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Research

Hepatoprotective evaluvation of *galanga (alpinia* officinarum) rhizome extract against paracetamol induced hepatotoxicity in rats

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Abstract

Herbal drugs classification system represent as an important system of medicine for the treatment of a wide array of diseases. The medicinal plants from India provide a diverse source for health care moieties in order to prevent different pathological states. *Alpinia officinarum,* known as lesser galangal. *Alpinia officinarum*, a plant from ginger family.The paracetamol 640mg/kg BW P.O induced injuries of liver in animal are mostly used to screen out the hepatoprotective effect of extract. In the present study total phenolic and flavonoid contents, *in vitro* antioxidant, and *in vivo* hepatoprotective (on paracetamol induced intoxication in experimental male Sprague Rats) Potentials of the *Alpinia officinarum* rhizome ethanolic extract were determined. For the identification of possible phytochemical test. Glycoside, Phenol, Tannins, Steroids, Flavonoids were identified *Alpinia officinarum* extract at dose of 200mg/kg BW P.O and 400 mg/kg BW P.O were given for 14days to paracetamol intoxicated rats and observed results were compared with standard silymarin 50 mg/kg. The level of lever enzymes like aspartate aminotransferase, alanine aminotransferase, alkaline phosphate, total protein and total bilirubin. Furthermore histopathological analysis of the liver tissues of control and treated groups also confirmed hepatoprotective effect of the *Alpinia officinarum* which was most probably due to its high antioxidant phenolic and flavonoids phytoconstituents.

Keywords: *Alpinia officinarum,* , Hepatoprotective, Pharacetamol, Silymarin.

INTRODUCTION

Global Epidemiology of Chronic Liver Disease: (Shantan cheemerla, M.D *et.al.* 2021)

Cirrhosis is a leading cause of mortality and morbidity across the world. It is 11th leading cause of death. Historically, viral hepatitis has been the leading etiology for chronic liver disease. However, improved prevention strategies (in the case of hepatitis B) and treatment (in the case of hepatitis C) have led to improving chronic liver disease trends. This is reflected in global declines that have been observed in liver disease mortality rates over the past 30 years. Meanwhile, obesity and alcohol consumption, which are common and increasing in many parts of the world, have become key liver disease risk factors. They are anticipated to drive chronic liver disease epidemiology going forward.

Hepatotoxicity

Liver diseases are the major medical problems faced by the people all over the world[30]. About 20,000 deaths occur every year due to liver disorders (Muhammed Shanavas VK *et.al.,*). In Africa and in Asia, the main causes of liver diseases are viruses and parasitic infections, whereas in Europe and in North America, a major cause is alcohol abuse[30]. Liver diseases are mainly caused by toxic chemicals, excessive intake of alcohol, infections and autoimmune disorders (Bhawna S *et.al.,* 2009). Hepatotoxicity due to drug appears to be a common contributing factor. Liver is expected not only to carryout physiological functions but also to protect against the hazardous of harmful drugs and chemicals (Hegde K*et.al.,* 2010) Drug induced chemical injury is responsible for 5% of all hospital admissions and 50% of all acute liver failures. More than 75% of cases of immunological reaction of drugs leading to liver transplantation or death. Hepatotoxicity mainly implies chemical-driven liver damage. Certain drugs when taken in overdose and sometimes even when administered within therapeutic ranges may injure many organs. Some chemical agents including those that are used in laboratories (Ccl4, paracetamol) and industries (Lead, arsenic) and natural chemicals (microcystine, aflatoxins) and herbal remedies (cascara sagrada, ephedra) can also cause hepatotoxicity. Chemicals which cause liver injury are collectively known as hepatotoxins (Gujrati V *et.al.,* 2007).

Plant Profile

Alpinia officinarum

Synonym : *A.alba, A.bifida, A.carnea*
Family : Zingiberaceae
Scientific name : *Alpinia galangal (L.) Willdenow*
Common name : *Galangal, Greater galangal, Java galangal*
Parts used : Rhizome

Fig 1: Alpinia Officinarum

Vernacular Names

Sanskrit : *Dharnula tikshra mula, kulanjana, Mahabhara vachu*
English : *Great galangal, Lesser galangal*
Hindi : *Bara khulanjan, kulanjan*
Tamil : *Chitharathai*
Kannada : *Dhumarashmi*
Malayalam : *Aratta*
Telugu : *Dumparaastramu*

Plant Taxonomy

Kingdom : *Plantae*
Division : *Mangoliophyta*
Class : *Liliopsida*
Subclass : *Zingiberidae*
Order : *Zigiberales*
Family : *Zingiberaceae*
Subfamily : *Alpiniodeae*
Tribe : *Alpinieae*

Genus : *Alpinia*
Species : *Alpinia galangal*

MATERIALS AND METHODS

Collection And Authentication Of Plant

The dried rhizomes of the plant *Alpinia officinarum* was collected from Chennai, Tamil Nadu, India on 2022 and it was botanically identified and authenticated by Dr.V.Aravindhan, Assistant professor, Department of Botany, Kongunadu arts and Science College, Coimbatore – 641 029.

Phytochemical Studies

Presence of phenolic compounds, Carbohydrates, Flavanoids, Glycosides, Saponins, Alkaloids, Anthroquinones, Proteins and Tannins were qualitatively analyzed. The steps involved in the qualitative phytochemical analysis are given below,

Preparation Of Extract

Step 1: The rhizomes were collected, washed with distilled water thoroughly and shade dried then powdered.
Step 2: The coarse powder was defatted by using petroleum ether at 50^{o}C in soxhlet extractor.
Step 3: Then the powder was removed and dried until no trace of pet ether was observed.
Step 4: The powder was packed again in soxhlet apparatus and extracted with ethanol.
Step 5: The extract was concentrated using rotary evaporator and used for further studies.

The individual extract was subjected to the qualitative phytochemical for the presence of some chemical constituents. The detection of these active principles in medicinal plants plays a strategic role in the phytochemical investigation of crude drugs and extracts and is very important in regard to their potential pharmacological effects. These tests facilitate the quantitative estimation and qualitative separation of pharmacologically active chemical compounds and subsequently may lead to the drug discovery and development. Phytochemical test were carried out adopting standards procedure.

Pharmacological screening (wahed tb *et.al.,*2019).

Experimental Protocol

Guideline	: OECD – 423
CPCSEA Reg. No	: JKKMMRFCP/IAEC/2022/006
Animal	: Sprague Dawley rats
Number of animals	: 30 animal (5 groups each groups 6 animal)
Sex	: Male
Route of administration	: Paracetamol p.o, extract p.o, silymarin ,p.o.
Duration	: 14 days observation
Blood collection	: Needed (cardiac puncture), end of 14 Days
Anesthesia	: Thiopental sodium 120 mg/kg
Sacrifice	: On day 14 after oral administration

Evaluvation of hepatoprotective activity

Animals: (Janbaz KH *et.al.,*1995).

Healthy adult male Sprague dawley rats (10 to 12 week of age and 200±50 gram body weight) were procured and randomly assigned to 5 groups, each containing 6 animals in polypropylene cages layered with husk and maintained in a controlled room at a temperature (22±3°C) and light (12 hours light/dark cycle). Animals were allowed free access to water and standard pellet diet. Animals were cared in accordance with the "Guide for the care and use of laboratory animals" and study was conducted in accordance with CPCSEA. All animal experiments were conducted during the present study got prior permission from Institutional Animal Ethics Committee (IAEC approved) and following the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) constituted by the Animal Welfare Division, Government of India (Reg.No:- JKKMMRFCP/IAEC/2022/006).

Methodology

Paracetamol dose was designated according to the findings of Janbaz KH *et.al.,*1995. All of the oral administration were given in the morning between 09:30 am and 10:30 am and continued for 14 consecutive days. During experimentation, the rats were observed daily for any unusual behaviour and death while body weight change were observed on a weekly basis. After 14 days of treatment, rats in each group were injected with a deeply anesthetized drug (Thiopental sodium 120 mg/kg and sacrificed. After, blood samples and liver tissues were collected for biochemical and histopathological examination.(Wahed TB *et.al.,*2019).

Table 1: Animal Grouping

Groups	Animal
control group 1	Animal received 0.05% tween 80 dissolved in 0.9% NaCl solution at 0.5 ml.
Negative control group 2	Animal received paracetamol alone at 640 mg/kg BW (P.O) dissolved in the vehicle.
Low dose EEAO group 3	Animal received ethanolic extract of *alpinia officinarum* 200mg/kg + paracetamol (640mg/kg BW,P.O) dissolved on the vehicle
High dose EEAO group 4	Animal received ethanolic extract of *alpinia officinarum* 400mg/kg + paracetamol (640mg/kg BW,P.O) dissolved on the vehicle
Silymarin is a standard dose group 5	Animal received silymarin at a dose of 50mg/kg BW (P.O) and paracetamol (640mg/kg BW, P.O) dissolved on the vehicle.

Evaluation of hepatoprotective efficacy

Efficacy of the extract was assessed using biochemical marker enzymes such as,

- SGPT
- SGOT.
- ALP (Alkaline Phosphatase).
- Total Protein.
- Total Bilirubin.
- Change in body weight.

Sample collection and biochemical assay

The blood collection after 14 days. The blood samples obtained were collected into plain sample tubes and centrifuged at 2000 rpm for 5 minutes to separate serum. Serum was carefully collected and kept in eppendrof tubes for the determination of the biochemical parameters.

Histopathology Study: (Palanivel MG *et.al.,*2008)

- All rats were sacrificed at the end of the study period and subjected to detailed gross necropsy. After gross observation, liver was collected and fixed in 10% Neutral Buffer Formalin.
- Trimming: Tissues were trimmed from all the lobes of liver.
- Processing: Processing is done with the help of Automated Tissue Processor (ATP) (Leica ASP 300) for 16 hours.
- Embedding: Processed tissues were embedded in paraffin with the help of paraffin embedding station (Leica EG 1150 H).
- Sectioning: Initially blocks were trimmed at 25 microns and then sectioned at 4 microns with the help of semi-automatic Microtome (Leica RM 2245).
- Staining: Slides were stained by H&E stain at Multistainer (Leica ST 5020).
- All the H&E stained slides were observed for pathological findings.

RESULTS

Percentage yield ethanolic extract of the rhizome of alpinia offcinarum

S.No	Solvent	Method Of Extraction	Physical Nature	Colour	Yield % W/W
1	Ethanol	Soxhlet extraction	Semisolid	Dark brown	7.2

Phytochemical Screening Of Ethanolic Extract Of *A.Officinarum*

Phytoconstituents	Presence Or Obsence – In The Extract Of Rhizome
Alkaloid	-
Glycoside	+
Phenol	+
Carbohydrates	-
Tannins	+

Steroids	+
Flavonoids	+
Saponins	-
Proteins & Aminoacids	+
Terpenoids	+

Note: (+) indicates presence, (-) indicate absence

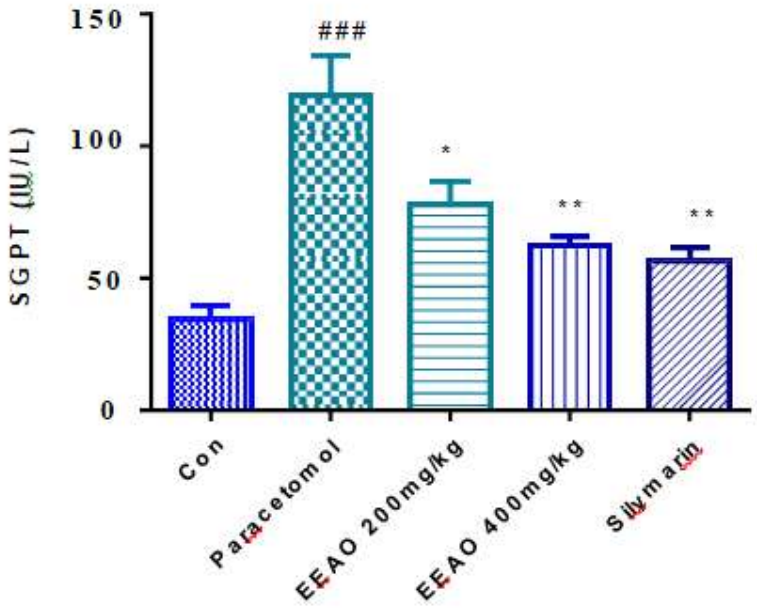
Hepato Protective Activity

Effect of *A.Officinarum* on SGPT Concentration in paracetamol model

The results of biochemical parameters revealed to the alteration of enzyme levels in paracetamol treated group indicating that paracetamol induced damage to the liver. Below the table show that paracetamol causes significant increase in SGPT level from control group 34.67±4.66 IU/L to 119.3 ±14.89### after paracetamol intoxication. Administration of EEAO 200 mg/kg and 400 mg/kg in paracetamol intoxicated rats caused reduction in SGPT level to 78.00±8.71* IU/L and 62.00 ±3.46** IU/L respectively the silymarin group almost same to the test group (EEAO group).

GROUPS	SGPT (IU/L)
NORMAL SALINE	34.67 ±4.66
PARACETAMOL 640mg/kg BW	119.3 ±14.89###
EEAO 200 mg/kg BW	78.00 ±8.71*
EEAO 400 mg/kg BW	62.00 ±3.46**
SILYMARIN 50mg/kg BW	56.67 ±4.66**

NOTE: Valve expressed as mean ±SEM

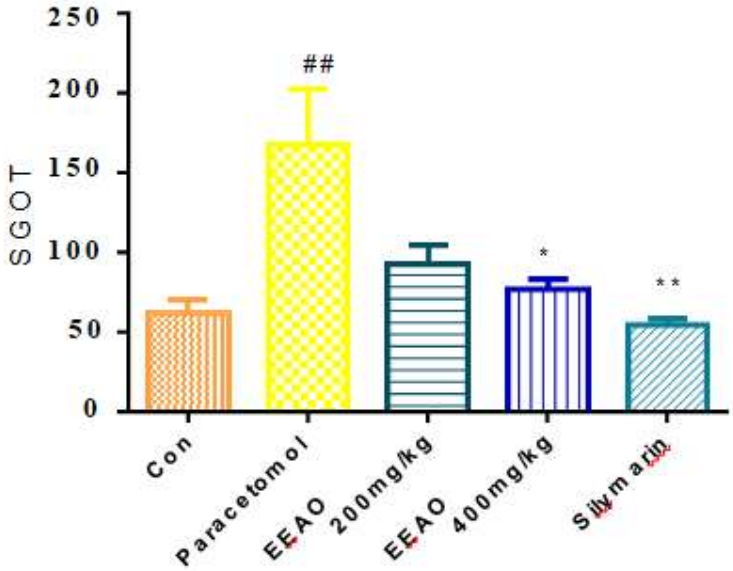


Effect of A.officinarum on SGOT concentration in paracetamol induced model

The below table show that paracetamol affect significant increase in SGOT concentration from control 62.00 ±8.08 IU/L to 167.3±34.65## IU/L after paracetamol intoxication. Administration of EEAO 200 mg/kg and 400 mg/kg in paracetamol intoxicated rats caused reduction in SGOT level up to 93.33±10.73* IU/L and 76.67 ±6.36** IU/L respectively (p□0.001).the extract in standard dose of silymarine 50mg/kg animal dose does not cause any significant change in SGOT level.

GROUPS	SGOT(IU/L)
NORMAL SALINE	62.00 ±8.08
PARACETAMOL 640 mg/kg BW	167.3±34.65##
EEAO 200 mg/kg BW	93.33±10.73*
EEAO 400 mg/kg BW	76.67 ±6.36**
SILYMARIN 50mg/kg BW	54.67±4.05**

NOTE: Valve expressed as mean ±SEM

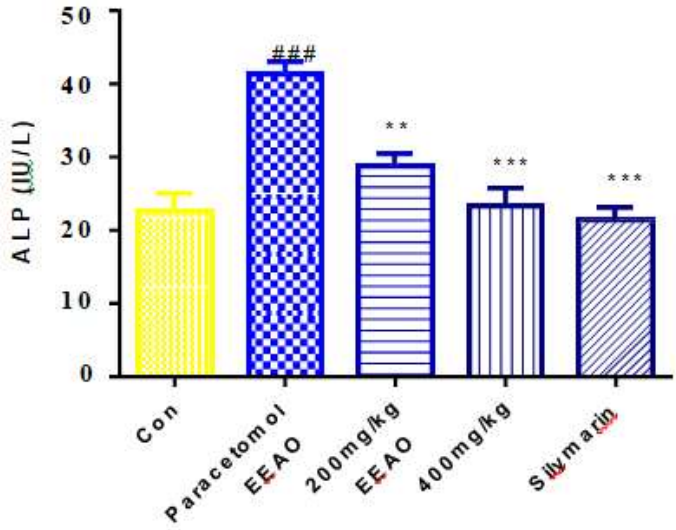


Effect of A.officinarum on ALP concentration in paracetamol induced model

ALP level in the control group increased from 22.67±2.40 IU/L to 41.33±1.76### IU/L in paracetamol intoxicated rat as shown in below the table. Administration of EEAO 200mg/kg and 400 mg/kg in paracetamol intoxicated rates led to lowering of the ALP 28.67±1.76** and 23.33±2.40*** respectively (p□0.001).the extract in control animals showed no significant alteration in ALP level.

GROUPS	ALP(IU/L)
NORMAL SALINE	22.67±2.40
PARACETAMOL 640mg/kg BW	41.33±1.76###
EEAO 200 mg/kg BW	28.67±1.76**
EEAO 400 mg/kg BW	23.33±2.40***
SILYMARIN 50mg/kg BW	21.33±1.76***

NOTE: Valve expressed as mean ±SEM



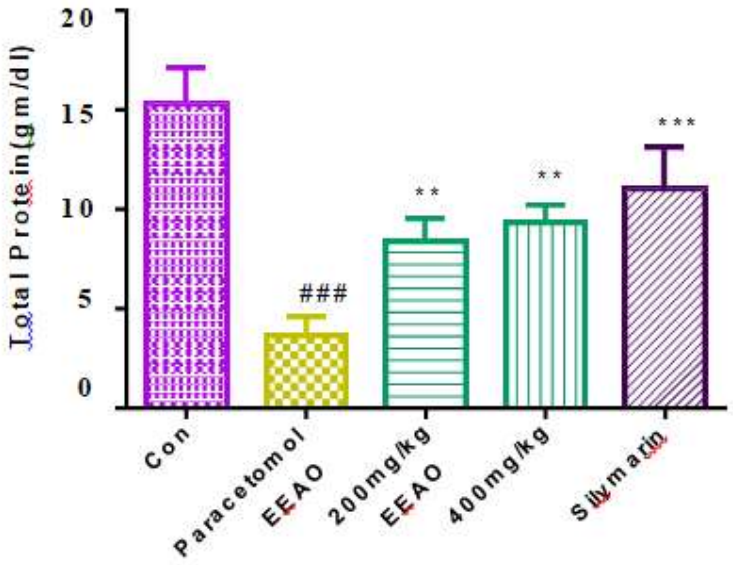
Effect of A.officinarum on total protein in concentration in paracetamol induced model

Total protein level in the control group reduced from 15.33±1.76 mg/dl to 3.66±0.88## mg/dl in paracetamol intoxicated rat as shown in below the table. Administration of EEAO 200mg/kg and 400 mg/kg in paracetamol intoxicated rats leadto increasing the concentration 7.66±1.20** and 9.33±0.88** respectively

(p□0.001).

GROUPS	TOTAL PROTEIN (mg/dl)
NORMAL SALINE	15.33±1.76
PARACETAMOL 640 mg/kg BW	3.66±0.88##
EEAO 200 mg/kg BW	7.66±1.20**
EEAO 400 mg/kg BW	9.33±0.88**
SILYMARIN 50mg/kg BW	11.00±2.08***

NOTE: Valve expressed as mean ±SEM

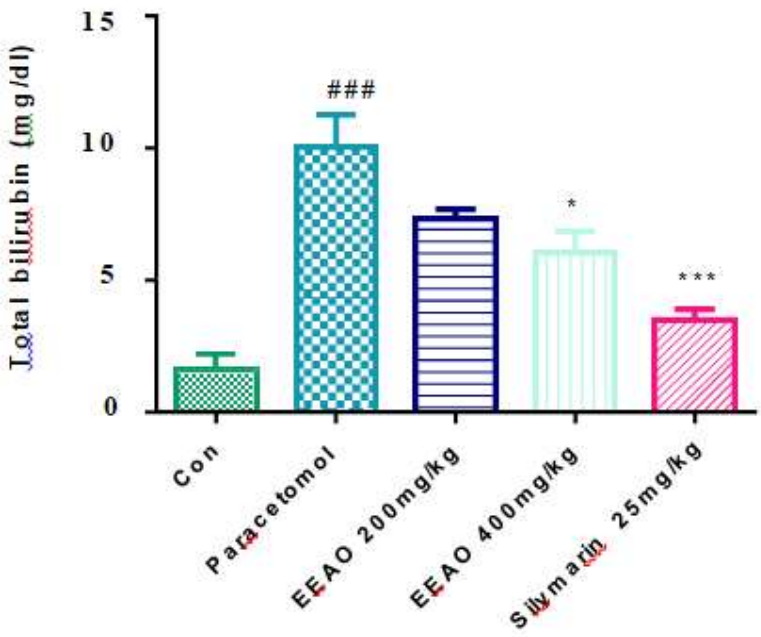


Effect of *A.officinarum* on total bilirubin in concentration in paracetamol induced model

Destruction of haemoglobin yuield bilirubin which is conjugated in the bile. Bilirubin accumulates in plasma when liver insufficiency exists or biliary obstruction is present or rat of hemolysis increases. The total bilirubin level increased from 1.63±0.58 mg/dl in the control group to 10.03±1.20### mg/dl after paracetamol intoxication as shown in below the table. Administration of EEAO 200mg/kg and 400 mg/kg in paracetamol intoxicated rats reduced the total bilirubin to 7.33±0.35 and 6.06±0.75* respectively (p□0.001). However, the extracts in control rats showed no such significant alteration in the serum bilirubin level.

GROUPS	TOTAL BILIRUBIN (mg/dl)
NORMAL SALINE	1.63±0.58
PARACETAMOL 640 mg/kg BW	10.03±1.20###
EEAO 200 mg/kg BW	7.33±0.35
EEAO 400 mg/kg BW	6.06±0.75*
SILYMARIN 50mg/kg BW	3.46±0.40

NOTE: Valve expressed as mean ±SEM



Topathology

Histology is the study of the microscopic anatomy of cells and tissues of plants and animals. Histology is an essential tool of biology and medicine. Histopathology, the microscopic study of diseased tissue, is an important tool in anatomical pathology, since accurate diagnosis of liver diseases usually requires histopathological examination of samples.

Control group: Photomicrograph of liver tissue of control rats showing normal hepatic cells with central vein and sinusoidal dilation

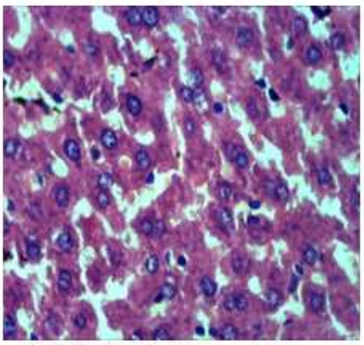
Paracetamol group: Showing severe centrilobular necrosis with disappearance of nuclei.

EEAO 200 mg/kg: showing mild degree of necrosis with mild inflammatory cells.

EEAO 400 mg/kg: showing normal hepatocytes with regenerating hepatocytes and mild inflammation in the portal area.

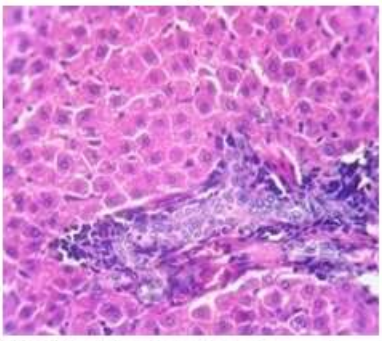
Silymarine: showing normal hepatocytes, portal vein and portal artery.

Group -1



CONTROLE: Normal saline

Group-2



NEGATIVE CONTROL: Paracetamol 640mg/kg BW

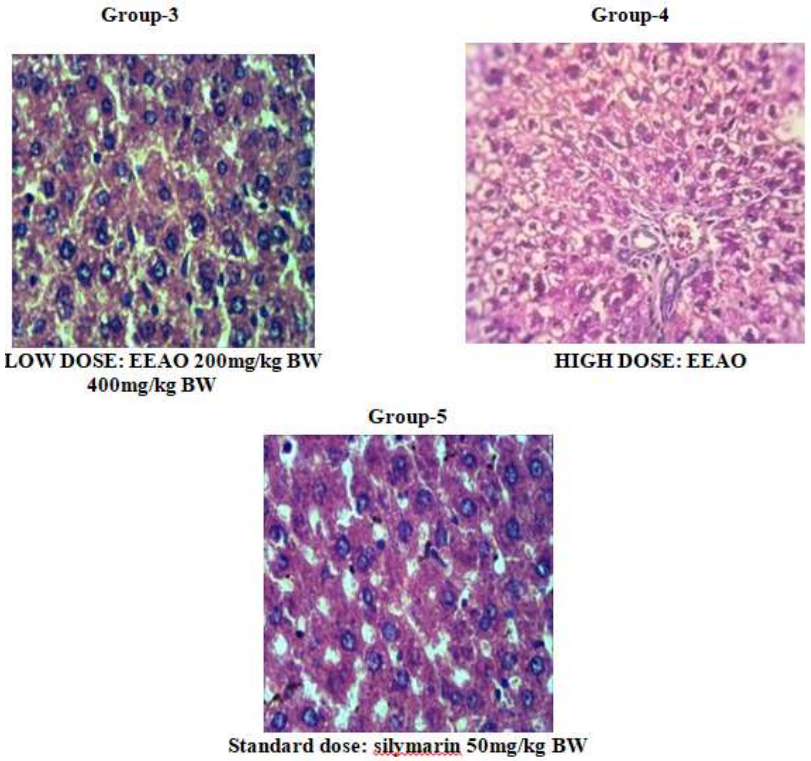


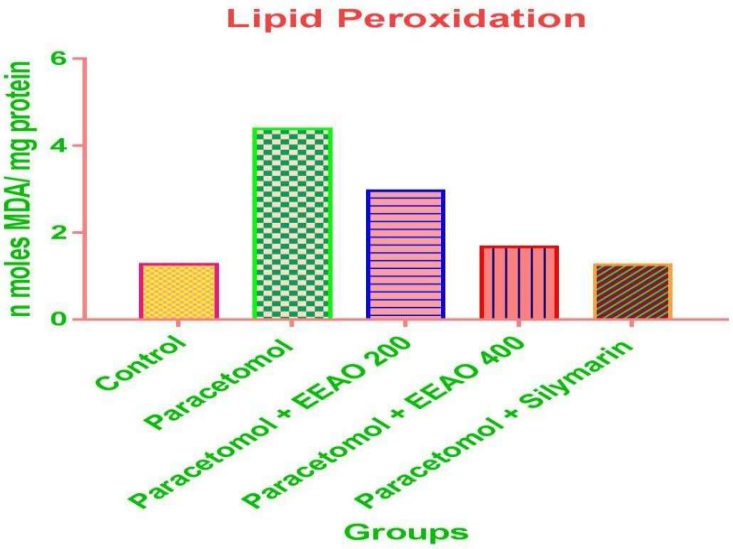
Fig 1: Histopathology

Antioxidant Activity Of Alpinia Officinarum

The result are summarized in below the table, the increase of the levels of MDA induced by paracetamol were significantly reversed by EEAO.

Table 2: Lipid peroxidation

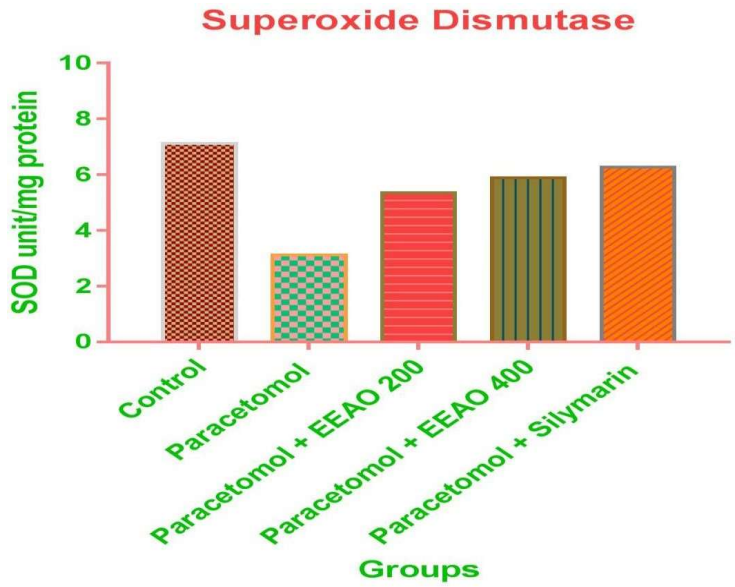
Groups	Normal Control	Paracetomol	EEAO 200 mg/kg	EEAO 400 mg/kg	Silymarin
Lipid Peroxidation (n moles of MDA/mg Protein)	1.27± 0.97	4.39 ± 0.56 a**	2.96 ± 0.76 b**	1.67 ± 0.46 b**	1.26 ± 0.78 b***



The result are summarized in below the table, the decreased of the levels of superoxide dismutase induced by paracetamol were significantly reversed by EEAO.

Table 3: Superoxide

Groups	Normal Control	Paracetomol	EEAO 200 mg/kg	EEAO 400 mg/kg	Silymarin
Superoxide dismutase (unit/mg protein)	7.12 ± 0.64	3.13 ± 0.42 a***	5.35 ± 0.33 b*	5.89 ± 0.69 b**	6.27 ± 0.58 b**



DISSCUSSION

The plant *Alpinia officinarum* is widely distributed in south Asia. The Rhizome part of plant have been studied its antibacterial activity but the hepatoprotective effect have been never studied. Hence the objective of the study is determining this effect from the dried rhizome extract of *Alpinia officinarum.* The preliminary phytochemical screening of whole plant extracts indicate in presence of Glycoside, Phenols, Tannins, Steroids, Flavonoid, Terpenoids , Protein and amino acid . May accounts antioxidant and hepatoprotective activity.

Liver is one of the important organs of body hence damage to liver leads to severe pathological problems or death. Liver diseases are mainly caused by toxic chemicals, excessive intake of alcohol, infections and autoimmune disorders. Liver injury caused by hepatotoxins such as carbon acetaminophen is characterized by varying degrees of hepatocyte degeneration and cell death by either apoptosis or by necrosis. SGOT, SGPT, ALP, Total Protein and Total Bilirubin levels are largely used as most common biochemical markers to evaluate liver injury.

Paracetamol (Acetaminophen), a widely used analgesic and antipyretic drug that produces acute liver damage in high doses. Paracetamol induced hepatotoxicity is thought to be caused by N-acetyl-p-benzoquinonemine (NAPQI), a cytochrome P-450 mediated intermediate metabolite. NAPQI can react with sulphydryl groups such as glutathione and protein thiols. The covalent binding of NAPQI to cell proteins is considered the initial step in a chain eventually leading to cell necrosis. In paracetamol (PCM) treated acute hepatic injury a significant difference in biochemical markers was observed between normal and PCM control groups. Results of present study shows that the levels of SGOT, SGPT, ALP and Total Bilirubin were significantly increased in paracetamol treated groups when compared with normal control group. While total protein is decreased in paracetamol treated groups due to damage produced in endoplasmic reticulum. Comparative analysis on the effect of SGOT, SGPT, ALP and Total Bilirubin revealed that extracts shows marked decrease in these enzymes when compared with toxic control group. While silymarine, and extract of Alpinia officinarum. Significantly restored total protein activities to normal level. Moreover, histopathological analysis showed that normal liver architecture was disturbed by Paracetamol treated rats oral feeding with extracts shows normal

architecture with mild hepatocyte degeneration compared with normal control group. Hepatoprotective study results shows that the levels of SGOT, SGPT, ALP and Total Bilirubin were significantly improvement may accounts hepatoprotective activity. As the results indicated that the extract possess significant hepatoprotective activity, after carrying out a thorough study of clinical trials, the plant can be considered as a low cost, potent, herbal medicine for liver disorders.

CONCLUSION

The present study was undertaken to determine the hepatoprotective activity of ethanolic extract from *Alpinia Officinarun*. The preliminary phytochemical investigation showed the presence of carbohydrates, Flavonoids, Terpenoids, phenolic compounds, fixed oils and fats,steroids in ethanol extract. Histopathological studies on isolated liver revealed that ethanolic extract of *Alpinia Officinarun* reversed the liver damage caused by Paracetamol. The normal pattern of histology of liver was observed. Based on the results obtained from the present study, it can be concluded ethanolic extract of *Alpinia Officinarun* (EEAO) is found to be more potent hepatoprotective.

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