄ had produced profound inflammation and congestion particularly in sinusoids. Pre-treatment of animals with Silymarin (100mg/kg) and *ROSA CENTIFOLIA* Extract 300, 600mg/kg) have not shown any pathological changes in histological study.

In curative model: here Group-I served as control group only up to 7 days, but Group-II served as control group for 14 days. From 8 to 14 days remaining groups (II-V) were treated with test drugs. Group-II (control) received 2% gum acasia-2ml/100g, Group-III (Standard) received Silymarin (100mg/kg), Group-IV and V received RC Extract 300mg/kg, 600mg/kg respectively. On 8th day blood was

withdrawn from Group-I rats for the estimation of enzymes and liver tissue was taken for histological study. Group-I significantly increased ($p<0.001$) the serum levels of SGPT, SGOT, ALP, TB and LW (liver weight). Liver histology shows marked level of fatty changes or degeneration and necrosis of the liver cells. Liver weight also increased due to inflammation. These results were compared with remaining all groups after 8 to 14 days treatment. In Silymarin treated groups, there was effectively reduced ($p<0.001$) the serum enzyme levels, liver cell inflammation and necrosis when compared with extract treated groups. Extract treated groups reduced the serum enzyme levels after treatment. Inflammation of the liver cells also recovered but not so effective when compared with preventive model groups. All the results were depicted in table no: 2 & 3

Table3: Effect of CCl₄ (1ml/kg) in Control group up to 7 days

GROUP-I	SGOT (IU/ml)	SGPT (IU/ml)	ALP (IU/ml)	TOTEL BILIRUBIN (Mg/dl)	LIVER WEIGHT-g/100gm bw
Control	278.5±0.29**	214.65±1.65**	255.5±0.87**	2.09±0.05**	3.71±0.07***

*P<0.05, **P<0.01, ***P<0.001 compared to Control.

Table 4: Effect of ROSA CENTIFOLIA on Curative model (8-14days treatment)

(n=6, Mean±SEM)

GROUPS (II-V)	SGOT (IU/ml)	SGPT (IU/ml)	ALP (IU/ml)	TOTEL BILIRUBIN (Mg/dl)	LIVER WEIGHT-g/100gm bw
Control (CCl ₄ 1ml/kg)	295.6±0.10	266.27±1.15	201.6±0.12	2.17±0.04	3.54±0.03
Standard (Silymarin-100mg/kg)	155.6±0.11***	167.64±0.21***	141.5±0.20***	0.91±0.04***	1.59±0.01***
Test-1 (Extract-300mg/kg)	211.5±0.15*	192.51±1.02*	211.9±0.07	1.74±1.11*	3.01±0.06*
Test-2 (Extract-600mg/kg)	190.4±0.06**	182.45±3.51**	164.7±0.11***	1.63±0.02**	2.01±0.51*

*P<0.05, **P<0.01, ***P<0.001 compared to Control.

DISCUSSION

In spite of tremendous development in the field of synthetic drugs during recent era, they are found to have some or other side effects, whereas plant products or homeo drugs still hold their own unique place by the way of having no side effects. According to literature available, the plant *ROSA CENTIFOLIA* have anti oxidant property and hepatoprotective activity [9]. but extensive scientific study was not done on this plant. So, in the present study, this plant was selected to prove its hepatoprotective activity scientifically by using experimental animal models. CCl₄ induced hepatic damage in rats model was used for the study. CCl₄ is commonly used drug to induce hepatotoxicity by generating free radicals in the experimental study [10]. Liver damage was confirmed by high serum enzymes (SGOT, SGPT, ALP and TB) levels because they are cytoplasmic in location and released into circulation after hepatocyte damage. Liver weight also increased due to toxic effect of CCl₄. [11, 12]. Due to anti oxidant property of *ROSA CENTIFOLIA*; its extract was used to inhibit generation of free radicals in hepatotoxicity induced by CCl₄ in rats. The ethanolic extract of leaves of *ROSA CENTIFOLIA* plant has shown hepatoprotective activity at 600mg/kg dose ($P < 0.01$) and also at 300mg/kg ($P < 0.05$) when given orally. High dose of extract (600mg/kg) is most effective, and almost nearer to standard drug Silymarin (100mg/kg) than low dose (300mg/kg) when compared to control group (CCl₄ treated group). Hepatoprotective and anti oxidant activity of *ROSA CENTIFOLIA* was most potent in preventive model than in curative animal models. Its probable mechanism in hepatic damage was anti oxidant effect by preventing free radical releasing. In histological examination also, no inflammatory cells and very less necrotic cells were appeared in high dose (600mg/kg) extract treated, Silymarin treated groups. Few inflammatory cells were appeared in low dose (300mg/kg) treated groups. Serum enzymes levels were also comes to normal in preventive models than curative models. The weight of the Liver also in both the models was effectively reduced in extract and Silymarin treated groups when compared with control group.

CONCLUSION

The present study indicates that the plant *ROSA CENTIFOLIA* has potential hepatoprotective activity of plant probably due to the compounds present such as Tannins and its anti oxidant property. So the plant *ROSA CENTIFOLIA* can be used for both Ayurvedic and Modern drug development areas because of its phyto-medicinal uses but it needs further clinical trials before complete trust and usage.

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REFERENCES

1. Ward, F.M. and M.J. Daly, Hepatic disease. In clinical pharmacy and Therapeutics (Walker R. and C.Edwards Eds). Churchill Livingstone, New York, 1999:195-212
2. Sharma, A. K.K Chakraborti and S.S Handa. Anti-hepatotoxicity activity of some Indian herbal formulations as compared to Silymarin. *Fitoterapia*, 1991; 62, (229-235).
3. Jena Jitendra, Tripathi Vineeta. *ROSA CENTIFOLIA*: plant review. *IJRPC*:2012; 2(3).
4. Vaidyaratnam P.S. Varier. Indian medicinal plants (compendium of 500 species).Orient Longman.1997. Vol: 5 (8).
5. Asli Semiz – African Journal of Biotechnology. 2007; Vol: 6(3): 273-277.
6. Health Effects Test Guidelines, Acute Oral Toxicity (computer program). OPPTS 870, 1100 United States Office of prevention, Pesticides and Toxic Substances Environmental ProtectionAgency.(7101).(Availablefrom-URL; <http://www.epa.gov/opptsfrs/home/guideline.htm>. 5/6/2004.
7. Sahu N, Das S. Hepatoprotective activity of *EUGENIA JUMBOSE* Linn. On albino rats. *IJP*: 2006.5(22-26).
8. Galighor. A.E. and E.N. Kozloff. Essentials of practical Micro Technique. 1976.2nd edi. New York: Lea and Fibiger.
9. Jena Jitendra, Tripathi Vineeta. *ROSA CENTIFOLIA*: plant review. *IJRPC*:2012; 2(3).
10. Shenoy K.A. Somayaji S.N. and Bairy.K.L. Hepatoprotective effect of *Ginkgo biloba* in Carbon tetrachloride induced hepatic injury in rats. *IJP*: 2001, 33(260).
11. Slater T.F. Biochemical mechanism of liver injury. London: 1965, Academic press.
12. Sallie R, J.M. Tredger and R. William, Drugs and the liver. Part-1.Testing liver functions. *Biopharm Drug Disp.*, 1991:12 (251-259).