WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 12, Issue 21, 631-661. Research Article ISSN 2277– 7105

HEPATOPROTECTIVE AND ANTI OXIDANT ACTIVITY OF *RHODODENDRON FERRUGINEUM* LEAVES

Mohammed Vazir*, Priyanka R., Tejashwini V. D. and Aishwarya Sadanand Teli

Hillside College of Pharmacy and Research Centre, #09 Kanakapura Road, Raguvanahalli,Subramanyapura, Bengaluru, Karnataka, 560062.

Article Received on
09 Oct. 2023,

Revised on 30 Oct. 2023,
Accepted on 20 Nov. 2023

DOI: 10. 20959/wjpr202321-30368

***Corresponding Author**
Mohammed Vazir
Hillside College of Pharmacy And Research Centre, #09 Kanakapura road, Raguvanahalli, Subramanyapura, Bengaluru, Karnataka, 560062.

ABSTRACT

Since ancient times, pla macological aspect of *Rhododendron ferrugineum L.* (Ericaceae) leaf, nor any scientific data, which could corroborate the claims. Therefore with all this raw data it is thought worthwhile to investigate and scientifically evaluate its hepatoprotective and antioxidant activity using experimental models in rats.

KEYWORDS: *Rhododendron ferrugineum*; leaves part, Pharmacological activities; Hepatoprotective activity: Antioxidant activity.

INTRODUCTION

Since ancient times, plants have been an exemplary source of medicine. Research conducted in last few decades on the plant mentioned in ancient literature or used traditionally.[1] Ornamental plants are grown for decorative purposes in gardens and landscape design projects, as house plants,for cut flowers and specimen display. Ornamentals and flowers crops are not only grown for the display of aesthetic features, but also have some nutritive and medicinal properties. There has been renewed interest in utilizing garden environments as therapeutic entities to enhance the process of healing that occurs in healthcare environments. By minimizing the stress response, therapeutic gardens can promote recovery from illness or preserve health. From centuries roses have been valued for their culinary, medicinal, cosmetic and aromatic properties.[2] The use of plant compounds for pharmaceutical purpose has gradually increased in India, about 80% of individuals from developed countries use traditional medicine, which

involves compounds derived from medicinal plant. Therefore, such plants should be investigated to better understand their properties, safety and efficiency.[3] Herbs are widely exploited in the traditional medicineand their curative potentials are well documented.[4]

Medicinal plant possesses an important role in human health care system. Herbal medicine has a tremendous demand in primary health sector because of their safety, efficacy, and least side effect.

It is now established that herbal remedies offer natural healing phenomenon via antagonizing degenerative pathological process.[5] So depending upon the scenario, we have chosen the leaves of *Rhododendron ferrugineum L.* (Ericaceae). It is one such plant, which is claimed in many ayurvedic text to possess hepatoprotective and antioxidant activity. However scientific data is not available regarding pharmacological aspect of *Rhododendron ferrugineum L.* (Ericaceae) leaf, nor any scientific data, which could corroborate the claims. Therefore with all this raw data it was thought worthwhile to investigate and scientifically evaluate its hepatoprotective and antioxidant activity.

Rhododendron ferrugineum L. (Ericaceae) is a sub-alpine shrub found throughout the Pyrenees and the European Alps at elevations of 1700 to 2300 m. The plant grows only in areas with silica- enriched soil and is found in large communities spreading over wide areas. The species is self- compatible and reproduces by selfing, out-crossing and through large vegetative spread down slopes.

Fig. 1: Leaves and flowers of *Rhododendron ferrugineum* L.

Rhododendron ferrugineum is an evergreen shrub that grows just above the tree line in the Alps, Pyrenees, Jura and northern Apennines, on acid soils. It is the type species for the genus *Rhododendron*. It may grow up to 1 m tall and produces clusters of pinkish-red, bell-shaped flowers throughout the summer. The undersides of the leaves are

covered in rust-brown spots, which give the species the second part of its binomial name (*ferrugineum*, Latin for 'rust-coloured, ferruginous'). This is in contrast to *Rhododendron hirsutum*, which has no such brown colouring, has hairy edges to the leaves and grows over limestone. Where the two species co- occur (usuallyon soils of intermediate pH), the hybrid *Rhododendron intermedium* may occur; as its name suggests, it is intermediate in form between the two parental species. The leaves are spirally arranged; leaf size can range from 1–2 cm (0.4–0.8 in) to over 50 cm (20 in). They may be either evergreen or deciduous. *R. ferrugineum* is moderately toxic, containing arbutin, arecoline and rhodoxanthin, and can cause vomiting, and difficulties of the digestive, nervous, respiratory and circulatory systems.[6]

List of Phytochemicals of *Rhododendron ferrugineum* L. leaves

From the phytochemical point of view the description of the plant is incomplete and rudimen-tal. The following compounds have been de-scribed as secondary metabolites from the leaves: triterpenes (ursolic acid[1], campanulin, friede-lin, epifriedelin, α- and β-amyrin[2]), phenols (rhododendron[3], phloracetophenon and phlor-acetophenone 4-O-glucoside[4], flavonoids (hy- peroside, myricetin 3-O-β-galactopyranoside, kaempferol 3-O-[6″-O-acetyl]-glucoside, querce- tin 3-O-[6″-O-acetyl]-glucoside, quercetin 3-O-[6″-O-acetyl]-galactoside, quercetin 3-O-[3″,6″- O-diacetyl]-galactoside, cis- and trans-taxifolin 3-O-α-L-arabinopyranoside[7]), volatile oil monoterpenes (geraniol, α- and β-pinene[8]), and short-chain organic acids. Andromedotoxin andhydroquinone derivatives are reported to be absent.[9,10]

Hepatotoxicity

Liver is the largest internal organ which plays an important role in maintaining the biological equilibrium of vertebrates because it synthesizes, secretes, excretes stores, generates, metabolize, protects and detoxicates s substances. The spectrum of its functions include, metabolism and disposition of chemicals (xenobiotics) to which the organ is exposed directly or indirectly; metabolism of lipids, carbohydrates and proteins; blood coagulation and immunomodulation.[11] It plays an important role in detoxification processes and also an important role in synthesizing useful principles. Therefore,damage to the liver inflicted by hepatotoxic agents is of grave consequences. There is an ever increasing need of an agent which could protect it from such damage.[12]

Liver disease is still a worldwide health problem. Unfortunately, conventional or

synthetic drugs used in the treatment of liver diseases are inadequate and sometimes can have serious side effects.[13] In the absence of a reliable liver protective drug in modern medicine there are a numberof medicinal preparations in Ayurveda recommended for the treatment of liver disorders.[14] Liver regulates many important metabolic functions. Hepatic injury is associated with distortion of these metabolic functions.[7] Along with that liver is the key organ of metabolism and excretion; itis continuously exposed to xenobiotics because of its strategic placement in the body. Many hepatotoxicants suchas carbon tetrachloride, nitrosamine and polycyclic aromatic hydrocarbon require metabolic activation, particularly by liver cytochrome P450 (CYP) enzymes, to form reactive, toxic metabolites, which in turn cause liver injury in animals and humans.[15]

Diseases of Liver

The response of the liver injury may take several forms and involve the hepatocytes, vascularcells or bile ducts. The most important disease are:

A. Biliary obstruction
B. Metabolic lesions caused by genetic disease or exogenous substance,such as alcohol.
C. Inflammation, especially that caused by hepatitis viruses.
D. Cirrhosis
E. Neoplasia

ANTI-OXIDANT

Antioxidants are substances which help to defend the body against cell damage caused by various free radicals. Free radicals are unstable oxygen molecules containing unpaired electrons. Broadly possible mechanisms by which antioxidants may protect against ROS toxicity are (i) prevention of ROS formation (ii) interception of ROS attack by scavenging the reactive metabolites and converting them to less reactive molecules and/or by enhancing the resistivity of sensitive biological targets to ROS attack (iii) facilitating the repair of damage caused by ROS (iv) providing a favorable environment for effective functioning of other antioxidants. The capacity to detoxify ROS is of critical importance in all aerobes. In the human body a complex combination of enzymatic and non-enzymatic systems function to minimize the stress induced by ROS. These antioxidants may be classified as endogenous antioxidants, those which are physiological origin and exogenous antioxidants are those which cannot be produced by the human body but may protect against pro-oxidant forces when administered as supplements.[16]

Types of Hepatotoxic Agents

Tabl 1&2: Types of Hepatotoxic Agents.

Compounds	Examples
INORGANIC AGENTS	Metals and metalloids: antimony, arsenic, beryllium, bismuth,boron, cadmium, chromium, cobalt, copper, iron, lead,manganese, mercury, gold, phosphorous, selenium, tellurium, thallium, zinc, hydrazine derivative, iodides.
ORGANIC AGENTS	
Natural : Plant toxins	Albitocin, cycasin, nutmeg, tannic acid, icterogenin, pyrrolidizines, saferole, indospicine.
Mycotoxins:	Aflatoxins, cyclochlorotine, ethanol, luteoskyrin, griseofulvin,sporidesmin, tetracycline, and other antibiotics.
Bacterial toxins:	Exotoxins(C.diphtheria, Clostridium botulinus), endotoxins, ethionine.
Synthetic:Non-medicinal	Haloalkanes and haloolephins, Nitroalkanes, Chloroaromatic compounds, Nitroaromatic compound, organic amines, Azo compounds. Phenol and derivatives, various other organic compounds.

MEDICINAL AGENTS

Category of drugs	Examples
1) Neuro psychotropic	Hydrazine, tranylcypromine anticonvulsants, antidepressants.
2) Anti-inflammatory and anti-muscle spasm agents	Cinchopen, cholchicine, ibuprofen, salicylates, indomethacin.
3)Hormonal derivatives and other drugsNused in endocrine disease	Acetohexamide, Azepinamide, Carbutamide, Tolbutamide.
4) Antimicrobials	Clindamycin, novobiocin, penicillin, tetracycline,sulfonamide, amodiaquine, isoniazid, rifampin.
5) Antineoplastic	L-Asparaginase, azacytidine, methotrexate,

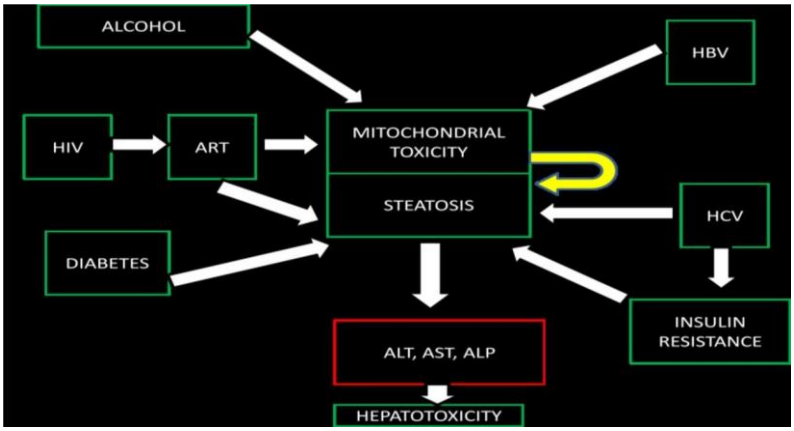


Fig. No. 2: Induction of hepatotoxicity by various agents.

Materials and Methods Collection of plant material

The leaves of *Rhododendron ferrugineum* was collected from the local region Darjeeling and authenticated by Taxonomist. The herbarium of the plant was kept in our institution. The leaves was dried in the shade for 7 days at room temperature (28 ± 2 ^{o}C) and ground to a coarse powder. The powder samples were kept at room temperature in a covered glass containers to protect themfrom humidity and light prior to extraction.

Successive solvent extraction

The powdered leaf (250g.) of *Rhododendron ferrugineum* was successively extracted with petroleum ether, chloroform, ethanol, distilled water with series of organic solvent with increasing polarity by using Soxhlet's apparatus for 12-14 hours on a water bath separately. The organic extracts were separately filtered with Whatmann filter paper and evaporated to dryness on waterbath to obtain semi-solid mass.

Preparation of various solvent extracts[17,18,19]

The powder obtained was subjected to successive Soxhlet extraction with the solvents with increasing order of polarity i.e. petroleum ether (60-80^{o}C), chloroform (59.5-61.5^{o}C), ethanol (64.5-65.5^{o}C) and water. If further required the shade- dried powder was extracted directly with ethanol (hydro-alcoholic extract). The extract was allowed to concentrate under reduced pressure(bath temperature 5^{o}C) and store in air tight container in refrigerator below 10^{o}C. All these extracts were used for biological investigations and in vivo studies. The extracts of *Rhododendron ferrugineum* leaves were planned for the following investigation.

Pharmacological activities

1. Toxicity studies (LD50)

Determination of acute toxicity (LD50)

The acute toxicity of a *Rhododendron ferrugineum* extracts was determined by using female albino mice (20-30g) those maintained under standard husbandry conditions. The animals were fasted 4hrs prior to the experiment and Acute Oral Toxicity-Acute Toxicity as per OECD guideline no. 423 was determined. Animals were administered with single dose 2000 mg/kg of extract and observed individually for once during the first 30 minutes, periodically during thefirst 24 hours, with special attention given during the first 4 hours and daily thereafter, for a total of 14 days. During this period the mortality and/or the

moribund status of the animals were noted.

2. Hepatoprotective ActivityExperimental animals

Albino rats (Wistar strain) of either sex weighing between 150-220g were procured from CentralAnimal House, Hyderabad for experimental purpose. After procuring, the animals were acclimatized for seven days under standard husbandry conditions with 12 hours light/dark cycle. The animals were fed with standard diet manufactured by Fischers chemical and Scientifics and stored in Alpha Omega Hi-tech Bio Research Centre (Reg. 962/2012). Water was allowed *ad libitum* under strict hygenic conditions. After obtaining prior permission from Institutional Animal Ethical Committee (IAEC) registration number **AOHB/PW/178/2017**, studies were performed inaccordance to guidelines of CPCSEA.

Materials

Successive extracts of *Rhododendron ferrugineum* leaves were extracted by Soxhlet apparatus with respective solvents. Silymarin was a gift sample from Sumee Pharma Limited, Hyderabad. Ethanol was procured from bonded laboratory. Paracetamol was purchased from Apollo Pharmacy, Hyderabad. Animal feed was supplied by Central Animal House, Hyderabad. Kits for estimationof selected biochemical parameters such as SGPT, SGOT, ALP and BIT were purchased from Sumee Pharma Limited, Hyderabad.

A) Paracetamol induced hepatotoxicity

The rats were selected and divided into 5 groups each containing six animals. Silymarin was dissolved in 2% gum acacia suspension and herbal extract was dissolved in water. The treatmentprotocol was planned to study the effect of herbal extract in preventive aspect of paracetamol induced hepatotoxicity. The dose of PCM to induce hepatic damage was selected as 2 g/kg bodyweight for three days. The dose of Silymarin used was 100 mg/kg body weight. The doses of *Rhododendron ferrugineum* aqueous extract selected were 300 mg/kg and 600 mg/kg bodyweight. The treatment protocol is summarized and given below.

Group 1- Control: 2% w/v gum acacia (1ml/kg) p.o. once daily for 3 days

Group 2- Toxicant: Paracetamol (2 g/kg) p.o. once daily for 3 days

Group 3- Standard: Silymarin (100 mg/kg) p.o. + after 30 min Paracetamol (2 g/kg) p.o. for 3 days

Group 4- *Rhododendron ferrugineum* aqueous extract (300 mg/kg) p.o. + after 30 min Paracetamol (2 g/kg) p.o. for 3 days.

Group 5- *Rhododendron ferrugineum* aqueous extract (600 mg/kg) p.o. + after 30 min Paracetamol (2 g/kg) p.o. for 3 days

On 0 and 4th day blood was collected from all animals by retro orbital puncture method. Serum was separated by centrifugation (3000 rpm for 15 min) and subjected for estimation of biochemical parameters (SGPT, SGOT, ALP and BIT).

Statistical analysis: Results were expressed as mean ± SEM. The difference among means was analysed by unpaired Student's t-test.

B) Alcohol induced hepatotoxicity

The rats were selected and divided into 5 groups each containing six animals. Silymarin was dissolved in 2% gum acacia suspension and herbal extract was dissolved in water. The treatment protocol was planned to study the effect of herbal extract in preventive aspect of ethanol induced hepatotoxicity. The dose of ethanol to induce hepatic damage was selected as 3.76g/kg body weight. The dose of Silymarin used was 100 mg/kg body weight. The dose of *Rhododendron ferrugineum* aqueous and successive extracts selected was 300 mg/kg body weight of each. The treatment protocol is summarized and given below.

Group 1- Control: 2% w/v gum acacia (1ml/kg) p.o. once daily for 25 days

Group 2- Toxicant: 40% Ethanol (3.76 g/kg) twice daily, p.o. for 25 days

Group 3- Standard: Silymarin (100 mg/kg) p.o. + after 30 min Ethanol (3.76 g/kg) p.o. for 25 days

Group 4- *Rhododendron ferrugineum* aqueous extract (300 mg/kg) p.o. + after 30 min Ethanol (3.76 g/kg) p.o. for 25 days

Group 5- *Rhododendron ferrugineum* successive extract (300 mg/kg) p.o. + after 30 minEthanol (3.76 g/kg) p.o. for 25 days

On 0 and 26th day blood was collected from all animals by retro orbital puncture method. Serum was separated by centrifugation (3000 rpm for 15 min) and subjected for estimation of biochemical parameters (SGPT, SGOT, ALP and BIT).

Statistical analysis: Results were expressed as mean ± SEM. The difference among means was analysed by unpaired Student's t-test.

C) Biochemical parameters

The biochemical parameters were estimated as per the standard procedure prescribed by the manufacturer's instruction manual provided in the kit using semi-autoanalyser.

a) Estimation of serum SGPT (UV – Kinetic method)

Principle: SGPT catalyses the transfer of amino group from L-Alanine to 2-Oxoglutarate with the formation of Pyruvate and L-Glutamate. The pyruvate so formed is allowed to react with NADH to produce L-Lactate. The rate of this reaction is monitored by an indicator reaction coupled with LDH in the presence of NADH (nicotinamide adenine dinucleotide). The oxidation of NADH in this reaction is measured as a decrease in the absorbance of NADH at 340 nm, which is proportional to SGPT activity.

Reagent contents

Reagent R1: Ready – to – useReagent R2: Ready – to – use

Reagent preparation: Mix 4 parts of R1 with 1 part of R2. The combined reagent is stable for 4weeks at 2 °C to 8 °C.

Sample: Blood was collected by retro orbital puncture in eppendorf's tubes and allowed to settlefor 30 min and serum was withdrawn using micropipette for estimation.

Procedure

Pipette into cuvettes	Sample
Reagent	500 µl
Sample	50 µl

Mix well and aspirate.

b) Estimation of serum SGOT (UV – Kinetic method)

Principle: SG0T catalyses the transfer of amino group from L-Aspartate to 2-Oxoglutarate with the formation of Oxaloacetate and L-Glutamate. The rate is monitored by an indicator reaction coupled with Malate dehydragenase (MDH) in which the Oxaloacetate formed is converted to Malate ion in the presence of NADH (Nicotinamide adenine dinucleotide). The oxidation of NADH in this reaction is measured as a decrease in the absorbance of NADH at 340 nm, which is proportional to SGOT activity.

Reagent contents

Reagent R1: Ready – to – useReagent R2: Ready – to – use

Reagent preparation: Mix 4 parts of R1 with 1 part of R2. The combined reagent is stable

for 4weeksat 2 °C to 8 °C.

Sample: Blood was collected by retro orbital puncture in eppendorf's tubes and allowed to settlefor 30 min and serum was withdrawn using micropipette for estimation.

Procedure

Pipette into cuvettes	Sample
Reagent	500 µl
Sample	50 µl

Mix well and aspirate.

c) Estimation of serum Alkaline phosphatase(ALP)

Principle: Estimation of Serum alkaline phosphatase hydrolyses p-Nitrophenyl phosphate in the presence of oxidizing agent Mg^{+2}. This reaction is measured as absorbance is proportional to theALP activity.

Reagent: ALP Reagent: Ready – to – use

Sample: Blood was collected by retro orbital puncture in eppendorf's tubes and allowed to settlefor 30 min and serum was withdrawn using micropipette for estimation.

Procedure

Pipette into cuvettes	Sample
Reagent	500 µl
Sample	10 µl

Mix well and aspirate.

d) Estimation of Serum bilirubin

Principle: Bilirubin reacts with diazotized Sulphanilic acid in acidic medium to form a pink coloured azobilirubin which has maximum absorbance at 546 nm in the aqueous solution. The intensity of the color produced is directly proportional to the amount of direct or total bilirubin concentration. Direct bilirubin, being water soluble directly reacts in acidic medium. However, indirect and unconjugated bilirubin is solubilised using a surfactant and then it is reacted similar to that of direct bilirubin.

Reagents: Total Bilirubin Reagent: Ready – to – useBilirubin Activator: Ready – to – use

Sample: Blood was collected by retro orbital puncture in eppendorf's tubes and allowed to settlefor 30 min and serum was withdrawn using micropipette for estimation.

Procedure

Pipette into cuvettes	Test Sample	Blank
Total Bilirubin Reagent	500 µl	500 µl
Bilirubin Activator	50 µl	–
Distilled Water	–	50 µl
Sample	50 µl	50 µl

Mix and incubate for 10 minutes at 37^{o} C. Aspirate serum blank and then test sample immediately.

3. *In-vivo* Antioxidant Activity

An attempt was made to assess the influence of pre-treatment with successive extract of *Rhododendron ferrugineum* leaves on the levels of glutathione and lipid peroxidation *in-vivo* in paracetamol induced hepatotoxicity.

a) Glutathione (GSH) and lipid peroxidation estimation in paracetamol induced hepatotoxicity in albino rats of either sex.

Glutathione is present in all type of living cells. Tissue such as mammalian liver normally contains high levels of reduced Glutathione. It has been suggested that GSH protects thiol groups in protein from oxidation, functions as an intracellular redox buffer and serves as a reservoir of cysteine.

The role of GSH in determining the extent of liver damage has been demonstrated in experiments where the hepatic concentration of GSH is altered by toxin treatments. Depletion of GSH contents has been reported to potentiate hepatic necrosis and covalent binding of toxic metabolites to cellular macromolecules.

Determination of reduced Glutathione (GSH) and Lipid peroxidation activity

In the dose response experiment, albino rats were randomly assigned into 5 groups of 6 rats in ech group.

Group-I: Animals (-ve Control) were administered normal saline 1ml/kg p.o, for 7 days.

Group-II: Animals (+ve Control) were administered normal saline 1ml/kg p.o, for 7days.

Group-III: Animals were administered with silymarin 100mg/kg p.o., for 7 days.

Group-IV: Animals were administered with 70% successive extract of *Rhododendronferrugineum* 200mg/kg p.o, for 7 days.

Group-V: Animals were administered with 70% successive extract of *Rhododendronferrugineum* 400mg/kg p.o., for 7 days.

Procedure

On 5th day, 30 min after normal saline, 100 mg/kg silymarin, 200mg/kg 70% successive extract and 400 mg/kg, 70% successive extract of *Rhododendron ferrugineum* leaves administration to group-II, III, IV and V respectively, received paracetamol 2g/kg p.o. After 48 hours of paracetamol feeding, rats were sacrificed under mild ether anesthesia. Hepatic tissues were collected and assessed.

A) Estimation of GSH

Tissue samples were homogenized in ice cold Trichloroacetic acid (1 gm tissue plus 10 ml 10% TCA) in a ultra turrax tissue homogenizer. Glutathione measurements were performed using a modification of the Ellamn procedure[50] briefly, after centrifugation at 3000 rpm for 10 minutes, 0.5 ml supernatant was added to 2 ml of 0.3 M disodium hydrogen phosphate solution. A 0.2 ml. solution of dithiobisnitrobenzoate (0.4 mg/ml in 1% sodium citrate) was added and the absorbance at 412 nm. was measured immediately after mixing. Percentage increase in OD is directly proportional to the increase in the levels of glutathione; hence the % increase in OD was calculated.

B) Estimation of lipid peroxidation

Combine 1 ml of biological sample (0.1 -0.2 mg of membrane protein) with 2 ml of TCA-TBA-HCl and mix thoroughly. The solution is heated for 15 min. in a boiling water bath. After cooling, the flocculent precipitate was removed by centrifugation at 1000 rpm for 10 min. The absorbance of the sample is determined at 535 nm. against a blank that contains allthe reagents minus the lipid.

RESULTS AND DISCUSSION RESULTS

Acute toxicity study

LD_{50} studies were conducted in albino rat by using OECD guidelines No- 423 for *Rhododendron ferrugineum* extracts. It was found that the extract even at 2000 mg/kg dose had not shown any mortality confirming it's practically non-toxic in nature and it falls in GHS category 5.

Hepatoprotective activity

Paracetamol induced hepatotoxicity

Alanine aminotransperase levels (ALT or SGPT)

The dose of 2 g/kg body weight of paracetamol induced significant increase in serum SGPT levels with an increase of 268.31% (44.53 IU/L to 164.01 IU/L) compared to normal control where the increase was 0.86% (40.51 IU/L to 40.86 IU/L). Paracetamol induces serum rise of SGPT was protected by 100mg/kg body weight dose of Silymarin and 300 and 600mg/kg body weight doses of *Rhododendron ferrugineum* aqueous extract. The rise was only 22.01% (43.93 IU/L to 53.6 IU/L), 36.78% (43.06 IU/L to 58.9 IU/L) and 32.94% (42.71 IU/L to 56.78 IU/L) respectively after three days treatment. The results are given in table no. from 3 to 13 and Fig. 3

Aspartate aminotransferase levels (AST or SGOT)

The dose of 2 g/kg body weight of paracetamol induced significant increase in serum SGOT levels with an increase of 463.61% (64.78 IU/L to 365.11 IU/L) compared to normal control where the increase was 0.52% (63.38 IU/L to 63.05 IU/L). Paracetamol induces serum riseof SGOT was protected by 100mg/kg body weight dose of Silymarin and 300 and 600mg/kg body weight doses of *Rhododendron ferrugineum* aqueous extract. The rise was only 10.09% (68.08 IU/L to 74.95 IU/L), 42.25% (68.35 IU/L to 97.23 IU/L) and 35.51% (66.46 IU/L to 90.06 IU/L) respectively after three days treatment. The results are given in table no. from 3 to 13 and Fig. 3 and 5.

Alkaline phosphatase levels (ALP)

The dose of 2 g/kg body weight of paracetamol induced significant increase in serum ALP levels with an increase of 344.24% (139.03 IU/L to 617.63 IU/L) compared to normal control where the increase was 0.4% (143.71 IU/L to 143.13 IU/L). Paracetamol induces serum rise of ALP was protected by 100mg/kg body weight dose of Silymarin and 300 and600mg/kg body weight doses of *Rhododendron ferrugineum* aqueous extract. The rise was only 31.81% (142.91 IU/L to 188.38 IU/L), 48.67% (141.9 IU/L to 210.96 IU/L) and 40.98% (140.8 IU/L to 198.5 IU/L) respectively after three days treatment. The results are given in table no. from 3 to 13 and Fig. 3 and 6.

Bilirubin total levels (BIT)

The dose of 2 g/kg body weight of paracetamol induced significant increase in serum BITlevels with an increase of 371.01% (0.69 IU/L to 3.25 IU/L) compared to normal control where the increase was 11.84% (0.76 IU/L to 0.85 IU/L). Paracetamol induces serum rise of BIT was protected by 100mg/kg body weight dose of Silymarin and 300 and 600mg/kg body weight doses of *Rhododendron ferrugineum* aqueous extract. The rise was only 3.33% (0.6 IU/L to 0.58 IU/L), 30.77% (0.65 IU/L to 0.45 IU/L) and 11.63% (0.43 IU/L to 0.48 IU/L) respectively after three days treatment.

The results are given in table no. from 3 to 13 and Fig. 3 and 7.

Table No. 3: Basal levels of selected serum biochemical parameters in rats, group-1 on day 0 (N=6).

Parameters	R1 (160g)	R2 (150g)	R3 (150g)	R4 (170g)	R5 (150g)	R6 (160g)	Average ±SEM
SGPT (IU/L)	40.8	39.2	39.8	41.8	41.3	40.2	40.51 ± 0.40
SGOT (IU/L)	61.0	64.8	64.1	67.0	63.2	60.2	63.38 ± 1.05
ALP (IU/L)	144.4	143.7	143.2	145.3	142.6	143.1	143.71 ± 0.45
BIT (mg/dL)	0.5	0.48	0.86	1.1	0.9	0.77	0.76 ± 0.09

Table No. 4: Basal levels of selected serum biochemical parameters in rats, group-2 on day 0 (N=6).

Parameters	R1 (180g)	R2 (150g)	R3 (150g)	R4 (180g)	R5 (160g)	R6 (150g)	Average ±SEM
SGPT (IU/L)	46.8	44.1	41.9	44.8	45.5	44.1	44.53 ± 0.72
SGOT (IU/L)	61.5	63.3	70.7	65.2	63.9	64.1	64.78 ± 1.31
ALP (IU/L)	133.1	143.8	142.1	135.5	141.3	138.4	139.03 ± 1.71
BIT (mg/dL)	0.45	0.63	1.11	0.9	0.65	0.45	0.69 ± 0.10

Table No. 5: Basal levels of selected serum biochemical parameters in rats, group-3 on day 0 (N=6).

Parameters	R1 (150g)	R2 (150g)	R3 (190g)	R4 (170g)	R5 (170g)	R6 (150g)	Average ± SEM
SGPT (IU/L)	43.5	44.2	44.6	43.9	44.8	42.6	43.93 ± 0.39
SGOT (IU/L)	75.2	73.1	62.8	68.3	63.5	68.6	68.08 ± 2.04
ALP (IU/L)	151.4	143.2	145.4	138.2	138.8	140.5	142.91 ± 1.98
BIT (mg/dL)	0.53	0.52	0.77	0.46	0.92	0.41	0.60 ± 0.26

Table No. 6: Basal levels of selected serum biochemical parameters in rats, group-4 on day 0 (N=6).

Parameters	R1 (170g)	R2 (160g)	R3 (150g)	R4 (170g)	R5 (170g)	R6 (150g)	Average ± SEM
SGPT (IU/L)	43.2	44.1	40.3	48.7	39.8	42.3	43.06 ± 1.09
SGOT (IU/L)	73.6	68.8	70.6	63.8	65.2	68.1	68.35 ± 1.38
ALP (IU/L)	141.3	143.8	145.5	135.8	141.6	143.4	141.9 ± 1.3
BIT (mg/dL)	0.37	1.2	0.5	0.63	0.3	0.92	0.65 ± 0.16

Table No. 7: Basal levels of selected serum biochemical parameters in rats, group-5 on day 0 (N=6).

Parameters	R1 (150g)	R2 (170g)	R3 (150g)	R4 (160g)	R5 (160g)	R6 (150g)	Average ±SEM
SGPT (IU/L)	43.6	45.8	41.0	39.1	42.6	44.2	42.71 ± 0.92
SGOT (IU/L)	75.6	63.8	65.2	63.7	61.4	69.1	66.46 ± 2.07
ALP (IU/L)	138.4	148.3	132.9	137.6	152.3	135.3	140.8 ± 3.17
BIT (mg/dL)	0.2	0.39	0.62	0.71	0.52	0.16	0.43 ± 0.11

Table No. 8: Levels of selected serum biochemical parameters in rats, group-1 on 4th day treatedwith2% w/v Gum acacia (1ml/kg).

Parameters	R1 (160g)	R2 (150g)	R3 (150g)	R4 (170g)	R5 (150g)	R6 (160g)	Average ±SEM
SGPT (IU/L)	41.6	36.2	42.8	42.4	39.2	43.0	40.86 ± 1.12
SGOT (IU/L)	57.7	65.9	65.0	69.4	61.8	58.5	63.05 ± 1.94
ALP (IU/L)	143.1	145.6	138.9	142.8	145.4	143.0	143.13 ± 1.01
BIT (mg/dL)	0.72	0.76	0.52	1.13	1.1	0.9	0.85 ± 0.10

Table No. 9: Levels of selected serum biochemical parameters in rats, group-2 on 4th day treatedwithParacetamol (2g/kg).

Parameters	R1 (180g)	R2 (150g)	R3 (150g)	R4 (180g)	R5 (160g)	R6 (150g)	Average ±SEM
SGPT (IU/L)	155.2	172.3	165.8	165.3	150.1	175.4	164.01 ± 3.99
SGOT (IU/L)	356.2	368.1	375.3	356.8	349.3	385.0	365.11 ± 5.60
ALP (IU/L)	598.2	640.2	648.3	610.4	623.6	585.1	617.63 ± 9.99
BIT (mg/dL)	2.4	3.3	4.1	3.6	3.1	3.0	3.25 ± 0.21

Table No. 10: Levels of selected serum biochemical parameters in rats, group-3 on 4th day treatedwith Paracetamol (2g/kg) + Silymarin (100mg/kg).

Parameters	R1 (150g)	R2 (150g)	R3 (190g)	R4 (170g)	R5 (170g)	R6 (150g)	Average ± SEM
SGPT (IU/L)	50.3	49.8	58.2	60.7	51.5	51.1	53.6 ± 1.90
SGOT (IU/L)	76.6	72.2	80.3	74.5	73.0	73.1	74.95 ± 1.18
ALP (IU/L)	183.9	210.2	185.6	193.4	171.0	186.2	188.38 ± 5.27
BIT (mg/dL)	0.52	0.5	0.72	0.51	0.88	0.34	0.58 ± 0.16

Table No. 11: Levels of selected serum biochemical parameters in rats, group-4 on 4th day treated withParacetamol (2g/kg) + *Rhododendron ferrugineum* aqueous extract (300mg/kg).

Parameters	R1 (170g)	R2 (160g)	R3 (150g)	R4 (170g)	R5 (170g)	R6 (150g)	Average ±SEM
SGPT (IU/L)	61.9	54.8	60.2	59.1	57.0	60.4	58.9 ± 1.18
SGOT (IU/L)	98.4	94.2	98.6	99.9	98.7	93.6	97.23 ± 1.10
ALP (IU/L)	224.4	202.1	201.2	215.8	209.0	213.3	210.96 ± 3.62
BIT (mg/dL)	0.45	0.33	0.32	0.84	0.61	0.16	0.45 ± 0.10

Table No. 12: Levels of selected serum biochemical parameters in rats, group-5 on 4th day treated withParacetamol (2g/kg) + *Rhododendron ferrugineum* aqueous extract (600mg/kg).

Parameters	R1 (150g)	R2 (170g)	R3 (150g)	R4 (160g)	R5 (160g)	R6 (150g)	verage ±SEM
SGPT (IU/L)	54.9	60.4	61.8	53.1	56.3	54.2	56.78 ± 1.46
SGOT (IU/L)	98.8	78.3	86.4	86.6	97.0	93.3	90.06 ± 3.23
ALP (IU/L)	198.8	205.9	190.2	197.8	189.5	208.8	198.5 ± 3.23
BIT (mg/dL)	0.12	0.41	0.53	1.1	0.41	0.29	0.48 ± 0.14

Table No. 13: Average percentage change in selected serum biochemical parameters in rats inParacetamol induced hepatotoxicity.

Group	Treatment	SGPT	SGOT	ALP	BIT
1.	Gum acacia (1ml/kg)	0.86 ± 2.55	0.52 ± 1.51	0.4 ± 0.84	11.84 ± 15.70
2.	PCM (2g/kg)	268.31±12.91***	463.61±11.31***	344.24±4.82***	371.01±32.08***
3.	PCM + SIL (100mg/kg)	22.01 ± 4.24***	10.09 ± 4.03***	31.81 ± 4.05***	3.33 ± 4.84***
4.	PCM + *R. ferrugineum* (300mg/kg)	36.78 ± 5.29***	42.25 ± 3.69***	48.67 ± 3.61***	30.77 ± 30.4***
5.	PCM + *R. ferrugineum* (600mg/kg)	32.94 ± 3.73***	35.51 ± 4.94***	40.98 ± 4.01***	11.63±14.98***

***P < 0.001 significant when compared group-1 with group-2 and group-2 with group-3, 4 and 5. PCM- Paracetamol, SIL-Silymarin, *R.ferrugineum-Rhododendron ferrugineum* aqueousextract.

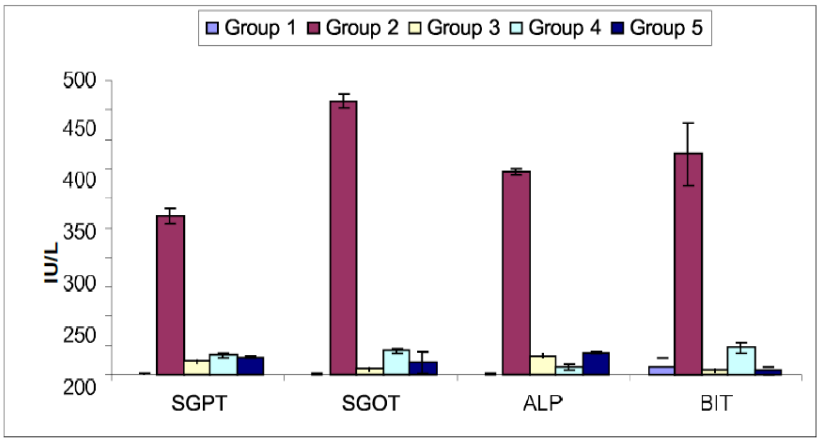


Fig No. 3: Average percentage change in selected serum biochemical parameters in PCM inducedhepatotoxicity in rats.

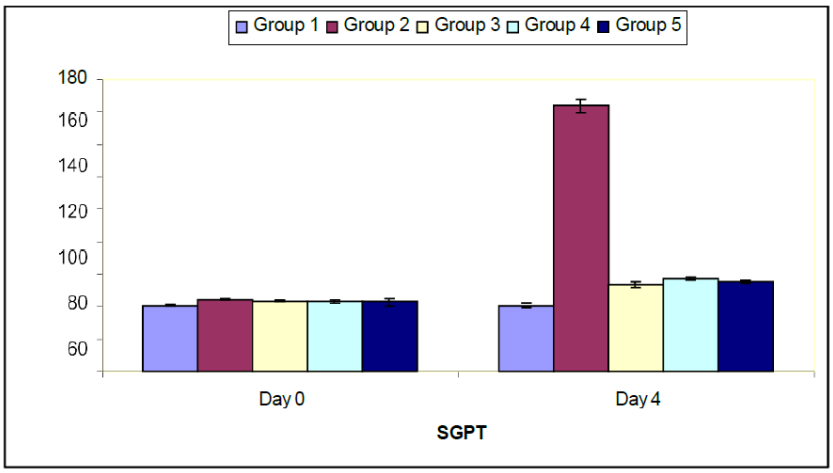


Fig No. 4: Influence of *Rhododendron ferrugineum* extract on SGPT in paracetamol inducedhepatotoxicity.

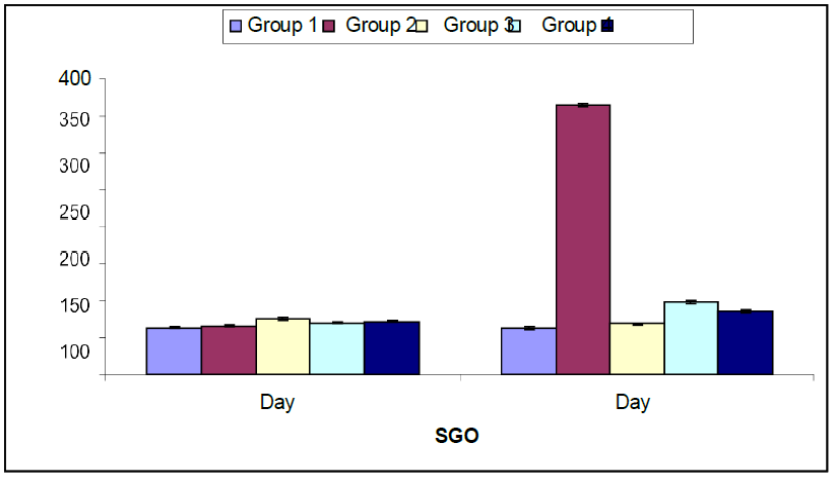


Fig No. 5: Influence of *Rhododendron ferrugineum* extract on SGOT in paracetamol inducedhepatotoxicity.

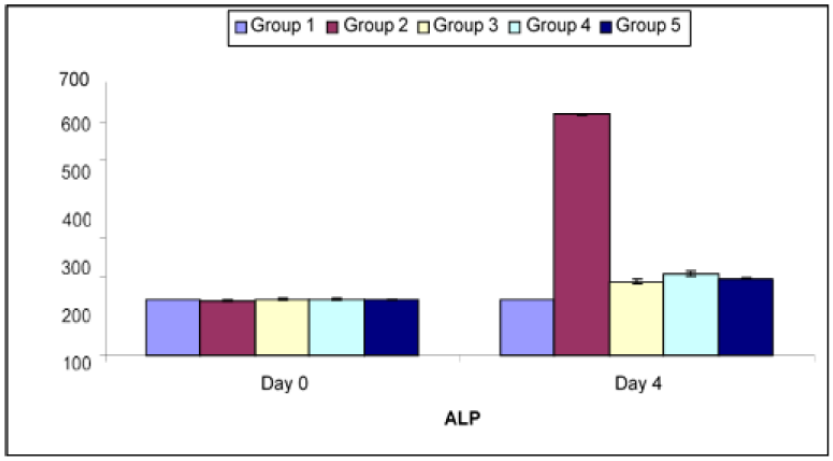


Fig No. 6: Influence of *Rhododendron ferrugineum* extract on ALP in paracetamol inducedhepatotoxicity.

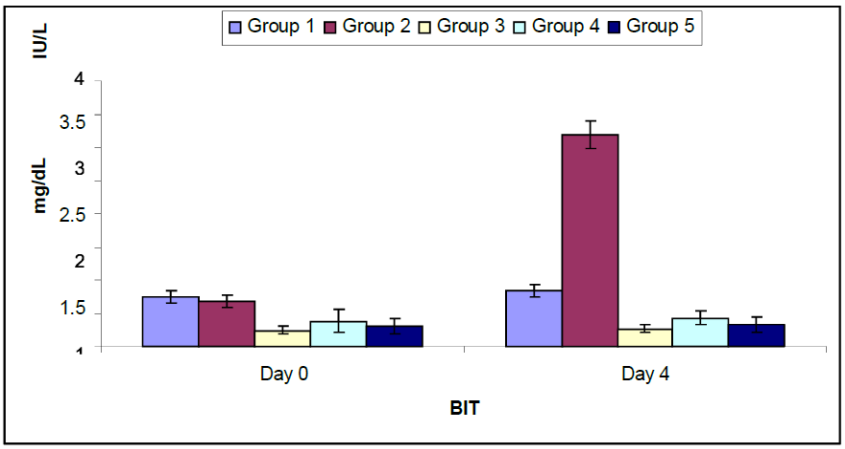


Fig No. 7: Influence of *Rhododendron ferrugineum* extract on BIT in paracetamol inducedhepatotoxicity.

Alcohol induced hepatotoxicity

i) Alanine aminotransperase levels (ALT or SGPT)

The dose of 3.76 g/kg body weight of ethanol induced significant increase in serum SGPT levels with an increase of 69.06% (44.55 IU/L to 144.03 IU/L) compared to normal control where theincrease was 0.92% (42.88 IU/L to 43.28 IU/L). Ethanol induced serum rise of SGPT was protected by 100mg/kg body weight dose of Silymarin and 300mg/kg body weight dose of aqueous and successive extract of *Rhododendron ferrugineum*. The rise was only 16.82% (40.81 IU/L to 49.06 IU/L), 17.32% (42.96 IU/L to 51.96 IU/L) and 41.01% (42.56 IU/L to 72.15 IU/L) respectively after 25 days treatment.

The results are given in table no. from 14 to 24 and Fig. 8 and 9.

ii) Aspartate aminotransferase levels (AST or SGOT)

The dose of 3.76 g/kg body weight of ethanol induced significant increase in serum SGOT levels with an increase of 72.73% (64.33 IU/L to 235.88 IU/L) compared to normal control where the increase was 1.2% (62.2 IU/L to 62.96 IU/L). Ethanol induced serum rise of SGOT was protected by 100mg/kg body weight dose of Silymarin and 300mg/kg body weight dose of aqueous and successive extract of *Rhododendron ferrugineum* . The rise was only 5.38% (66.45 IU/L to 70.23 IU/L), 7.75% (65.06 IU/L to 70.53 IU/L) and 19.05% (66.46 IU/L to 82.1 IU/L) respectively after 25 days treatment.

The results are given in table no. from 14 to 24 and Fig. 8 and 10.

iii) Alkaline phosphatase levels (ALP)

The dose of 3.76 g/kg body weight of ethanol induced significant increase in serum ALP levels with an increase of 68.42% (139.41 IU/L to 441.46 IU/L) compared to normal control where the increase was 0.11% (145.0 IU/L to 144.83 IU/L). Ethanol induced serum rise of ALP was protected by 100mg/kg body weight dose of Silymarin and 300mg/kg body weight dose of aqueous and successive extract of *Rhododendron ferrugineum* . The rise was only 5.34% (139.2 IU/L to 147.06 IU/L), 8.16% (142.03 IU/L to 154.65 IU/L) and 21.97% (141.96 IU/L to 181.95 IU/L) respectively after 25 days treatment.

The results are given in table no. from 14 to 24 and Fig. 8 and 11.

iv) Bilirubin total levels (BIT)

The dose of 3.76 g/kg body weight of ethanol induced significant increase in serum BIT levels with an increase of 84.21% (0.39 IU/L to 2.47 IU/L) compared to normal control where the increase was 0.38% (0.261 IU/L to 0.26 IU/L). Ethanol induced serum rise of BIT wasprotected by 100mg/kg body weight dose of Silymarin and 300mg/kg body weight dose of aqueous and successive extract of *Rhododendron ferrugineum*. The rise was only 16.98% (0.44 IU/L to 0.53 IU/L), 8.33% (0.52 IU/L to 0.48 IU/L) and 64.21% (0.34 IU/L to 0.95 IU/L) respectively after 25 days treatment.

The results are given in table no. from 14 to 24 and Fig. 8 and 12.

Table No. 14: Basal levels of selected serum biochemical parameters in rats, group-1 on day 0 (N=6).

Parameters	R1 (170g)	R2 (150g)	R3 (180g)	R4 (170g)	R5 (150g)	R6 (160g)	Average ± SEM
SGPT (IU/L)	44.2	41.6	45.2	39.7	42.2	44.4	42.88 ± 0.84
SGOT (IU/L)	60.6	57.7	67.3	63.2	60.1	64.3	62.2 ± 1.42
ALP (IU/L)	148.2	145.6	138.7	146.4	149.3	141.8	145.0 ± 1.70
BIT (mg/dL)	0.19	0.12	0.42	0.17	0.33	0.34	0.26 ± 0.05

Table No. 15: Basal levels of selected serum biochemical parameters in rats, group-2 on day 0 (N=6).

Parameters	R1 (190g)	R2 (160g)	R3 (160g)	R4 (170g)	R5 (150g)	R6 (180g)	Average ± SEM
SGPT (IU/L)	41.9	44.6	47.4	43.8	45.5	44.1	44.55 ± 0.74
SGOT (IU/L)	62.5	64.1	73.2	60.6	63.5	62.1	64.33 ± 1.91
ALP (IU/L)	141.8	138.9	143.0	145.2	134.9	132.7	139.41 ± 1.99
BIT (mg/dL)	0.44	0.38	0.1	0.62	0.7	0.11	0.39 ± 0.11

Table No. 16: Basal levels of selected serum biochemical parameters in rats, group-3 on day 0 (N=6).

Parameters	R1 (180g)	R2 (180g)	R3 (150g)	R4 (170g)	R5 (170g)	R6 (150g)	Average ± SEM
SGPT (IU/L)	40.5	40.1	42.0	40.8	43.1	38.4	40.81 ± 0.73
SGOT (IU/L)	61.4	75.5	60.3	59.8	73.6	68.1	66.45 ± 2.80
ALP (IU/L)	141.9	136.6	141.3	133.1	138.4	143.9	139.2 ± 1.62
BIT (mg/dL)	0.53	0.16	0.40	0.38	0.69	0.52	0.44 ± 0.08

Table No. 17: Basal levels of selected serum biochemical parameters in rats, group-4 on day 0 (N=6).

Parameters	R1 (150g)	R2 (170g)	R3 (150g)	R4 (170g)	R5 (150g)	R6 (160g)	Average ± SEM
SGPT (IU/L)	44.1	41.5	43.7	44.2	45.4	38.9	42.96 ± 0.92
SGOT (IU/L)	63.6	68.7	60.8	63.1	65.3	68.9	65.06 ± 1.22
ALP (IU/L)	136.1	143.6	141.9	145.5	136.3	148.8	142.03 ± 2.06
BIT (mg/dL)	0.36	1.3	0.42	0.66	0.31	0.13	0.52 ± 0.17

Table No. 18: Basal levels of selected serum biochemical parameters in rats, group-5 on day 0 (N=6).

Parameters	R1 (160g)	R2 (160g)	R3 (150g)	R4 (180g)	R5 (150g)	R6 (180g)	Average ± SEM
SGPT (IU/L)	43.9	46.2	39.1	39.6	41.5	45.1	42.56 ± 1.22
SGOT (IU/L)	75.4	63.2	63.9	61.6	69.3	65.4	66.46 ± 2.08
ALP (IU/L)	145.6	138.6	140.1	143.2	142.3	142.0	141.96 ± 0.96
BIT (mg/dL)	0.1	0.39	0.35	0.5	0.16	0.55	0.34 ± 0.08

Table No. 19: Levels of selected serum biochemical parameters in rats, group-1 on 26th day treated with 2% w/v Gum acacia (1ml/kg).

Parameters	R1 (180g)	R2 (150g)	R3 (180g)	R4 (180g)	R5 (160g)	R6 (160g)	Average ± SEM
SGPT (IU/L)	45.8	42.2	45.9	39.0	41.8	45.2	43.28 ± 1.17
SGOT (IU/L)	62.5	61.1	65.3	64.3	60.2	64.4	62.96 ± 0.99
ALP (IU/L)	147.3	145.2	138.7	146.5	148.2	143.1	144.83 ± 1.55
BIT (mg/dL)	0.21	0.19	0.36	0.21	0.28	0.31	0.26 ± 0.03

Table No. 20: Levels of selected serum biochemical parameters in rats, group-2 on 26th day treated with Ethanol (3.75g/kg).

Parameters	R1 (170g)	R2 (160g)	R3 (160g)	R4 (160g)	R5 (150g)	R6 (170g)	Average ± SEM
SGPT (IU/L)	125.6	144.8	168.2	133.5	153.1	139.0	144.03 ± 3.54
SGOT (IU/L)	210.4	263.7	238.4	215.5	228.7	258.6	235.88 ± 7.45
ALP (IU/L)	432.3	477.5	423.1	410.8	445.2	458.9	441.46 ± 9.98
BIT (mg/dL)	2.8	2.1	1.81	3.02	3.42	1.7	2.47 ± 0.29

Table No. 21: Levels of selected serum biochemical parameters in rats, group-3 on 26th day treatedwith Ethanol (3.75g/kg) + Silymarin (100mg/kg).

Parameters	R1 (180g)	R2 (170g)	R3 (150g)	R4 (170g)	R5 (170g)	R6 (150g)	Average ± SEM
SGPT (IU/L)	49.5	53.6	44.7	48.3	55.6	42.7	49.06 ± 1.88
SGOT (IU/L)	70.3	73.3	63.8	68.0	71.9	74.1	70.23 ± 1.66
ALP (IU/L)	146.1	146.2	153.2	142.9	137.8	156.4	147.06 ± 2.76
BIT (mg/dL)	0.5	0.65	0.48	0.66	0.4	0.57	0.53 ± 0.05

Table No. 22: Levels of selected serum biochemical parameters in rats, group-4 on 26th day treated with Ethanol (3.75g/kg) + *Rhododendron ferrugineum* aqueous extract (300mg/kg).

Parameters	R1 (160g)	R2 (170g)	R3 (150g)	R4 (180g)	R5 (160g)	R6 (160g)	Average ± SEM
SGPT (IU/L)	52.8	55.7	52.1	50.6	48.2	52.4	51.96 ± 1.02
SGOT (IU/L)	68.3	71.4	64.5	71.3	73.1	74.6	70.53 ± 1.48
ALP (IU/L)	149.3	152.8	151.7	160.3	151.2	162.6	154.65 ± 2.17
BIT (mg/dL)	0.4	0.71	0.49	0.6	0.42	0.33	0.48 ± 0.06

Table No. 23: Levels of selected serum biochemical parameters in rats, group-5 on 26th day treated withEthanol (3.75g/kg) + *Rhododendron ferrugineum* successive extract (300mg/kg).

Parameters	R1 (160g)	R2 (160g)	R3 (150g)	R4 (180g)	R5 (150g)	R6 (180g)	Average ± SEM
SGPT (IU/L)	73.1	72.8	68.7	68.2	73.5	76.6	72.15 ± 1.27
SGOT (IU/L)	90.0	78.5	76.6	74.5	85.8	87.2	82.1 ± 2.60
ALP (IU/L)	188.4	176.9	180.2	183.5	182.4	180.3	181.95 ± 1.54
BIT (mg/dL)	0.61	0.92	1.02	1.9	0.59	0.68	0.95 ± 0.20

Table No. 24: Average percentage change in selected serum biochemical parameters in rats in Ethanol induced hepatotoxicity.

Group	Treatment	SGPT	SGOT	ALP	BIT
1.	Gum acacia (1ml/kg)	0.92±0.79	1.2±0.8	0.11±0.24	0.38±3.83
2.	ETH (3.76g/kg)	69.06±0.8***	72.73±1.06***	68.42±1.09***	84.21±3.01***
3.	ETH (3.76g/kg) + SIL(100mg/kg)	16.82±3.19***	5.38±1.81***	5.34±1.31***	16.98±1.09***
4.	ETH (3.76g/kg) + *R. ferrugineum* (aq.) (300mg/kg)	17.32±3.04***	7.75±0.97***	8.16±1.22***	8.33±4.77***
5.	ETH (3.76g/kg) + *R. ferrugineum* (successive) (300mg/kg)	41.01±1.01***	19.05±1.44***	21.97±0.15***	64.21±3.29***

***P < 0.001 significant when compared group-1 with group-2 and group-2 with group-3, 4 and 5. ETH-Ethanol, SIL-Silymarin, *R. ferrugineum* (aq.)-*Rhododendron ferrugineum* aqueous extract, *R. ferrugineum* (successive)- *Rhododendron ferrugineum* succesive extract.

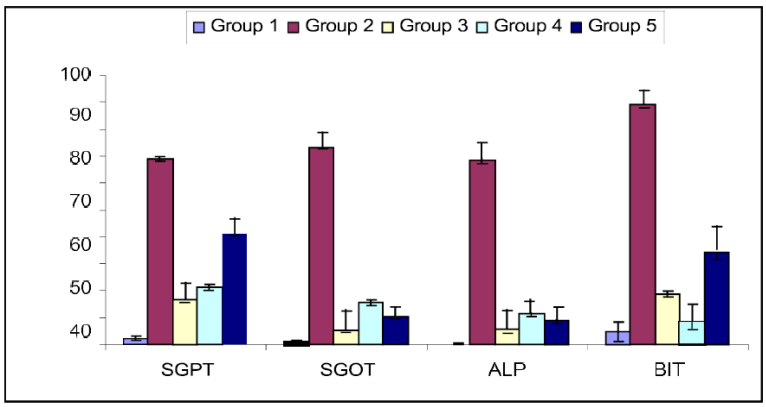


Fig No. 8: Average percentage change in selected serum biochemical parameters in ETH inducedhepatotoxicity in rats.

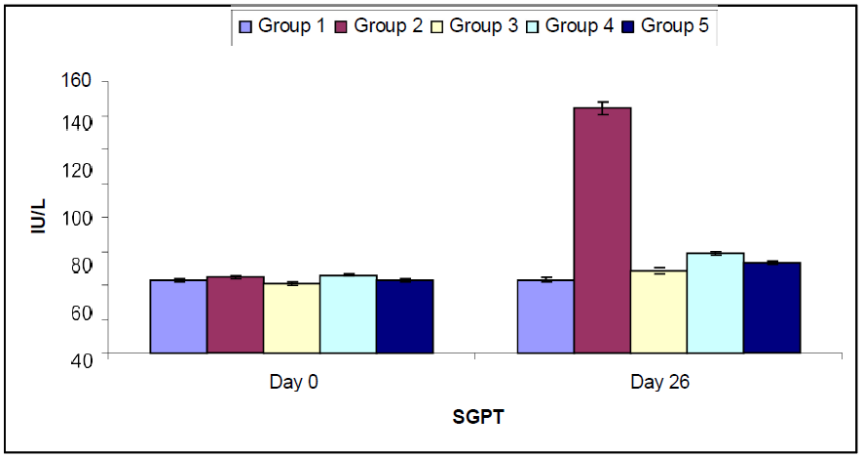


Fig No. 9: Influence of *Rhododendron ferrugineum* extract on SGPT in ETH induced hepatotoxicity.

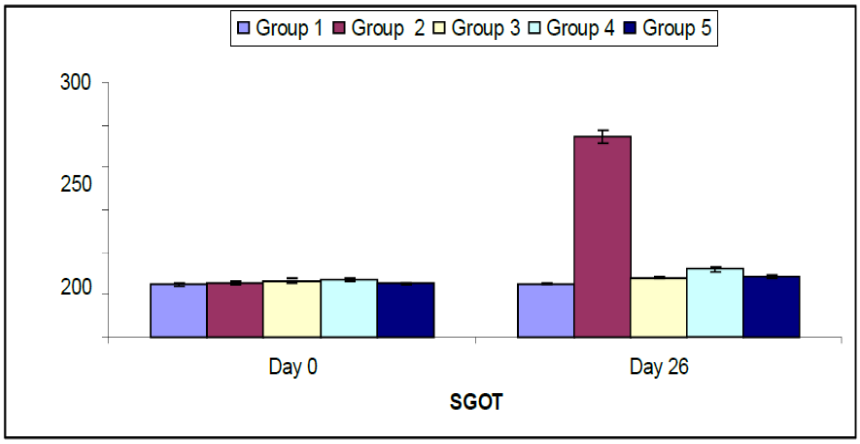


Fig No. 10: Influence of *Rhododendron ferrugineum* extract on SGOT in ETH inducedhepatotoxicity.

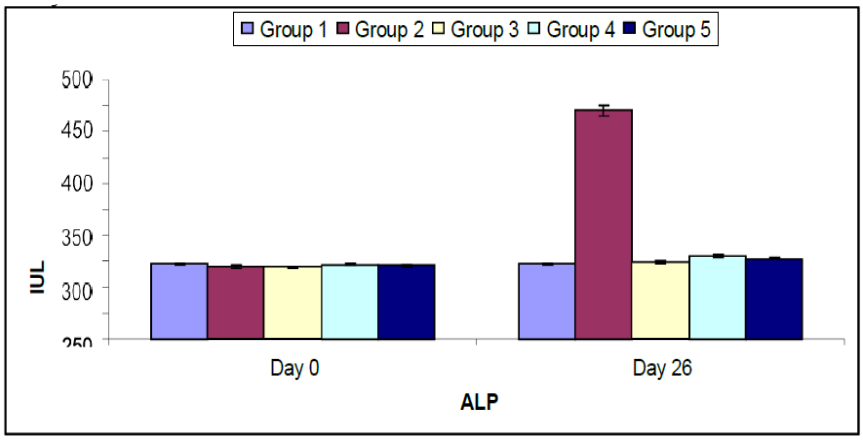


Fig No. 11: Influence of *Rhododendron ferrugineum* extract on ALP in ETH induced hepatotoxicity.

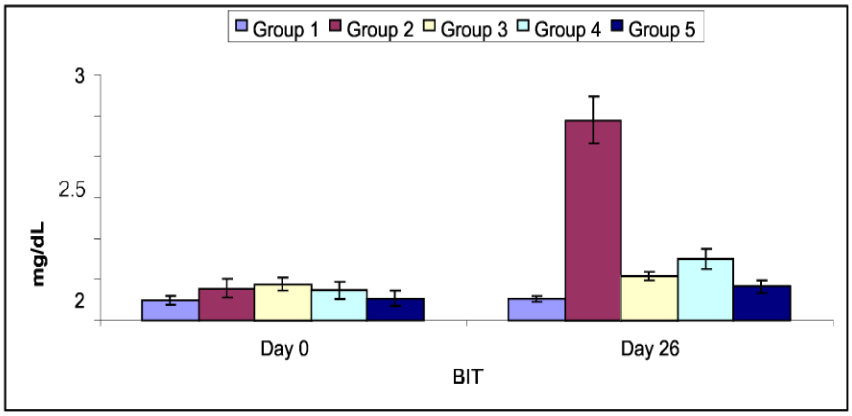


Fig No. 12: Influence of *Rhododendron ferrugineum* extract on BIT in ETH induced hepatotoxicity.

Antioxidant Activity

Influence of 70% successive extract of *Rhododendron ferrugineum* leaves on GSH levels in paracetamol induced hepatotoxicity in rats

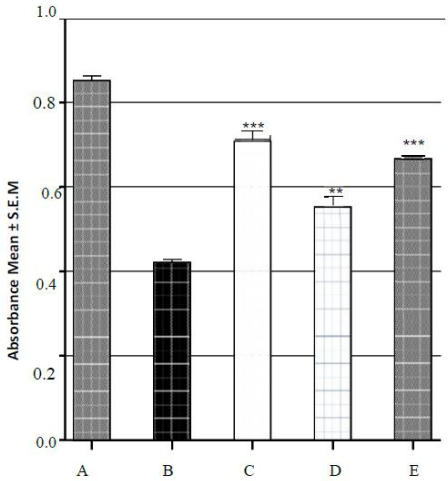
There is a marked depletion of GSH levels in paracetamol treated group. 100 mg/kg silymarin has increased it by 71.72%, 70% successive extract has shown a dose dependent increase in the levels of GSH, 400 mg/kg 70% successive extract has increased the GSH levels by 58.20% which is almost equal to standard silymarin.

The results are summarized in Table 25 and graphically depicted in Fig. 13

Table 25: Effect of 70% successive extract of *Rhododendron ferrugineum* leaves on tissue GSH levels inParacetamol induced hepatotoxicity.

Treatment	Absorbance Mean ± SEM	Percentage Increase
Normal Control (1ml vehicle)	0.848 ± 0.01	-
Paracetamol Treated (2ml/kg s.c)	0.422 ± 0.007	-
Paracetamol + Silymarin (2ml/kg s.c. + 100 mg/kg p.o)	0.724 ± 0.005***	71.72
Paracetamol + 70% successive extract (2ml/kg s.c. + 200 mg/kg p.o)	0.551 ± 0.005**	26.35
Paracetamol + 70% successive extract (2ml/kg s.c. + 400 mg/kg p.o)	0.667 ± 0.004***	58.20

Values are the mean ± S.E.M. of six rats/treatment. Significance ***P< 0.001, **P< 0.01, *< 0.05, compared to Paracetamol treatment.



Groups

A: Normal Control (1 ml vehicle)

B: Paracetamol treated (2 gm/kg p.o.).)

C: Paracetamol + Silymarin (2 gm/kg p.o. + 100mg/kg p.o.)

D: Paracetamol + 70% successive extract (2 gm/kg p.o. + 200mg/kg p.o.)

E: Paracetamol + 70% successive extract (2 gm/kg p.o. + 400mg/kg p.o.)

Fig No. 13: Effects of 70% successive extract of *Rhododendron ferrugineum* leaves on in vivo GSH levels in paracetamol induced hepatotoxicity.

Influence of 70% successive extract of Rhododendron ferrugineum leaves on lipid peroxidation levels in paracetamol induced hepatotoxicity in rats

There is a dose dependent inhibition of *in-vivo* lipid peroxidation by both doses of 70% successive extract. 100 mg/kg of silymarin has 62.11% inhibition where 400 mg/kg of 70% successive extract of the leaves has 58.76% inhibition, which is almost equal to standard silymarin.

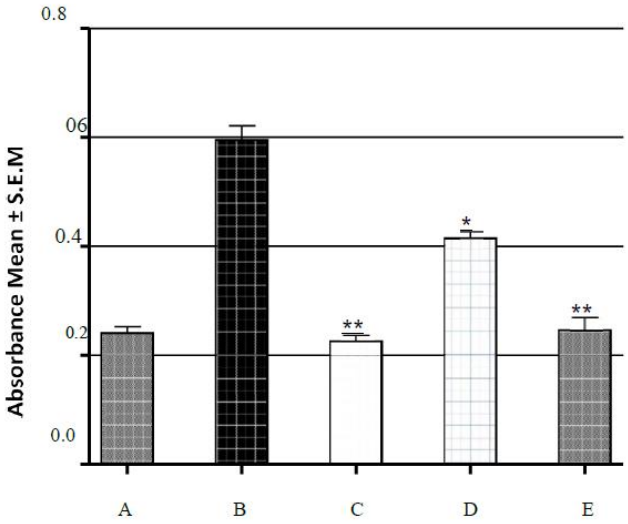
The results are summarized in Table 26 and graphically depicted in Fig. 14.

Table No. 26: Effect of 70% successive extract of *Rhododendron ferrugineum* leaves ontissue lipidperoxidation levels in Paracetamol induced hepatotoxicity.

Treatment	Absorbance Mean ± SEM	Percentage Increase
Normal Control (1ml vehicle)	0.241 ± 0.029	-
Paracetamol Treated (2ml/kg s.c)	0.595 ± 0.026	-
Paracetamol + Silymarin (2ml/kg s.c. + 100 mg/kg p.o)	0.224 ± 0.010***	62.11
Paracetamol + 70% successive extract (2ml/kg s.c. + 200 mg/kg p.o)	0.415 ± 0.012**	30.31

Paracetamol + 70% successive extract (2ml/kg s.c. + 400 mg/kg p.o)	0.246 ± 0.024***	58.76

Values are the mean ± S.E.M. of six rats/treatment. Significance ***P< 0.001, **P< 0.01, *< 0.05, compared to Paracetamol treatment



Groups

A: Normal Control (1 ml vehicle)

B: Paracetamol treated (2 gm/kg p.o.)

C: Paracetamol + Silymarin (2 gm/kg p.o. + 100mg/kg p.o.)

D: Paracetamol + 70% successive extract (2 gm/kg p.o. + 200mg/kg p.o.)

E: Paracetamol + 70% successive extract (2 gm/kg p.o. + 400mg/kg p.o.)

Fig No. 14: Effects of 70% successive extract of *Rhododendron ferrugineum* leaves on lipidperoxidationlevels in paracetamol induced hepatotoxicity.

DISCUSSION

Acute toxicity study

LD_{50} studies were conducted in albino mice by using OECD guidelines No-423 for *Rhododendron ferrugineum* extracts. It was found that the extracts even at 2000 mg/kg dose had not shown any mortality confirming its practically non-toxic nature.

Hepatoprotective study

Liver participates in a variety of metabolic activities and contain a lot of enzymes and in this process it could be injured by many toxicants, chemicals and drugs. In our study, ethanol and paracetamol were used as a hepatotoxicants to induce liver damage.

Paracetamol induced hepatotoxicity

Liver diseases arise due to liver cell damage and proliferation of fibrous tissue. The damage certainly becomes extensive and normal structure of the liver gets distorted and its function becomes impaired. Reactive oxygen species (ROS), such as superoxide anion, hydroxyl radical, and hydrogen peroxide, have a causal relationship with oxidative stress. Over production of ROS can aggravate the oxidative stress and the result is a unifying mechanism of injury that occurs in many developments of clinical disease processes, such as heart disease, diabetes, liver injury, cancer, aging, etc.

Paracetamol induced hepatotoxicity was used by several workers as model for screening of hepatoprotective agents. The dose used for induction of hepatotoxicity by different workers was found to vary. In the present study 2 g/kg body weight paracetamol was used as toxicant for 3 days in male albino rats of Wistar strain.

Paracetamol (acetaminophen) is a safe, effective and widely used analgesic– antipyretic drug however an overdose can induce severe hepatotoxicity in experimental animals and humans. Excessive administration of paracetamol can cause over production of ROS during formation of N-acetyl-p-benzoquinoneimine (NAPQI) by cytochrome P450. This mechanism has been suggested to participate in the development of oxidative stress and injury in paracetamol induced hepatotoxicity. The hepatic injury leads to elevation of serum levels of SGOT (AST), SGPT (ALT), ALP and BIT in rats and are used as markers for assessing toxicant effect and also hepatoprotective agents. These parameters are also used clinically for assessing hepatic damage and the effectiveness of therapeutic agents given. Hence paracetamol induced hepatotoxicity in rat represent an adequately established experimental model of liver cirrhosis (hepatic degeneration) in man and it is used for the screening of hepatoprotective drugs.

The main aim of any medication in the treatment of liver disorders is to prevent degeneration of hepatocytes and associated metabolic abnormalities and promote regeneration of hepatic cells. Inpresent study the hepatoprotective activity of *Rhododendron ferrugineum* extract was evaluated in paracetamol induced liver toxicity by the above mentioned biochemical parameters. Acute administration of paracetamol produced marked elevation of the serum levels of the parameters in treated rats (Group II) compared to that of the control group (Group I). Treatment with *Rhododendron ferrugineum* aqueous extract produce significant prevention in paracetamol induced rise

in the above biochemical parameters. Silymarin at dose of 100 mg/kg body weight significantly prevented such rise in study. The effect of Silymarin was found to be in between theeffect of selected doses of *Rhododendron ferrugineum* aqueous extract.

Ethanol induced hepatotoxicity

Recent study indicates that oxidative stress is involved in the pathogenesis of liver diseases including drug induced hepatic damage, alcohol hepatitis and viral hepatitis or ischaemic liver injury.

Ethanol induced hepatotoxicity was used by several workers as model for screening of hepatoprotective agents. The dose used for induction of hepatotoxicity by different workers was found to vary. In the present study 3.76 g/kg body weight Ethanol was used as toxicant for 25 daysin male albino rats of Wistar strain.

Increased formation of lipoperoxides, conjugated diens and malondialdehyde and reduced levels of antioxidants like vitamin E and glutathione in the tissues have been demonstrated in experimental animals administered with ethanol as well as in alcoholic human subjects. The increased level of AST, ALT, ALP, and bilirubin is conventional indicator of liver injury. Oxidative stress is one major factor in etiology of ethanol injury, mainly by Kupffer cell derived reactive oxygen species (ROS) and Ethanol activates Kupffer cells primarily through the action of a substance called endotoxin, which is released by certain gram-negative bacteria present in the intestine, activates Kupffer cell to generates ROS and pro inflammatory cytokines (TNF alpha, IL- 1), both of them can lead to liver damage.

In present study the hepatoprotective activity of *Rhododendron ferrugineum* aqueous and benzene extracts were evaluated in ethanol induced liver toxicity by the above mentionedbiochemical parameters. Acute administration of ethanol produced marked elevation of theserum levels of the parameters in treated rats (Group II) compared to that of the control group (Group I). Treatment with *Rhododendron ferrugineum* aqueous extract 300mg/kg produces significant prevent the ethanol induced rise in the parameters compared to *Rhododendron ferrugineum* benzene extract 300 mg/kg. Silymarin at 100 mg/kg body weight significantly prevented such rise in study.

Antioxidant activity

Role of free radicals has been implicated in the causation of several diseases such as liver cirrhosis, atherosclerosis, cancer, aging, arthritis, diabetes etc and the compounds that can scavenge free radicals have great potential in ameliorating these diseases processes.

Out of these extracts 70 % successive extract was found to be better than aqueous, hence this extract was selected for further studies. The 70% successive extract was subjected to *in- vivo* antioxidant activity. Effect on GSH and lipid peroxidation content upon paracetamol induced GSH and lipid peroxidation depletion. Per-treatment with 70% successive extract has increased the depleted GSH and decresed lipid peroxidation levels during paracetamol challenges in a dose dependent manner.

The lipids peroxidation is directly proportional to the tissue damage and extent of GSH depletion is also the indicator of severity of tissue damage. Therefore this extract was further subjected to screen for organ protective properties against various experimentally induced organ toxicities, e.g. hepatotoxicity.

CONCLUSION

- The present study is aimed to explore the hepatoprotective and antioxidant activity of *Rhododendron ferrugineum* succesive extracts in rats.
- The acute toxicity studies were conducted as per OECD guidelines 423 for 2000 mg/kg dose of *Rhododendron ferrugineum* extracts. It was found that the extracts even at 2000 mg/kg dose had not shown any mortality confirming it's practically non-toxic in nature.
- The *Rhododendron ferrugineum* extracts has hepatoprotective activity. *Rhododendron ferrugineum* extracts possesses a statistically significant hepatoprotective activity as evidenced by prevention in the elevation of serum biochemical parameters like SGPT, SGOT, ALP & BIT in ethanol and paracetamol induced hepatotoxicity in rats which were compared with standard Silymarin drug.
- The *Rhododendron ferrugineum* extracts has antioxidant activity. 70% of successive extract of the leaves of *Rhododendron ferrugineum* has demonstrated dose dependent increase in the depletion tissues GSH and decrease lipid peroxidation levels. The quantities of the *Rhododendron ferrugineum* extracts needed for the *in vivo*inhibition (IC50) of reduced glutathione and lipid peroxidation possesses significant antioxidant activity.

ACKNOWLWDEMENT

We would like to express our sincere gratitude to all those who contributed to the completion of this research. We are thankful for the support, guidance, and insights provided by our advisors. We also acknowledge the college that made this research possible. Lastly, we extend our appreciation to the study participants and all individuals who generously shared their time and expertise. Without their contributions, this research would not have been possible.

REFERENCES

1. Zahid Zaheer, Ajinkya Konale G, Khuman Patel A, Subur Khan, Rana Ahmed Z. Comparative phytochemical screening of flowers of *Rhododendron ferrugineum* and *plumeria rubra*. Asian Journal of Pharmaceutical and Clinical Research, 2010; 3(4): 88-89.
2. Sharma Yashashwini, Hegde RV, Venugopal CK. Health and nutrition from ornamentals. International Journal of Research in Ayurveda and Pharmacy, 2011; 2(2): 375-382.
3. Kumar RS, Sivakumar T, Sunderam RS, Gupta M, Mazumdar UK, Gomathi P, Rajeshwar Y, Saravanan S, Kumar MS, Murgesh K, Kumar A. Antioxidant and antimicrobial activities of *Bauhinia racemosa* L. stem bark. Brazilian Journal of Medicaland Biological Research, 2005; 38: 1015-1024.
4. Dubey NK, Kumar R, Tripathi P. Global promotion of herbal medicines. India's opportunity. Current Science, 2004; 86: 37-41.
5. Ashif Iqubala, Mohammad Kashif Iqubalb and Syed Ehtaishamul Haquea. Experimental hepatotoxicity Inducing agents: A Review. International Journal of Pharmacological Research, 2016; 6(11): 325-335.
6. http://herbarium.freehostia.com/plant.php/Rhododendron+ferrugineum/Rhododendron
7. Guntupalli M, Chandana V, Palpu P, Annie SI. Hepatoprotective effects of rubiadin, a major constituent of *Rubia cordifolia* Linn Journal of Ethnopharmacol, 2006; 103: 484- 490.
8. Chatterjee TK. Medicinal plants with hepatoprotective properties. In: Herbal Options. 3rd Edition, Calcutta: Books and Allied (P) Ltd., 2000; 135-137.
9. M. Sudheer, B. Suresh Babu, K. Divya, K. V. Himavani, M. Siva Prakash, T. V. Sai Srinivas. Journal of Pharmacy Research, 2011; 4(3): 647-649.

10. Gonzalez FJ. The molecular biology of cytochrome p450s. Journal of Pharmacology, 1998; 40: 243-288.
11. Rajesh MG, Latha MS. Preliminary evaluation of the anti hepatotoxic activity of Kamilari, a polyherbal formulation. J Ethnopharmacol, 2004; 91: 99–104.
12. Girish S Achliya, Sudhir G Wadodkar, Avinash K Dorle. Evaluation of hepatoprotective effect of *Amalkadi Ghrita* against carbon tetrachloride- induced hepatic damage in rats. J Ethnopharmacol, 2004; 90: 229–32.
13. Gulcin I, Buyukokuroglu ME, Oktay M, Kufrevioglu OI. On the *in- vitro* antioxidant properties of melatonin. Journal of Pineal Research, 2002; 33: 167-171.
14. Halliwell B, Gutteridge JM. Free Radicals in Biology and Medicine. *3rd edition,* UK; Oxford University Press Oxford, 1999; *936*.
15. Yildirim A, Oktay M, Kara AA, Algur OF, Bilaloglu V. Comparison of antioxidant and antimicrobial activities of tilia(*Tilia argenta* Desf Ex DC), sage(*Salvia triloba* L.) and black tea(*Camellia scinensis*) extracts. Journal of Agricultural and Food Chemistry, 2000; 48: 5030-5034.
16. Halliwel B, Guteridge JMC, Carrol EC. Free radicals, antioxidants and human diseases: where are we now? J Lab din Med., 1994; 119: 598-619.
17. Ohkawa H, Ohish N, yagi K. Assay for lipid peroxides in animal tissues by thiobarbituricacid. Anal Biochem, 1979; 95: 351-358.
18. Abeer Hanafy, Hibah. M. Aldawasi, Jihumm Badrm Amany K. Ibharm and Senam El Sayed Abdel-Hady, "Evaluation of hepato-protective activity of *Adansonia Digitata* extract on acetaminophen induced hepatotoxicity in rats" HPCECA, 2016; I: 7.
19. Viswanath. J, Preetham GB, Anil KB, Swark RJ, A review on hepato-protective plants, IJDDR, 2012; 4: 1-8.