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IN VITRO AND *IN VIVO* ANTI HEPATOTOXIC EVALUATION OF ALPINIA GALANGA ON D- GALACTOSAMINE INDUCED TOXICITY

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ABSTRACT

Alpinia galangal is a rhizome belongs to the family Zingeberaceae. Qualitative phytochemical analysis of plant extracts showed the presence of majority of the compound including terpenoids, flavonoids, alkaloids, tannins, saponins and glycosides. The hydro alcoholic hot extract has shown high total phenol content 14.50 ± 0.210 mg/g and hydro alcoholic cold shown 12.59 ± 0.295 mg/g. *Alpinia galangal* hydro alcoholic extract prepared by hot maceration showed potent antioxidant activity with IC value 7.7 ± 0.121 µg/ml. The antihepatotoxicity produced by the extract at concentration of 200 and 400 µg/ml was effective against the D- Galactosamine induced hepatotoxicity, whereas at the concentration of 600 and 800 µg/ml it was found to be cytotoxic for both the hydro alcoholic extracts prepared by cold and hot maceration process. A significant increase in the levels of ASAT, ALAT, ALP, total Bilirubin, direct Bilirubin ($P<0.001$) and a significant reduction in the levels of TGL, total proteins and albumin ($P< 0.001$) was observed in hepatocytes exposed to D- Galactosamine when compared to normal hepatocytes. These cells when treated alone with different extract of *Alpinia Galanga* showed a significant restoration of the altered biochemical parameters towards the normal ($P< 0.001$) when compared to D- Galactosamine treated group) and were dose dependent. A similar result was obtained with the silymarin. The antihepatotoxic effect of extracts of *Alpinia galangal* was found to be 200 – 400 µg/ml concentration.

KEYWORDS: Alpinia galangal, anti hepatoxicity, antioxidant, cytotoxic.

INTRODUCTION

The liver is a vital organ present in vertebrates and some other animals, and is typically the largest visceral organ. The liver plays a major role in transforming and clearing chemicals and is susceptible to the toxicity from these agents. Some medicinal agents, when taken in overdoses and sometimes even when introduced within therapeutic ranges, may injure the organ. Chemicals that cause liver injury are called hepatotoxins.[1] Chemicals often cause subclinical injury to liver which manifests only as abnormal liver enzyme tests. Drug-induced hepatotoxicity represents a major clinical problem accounting for 50% of all cases of acute liver failure. Although the majority of cases of acute liver failure are due to intentional or unintentional misuse, 16% are idiosyncratic.[2] Some of the inorganic compounds producing hepatotoxicity are arsenic, phosphorus, copper and iron. The organic agents include certain naturally occurring plant toxins such as pyrrolizidine alkaloids, In addition, exposure mycotoxins and bacterial toxins also produce hepatotoxicity. In addition, exposure to hepatotoxic compounds the other factors which produce hepatotoxicity may be occupational environmental or domestic that could be accidental, homicidal or suicidal ingestion.[3]

Drugs continue to be pulled from the market with disturbing regularity because of late discovery of hepatotoxicity.[4] The mechanism of hepatic injury has been proposed to involve 2 pathways-direct hepatotoxicity and adverse immune reactions. In most instances, hepatic injury is initiated by the bioactivation of drugs to chemically reactive metabolites, which have the ability to interact with cellular macromolecules such as proteins, lipids, and nucleic acids, leading to protein dysfunction, lipid peroxidation, DNA damage and oxidative stress. Additionally, these reactive metabolites may induce disruption of ionic gradients and intracellular calcium stores, resulting in mitochondrial dysfunction and loss of energy production. Its dysfunction releases excessive amount of oxidants which in turn injures hepatic cells.[5] Hepatic cellular dysfunction and death also have the ability to initiate immunological reactions, including both innate and adaptive immune responses.

Stress and damage to hepatocytes result in the release of signals that stimulate activation of other cells, particularly those of the innate immune system, including Kupffer cells (KC), natural killer(NK) cells, and NKT cells. These cells contribute to the progression of liver injury by producing proinflammatory mediators and secreting chemokines to further recruit inflammatory cells to the liver. It has been demonstrated that various inflammatory cytokines, such as tumor necrosis factor (TNF) α, interferon (IFN), and interleukin (IL)-1, produced

during hepatic injury are involved in promoting tissue damage.[6] However, innate immune cells are also the main source of IL-10, IL-6, and certain prostaglandins, all of which have been shown to play a hepatoprotective role.[7] In this paper the hepatoprotective activity of *Alpinia galangal* has been carried out in on D- galactosamine induced toxicity in albino rats of wister strain in both *in -vitro* and *in-vivo.*

MATERIALS AND METHODS

Alpinia galangal belongs to the family Zingiberaceae The creamy white rhizomes contain a volatile essential oil quite similar to that of the ginger. The chemical constituents of this oil are methyl cinnamate, cineole, eugenol, camphor and d-pinene. Present are also: phoblaphenes, starch, sulphates and chlorides. The essential oil is active again gram positive and gram negative microorganisms. Broncho-spasm induced by pilocarpine is counteracted by small doses of a tincture of galanga. The seeds have anti-ulcer activity. It is an excellent candidate for the development of a remedy for opportunistic fungal infections in AIDS patients. Anti-tumor and anti-dementia effects have been observed in rodents.

The rhizome is used against rheumatism, bronchial catarrh, bad breath and ulcers whooping colds in children, throat infections, to control incontinence and fever. It is a potent antioxidant. Alpinia species show promise as anti-fungal, hypertensive, enhancers of sperm count and motility. Homoeopaths use it as a stimulant. It has some reputation as a remedy for perineal relaxation with hemorrhoids and for a lax and pendulous abdomen. It is used as a snuff to treat cold and flu symptoms. Galangal Root has also been used as a digestive aid, especially in combating dyspepsia and flatulence.[8]

Collection and authentification of plant material

Both the plants were collected in the Kannur Kerala, during the month of May 2013. The plant was authenticated by Dr. S. Rajan, Botanical Survey of India, Medicinal Plant Survey and Collection Unit, Government Arts College, Ootacamund, Tamil Nadu, and India.

Preparation of plant extracts[9,10]

The collected rhizomes and leaves of the plant were dried under shade then they were chopped and coarsely powdered. Extraction was performed by soxhlet extraction, cold maceration and hot maceration process.

1) Soxhlet extraction

Coarsely powdered plant material was extracted by soxhlet apparatus with a range of solvents from non polar to polar.

2) *Cold maceration*

Shade dried coarsely powdered material (rhizome & leaves) were macerated for 7 days with 50% ethanol.

3) *Hot maceration*

The powdered (rhizome& leaves) were macerated with 50% ethanol for 6 hr at 60^{o}C.

Phytochemicals Studies of Extracts[11,12]

A) Qualitative Phytochemical analysis

A systematic and complete study of crude drugs should include a complete investigation of both primary and secondary metabolites derived from plant metabolism. The different qualitative chemical tests and to be performed for establishing profiles of given extracts for their nature of chemical composition. The ethanol extracts obtained as above were tested for the following qualitative chemical tests for the identification of various phytoconstituents.

B) Quantitative phytochemicals analysis[13]

Estimation of Total Phenolic Content

400 µl of the each dilution of the extracts were separately mixed with 2 ml of Folin-Ciocalteau reagent and 1.6 ml of sodium carbonate. After shaking, it was incubated for 2 h at 37^{0}C. The absorbance was measured at 750 nm (Shimadzu UV-160 A Spectrophotometer, Shimadzu Corporation, Japan). Using Gallic acid monohydrate, standard curve was prepared and linearity was obtained. The total phenol content was expressed as Gallic acid equivalent in mg/g or % w/w of the extracts.

Estimation of Total Flavanol Content

0.5 ml of the extracts was separately mixed with 1.5 ml methanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1M potassium acetate and 2.8 ml of distilled water. After incubation at room temperature for 30 min, the absorbance of the reaction mixture was measured at 415 nm with a Shimadzu UV-160A Spectrophotometer (Shimadzu Corporation, Japan). Using rutin, standard curve was prepared and linearity was obtained in the range of 1000-10 µg/ml. The total flavanol content was expressed as rutin equivalent in mg/g or % w/w of the extracts.

***In Vitro* Antioxidant Screening of Extracts**

DPPH radical scavenging activity[14,15]

The assay was carried out in a 96 well microtitre plate. To 200 µl of DPPH solution, 10 µl of each of the extract or standard solution was added separately in wells of the microtitre plate. The plates were incubated at 37 ^{o}C for 30 min and the absorbance was measured at 490 nm using ELISA reader.

Calculation

The absorbance was measured spectrophotometrically against the corresponding blank solution. The percentage inhibition was calculated by using the following formula.

$$\text{Radical scavenging activity (\%)} = \frac{\text{OD control - OD sample}}{\text{OD control}} \times 100$$

IC_{50}, which is the concentration of the sample required to scavenge 50% of free radicals was calculated.

Measurement of Reducing power ability

Measurement of reducing power ability was investigated in Fe^{3+} - Fe^{2+} transformation in the presence of the extracts.

1 ml of extract, 2.5 ml of phosphate buffer, 2.5 ml of $K_3Fe(CN)_6$ were incubated at 50^{0}C for 20 min, 2.5 ml of TCA was added to the mixture and centrifuged for 10 min at 3000 rpm. From the upper layer 2.5 ml was taken and diluted with 2.5 ml of distilled water and shaken with 0.5 ml fresh $FeCl_3$. The absorbance was measured at 700 nm after 20 min the blank solution contained distilled water instead of the samples.

Evaluation of Total Antioxidant capacity[16]

The total antioxidant capacity was determined by phosphomolybdenum method and is based on the reduction of Mo (VI) to Mo (V) by the antioxidant compounds and the formation of a green Mo (V) complex which has the maximal absorption at 695nm.

An aliquot of 0.1 ml of the sample solution containing a reducing species in DMSO was combined in an assay tube with 1 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). The tubes were capped and incubated in water bath at 95 °C for 90 min. The samples were cooled to room

temperature, and the absorbance of each solution was measured at 695 nm. The total antioxidant capacity was expressed as mM equivalent of ascorbic acid.

***In vitro* antihepatotoxic activity on isolated rat hepatocytes[17]**

The HEPES buffer and collagenase solution were warmed in a water bath (38°C-39°C to achieve 37^0 C in the liver) The pump flow rate was adjusted to 30 ml/min. The rat (180-200 g) was anaesthetized by intra peritoneal administration of Phenobarbital sodium 35 mg/kg b.w. The abdomen was opened and a loosely tied ligature was placed around the portal vein approximately 5 mm from the liver, and the cannula was inserted up to the liver and then the ligature was tightened, and heparin (1000 IU) was injected into the femoral vein. Subhepatic vessels were rapidly incised to avoid excess pressure and 600 ml of calcium free HEPES buffer was perfused at a low rate of 30 ml/min for 20 minutes. The liver swells during this time slowly changing color from dark red to greyish white. 300 ml of collagenase solution were perfused at a flow rate of 15 ml/min for 20 minutes during which the lobes swell. The lobes were removed and washed HEPES buffer, after disrupting the Glison capsule. The cell suspension was centrifuged at 1000 RPM to remove the collagenase, damaged cells and non-parenchymal cells. The hepatocytes were collected in Ham's F12 medium enriched with 0.2% bovine albumin, 10 µg/ml bovine insulin and 0.2% of dexamethasone.

Cell counting[18]

Tryphan Blue dye exclusion technique

Trypan blue is a dye, which is capable of penetrating the dead cells; dead cells take up the blue stain, where as viable cells do not. This method gives an exact number of dead cells and viable cells. In this procedure One drop of cell suspension was mixed with one drop of tryphan blue (0.4%) dye on a slide and mixed well for one minute. A drop of the mixture was loaded on a haemocytometer and the viable and non-viable count was recorded (live cells don't take stain where as dead cells get stained). The observations were recorded in a couple of minutes; else the live cells would degenerate and take up the stain.

The percentage viability was calculated as

$$\% \text{ Viability} = \frac{\text{Total cells} - \text{Dead cells}}{\text{Total cells}} \times 100$$

Determination of Hepatoprotective Activity on Freshly Isolated Rat Hepatocytes by Estimating the Bio-chemical Parameters[18]

The hepatocytes isolated were incubated for 30 minutes at 37°C for stabilization. The cells were then diluted in F12 Coons modified medium to obtain a cell count $5x10^5$ cells/ml. 100 µl of this cell suspension was seeded in each well of 96 well plates in each well. After 2 hours of pre-incubation, the medium was replaced with fresh medium. Then the hepatocytes were pretreated with extracts 15 min before galactosamine - induced treatment (50 µl of D-galactosamine and 50 µl of different extract concentration into each well). Hepatocytes injury was induced by incubation of hepatocytes with 30 mM D-galactosamine for 24 hours by incubating at 37°C. After incubation, the toxicant and drug treated cell suspensions were pooled into eppendroff tubes and centrifuged. The Asparate Aminotransferase Alanine Aminotransferase, Alanine Aminotransferase Alkaline Phosphatase enzyme levels as well as total protein and total bilirubin and total protein levels were determined in supernatant using Ecoline diagnostic kits.

***In vivo* antioxidant and hepatoprotective studies[19,20,21,22,23]**

Selection and maintenance of animals

Healthy adult albino rats of Wistar strain weighing 150-200 g were obtained from J.S.S. College of Pharmacy, Animal house, Ooty, India. The animal house was well ventilated and animals had 12 ± 1 hour day and night schedule with temperature between 20 ± 2^0C. The animals were housed in large spacious hygienic cages during the course of the experimental period. The animals were fed with rat feed supplied by M/S. Hindustan Lever Ltd., Bangalore. Experiments were carried out after getting permission from the institutional Ethical Committee of JSS college of Pharmacy, Ooty.

Preparation of extracts dose

S. No.	Plant name	Extract name	Concentration (mg/kg/b.w.)
1	*Alpinia galanga*	50% hydro alcoholic, hot maceration	200 and 400 mg/ml
2	*Alpinia galanga*	50% hydroalcoholic cold maceration	200 and 400 mg/ml

Preparation of standard

Single dose of 25mg/kg b.w. of silymarin product for 13 days.

Preparation and Induction of Hepatotoxicity

D-galactosamine HCl (400mg/kg b.w.) was administered intraperitonially on the 14th day to induce liver damage.

Randomization, Numbering and Grouping of Animals

The animals were divided into nine groups with four animals in each (equal number of both male and female) and they were treated as described below.

- **GROUP I** Served as solvent control which received double distilled water (1ml/kg.b.w) and 0.3% sodium carboxy methyl cellulose (CMC).
- **GROUPII** Served as negative control which received (1ml/kg.b.w) of double distilled water and 0.3% CMC orally once a day for 13 days. On the 14th day, D-galactosamine HCl (400mg/kg b.w.) was given.
- **GROUP III** Received a single dose of 25mg/kg b.w. of silymarin product for 13 days followed by treatment with the toxicant on the 14th day.
- **GROUP IV** Received a single dose of (200mg/kg b.w.) of hydro alcoholic hot macerated extract of *A. galanga* for 13 days followed by treatment with the toxicant on the 14th day.
- **GROUP V** Received a single dose of (400mg/kg b.w.) of hydro alcoholic hot macerated extract of *A. galanga* for 13 days followed by treatment with the toxicant on the 14th day.
- **GROUP VI** Received a single dose of (200mg/kg b.w.) hydro alcoholic cold macerated extract of *A. galanga* for 13 days followed by treatment with the toxicant on the 14th day.
- **GROUP VII** Received a single dose of (400mg/kg b.w.) hydro alcoholic cold macerated extract of *A. galanga* for 13 days followed by treatment with the toxicant on the 14th day.

Isolation of blood

The rats were anesthetized using thiopental and the blood was collected from abdominal artery and after collection, the blood was kept at 37^{0}C in the incubator for 30 min later, it was cold centrifuged at 2000 rpm for 15 min to get clear supernatant serum, which was used for the biochemical estimations.

Preparation of Liver and Kidney homogenates

The liver and kidneys were removed, weighed and homogenized immediately with Elvenjan homogenizer fitted with Teflon plunger, in ice chilled 10% KCl solution (10 ml/g of tissue). After centrifugation at 2000 rpm for 10 min, clear supernatant was used for the determination of SOD, CAT, TBARS and protein estimations. SOD and CAT levels were determined immediately after centrifugation and malondialdehyde (MDA) as TBA-RS and protein content were estimated in frozen samples.

***In vivo* antihepatotoxic and antioxidant evaluation[24]**

Estimation of in vivo antioxidant enzymes levels in homogenates

Catalase estimation

2.25 ml of potassium buffer (65mM, pH 7.8) and 100 µl of the tissue homogenate or sucrose (0.32 M) were incubated at 25^0C for 30 min. 0.65 ml of H_2O_2 (75mM) was added to initiate the reaction. The change in absorption at 240 nm was measured for 2-3 min, and *dy / dx* for 1 min for each assay was calculated and the results are expressed as CAT units / mg of tissue.

SOD estimation

Assay mixture contained 0.1mL of supernatant, 1.2mL of sodium phosphate buffer (pH 8.3; 0.052 M), 0.1mL of Phenazine methosulphate (186 µm), 0.3mL of nitro blue tetrazolium, 300 µM, 0.2mL of NADH (750 µM). Reaction was started by addition of NADH. After incubation at 30 ^{0} C for 90 s, the reaction was stopped by addition of 0.1mL of glacial acetic acid. Reaction mixture was stirred vigorously with 4.0mL of *n*-butanol. Colour intensity of the chromogen in the butanol was measured spectrophotometrically at 560 nm and concentration of SOD was expressed as U/mg of protein.

Thiobarbituric acid reactive substances (TBA-RS) estimation

Acetic acid 1.5mL (20%; pH 3.5), 1.5mL of thiobarbituric acid (0.8%) and 0.2mL of sodium lauryl sulphate (8.1%) were added to 0.1mLof supernatant and heated at 100^0C for 60 min. Mixture was cooled and 5mL of *n*-butanol-pyridine (15:1) mixture, 1mL of distilled water was added and vortexed vigorously. After centrifugation at 1200×*g* for 10 min, the organic layer was separated and absorbance was measured at 532 nm using a spectrophotometer. MDA is an end product of lipid peroxidation, which reacts with thiobarbituric acid to form pink chromogen–thiobarbituric acid reactive substance. It was calculated using a molar extinction coefficient of $1.56\times105M^{-1}$ $cm^{-1}$and expressed as nanomoles of TBARS/mg of protein.

Protein estimation

100 µl of homogenates, 900 µl of distilled water, and 5ml of Bradford reagent. Mix well and allow the color to develop for at least 5 min but no longer than 30 min. read the absorbance at 595 nm. Plot a standard curve using the standard protein absorbance Vs concentration. Calculate the protein in the experimental sample using the standard curve.

Estimation of biochemical parameters from serum

ASAT, ALAT, ALP, total protein, albumin, LDH, total bilirubin, direct bilirubin and triglycerides were assayed using Ecoline diagnostic kit.

Statistical analysis

The results of *in vitro* antihepatotoxic & *in vivo* antihepatotoxic and antioxidant activity were analyzed statistically using one way analysis of variance (ANOVA) followed by Dunnett's *t*-test.

RESULT AND DISCUSSION

Collection and authentification of plant

The Rhizomes of *Alpinia galangal* was collected from the Kerala, in the month of May 2013. The plant was authenticated by Dr. S. Rajan, Botanical Survey of India, Medicinal Plant Survey and Collection Unit, Government Arts College, Ootacamund, Tamil Nadu, and India.

Preparation of plant extracts

The dried parts of rhizome of ***Alpinia galanga*** were chopped and coarsely powdered, the powdered materials were extracted separately using different solvent according to polarity by Soxhletion and hot and cold maceration. All the extracts were stored in refrigerator till further use. The percentage yields were given in table 1**.**

Table 1. Percentage yield of rhizome of Alpinia galanga extracts

S. No.	Extraction process	Powder weight	Solvent used	Weight of extract	% Yield
1	Soxhlet extraction	100 g	Petroleum ether	3.85 g	3.8%
2		100 g	Chloroform	7.86 g	7.8%
3		100 g	Ethanol	9.50 g	9.5%
4		100 g	Methanol	9.45 g	9.4%
5	Cold maceration	100 g	Hydro alcoholic (50%)	8.54 g	8.5%
6	Hot maceration	200 g	Hydro alcoholic (50%)	19.25 g	9.7%

Phytochemicals studies of extracts

A) Qualitative Phytochemicals analysis

Qualitative Phytochemical analysis of plant extracts showed the presence of majority of the compounds including terpenoids, flavanoids, alkaloids, tannins, saponins and glycosides. The results from chemical tests are shown in table no 2.

Table 2. Analysis of *Alpinia galanga* extracts

Tests	*Alpinia galanga*					
	Pet. ether	**Chloroform**	**Ethanol**	**Methanol**	**Hot maceration**	**Cold maceration**
Alkaloids	_	_	+	_	+	+
Carbohydrates	_	_	_	+	+	+
Flavanoids	+	+	+	+	+	+
Terpenoids	+	+	+	_	+	+
Glycosides	_	_	+	_	+	+
Phytosterols	+	+	_	_	_	_
Proteins and aminoacids	+	_	_	_	+	+
Saponins	_	_	_	_	+	+
Phenolic compounds and Tannins	+	+	+	+	+	+

Estimation of Total Phenolic Content

Among the six extracts, the hydro alcoholic (hot) extract has shown high total phenol content 14.50 ± 0.210 mg/g, and hydro alcoholic (cold) shown 12.59 ± 0.295 mg/g. The other four extracts showed total phenol content in the range of 6.42 ± 0.058 mg/g to 10.59 ± 0.230 mg/g. The results from total phenol content are shown in table no.3.

Table 3. Total phenol estimation of different extracts of Alpinia galanga

S. No.	Extract	Total phenol (mg/g)
1	Petroleum Ether	6.42 ± 0.058
2	Chloroform	8.21 ± 0.078
3	Ethanol	11.43 ± 0.363
4	Methanol	10.59 ± 0.230
5	Hydro alcoholic (cold)	12.59 ± 0.295
6	Hydro alcoholic (hot)	14.50±0.210

Estimation of total Flavanol content

Among the six extracts, the hydro alcoholic (hot) extract has shown high total flavanol content 285.57 ± 0.188 mg/g, and hydro alcoholic (cold) shown 174.41 ± 0.383 mg/g. The

other four extracts showed total flavanol content in the range of 49.62 ± 0.342 mg/g to 169.84 ± 0.145 mg/g. The results from total flavanol content are shown in table 4.

Table 4. Total flavanol content of different extracts of Alpinia galanga*:*

S. No.	Extract	Total flavanol (mg/g)
1	Petroleum Ether	119.46 ± 0.453
2	Chloroform	169.84 ± 0.145
3	Ethanol	59.86 ± 0.115
4	Methanol	49.62 ± 0.342
5	Hydro alcoholic (cold)	174.41 ± 0.383
6	Hydro alcoholic (hot)	285.57 ± 0.188

***In Vitro* Antioxidant Screening of Extracts**

DPPH radical scavenging activity

Among the six extracts of *Alpinia galanga* hot extract showed potent antioxidant activity with IC_{50} value of 7.7 ± 0.121µg/ml The other five extracts showed antioxidant activity in the range of 12.35 ± 0.176 µg/ml to 64.03 ± 5.179 µg/ml. the results from DPPH activity are shown in table 5.

Table 5. DPPH radical scavenging activity of different extracts of Alpinia galanga

S. No.	Extract	IC_{50} (µg/ml)
1	Petroleum Ether	64.03 ± 5.179
2	Chloroform	33.44 ± 0.066
3	Ethanol	13.65 ± 0.136
4	Methanol	15.76 ± 0.237
5	Hydro alcoholic (cold)	12.35 ± 0.176
6	Hydro alcoholic (hot)	7.7 ± 0.121
7	Ascorbic acid	2.69 ± 0.05

Total antioxidant capacity

Among the six extracts of *A. galanga* hydro alcoholic (hot) extract showed potent antioxidant capacity 0.24 ± 0.016 mM equivalent of Ascorbic acid in comparison to other extracts.

Table 6 Total antioxidant capacity of different extracts of A. galanga

S. No.	Extract	TAC (mM equivalent of Ascorbic acid)
1	Petroleum Ether	0.72 ± 0.019
2	Chloroform	0.69 ± 0.018
3	Ethanol	0.34 ± 0.017
4	Methanol	0.38 ± 0.011
5	Hydro alcoholic (cold)	0.30 ± 0.012
6	Hydro alcoholic (hot)	0.24 ± 0.016

Reducing power ability

Among the six extract of *Alpinia galanga*, hydro alcoholic (hot) extract showed potent reducing power ability 8.9 ± 0.572 µg/ml in comparison to other extract.

Table 7. Reducing power ability of different extracts of Alpinia galangal

S. No.	Extract	IC $_{50}$(µg/ml)
1	Petroleum. Ether	67.28 ± 0.98
2	Chloroform	36.42 ± 2.91
3	Ethanol	17.63 ± 0.128
4	Methanol	19.32 ± 1.27
5	Hydro alcoholic (cold)	15.73 ± 0.126
6	Hydro alcoholic (hot)	8.9 ± 0.572
7	Ascorbic acid	1.62 ± 0.13

***In vitro* antihepatotoxicity activity**

The *in vitro* antihepatotoxicity activity of selected extracts was carried out on freshly prepared rat hepatocytes to estimate the different parameters.

The pathological condition of normal, silymarin treated and D-galactosamine induced hepatocytes are as below.

Table-8 Biochemical parameters of d-galactosamine intoxicated rats

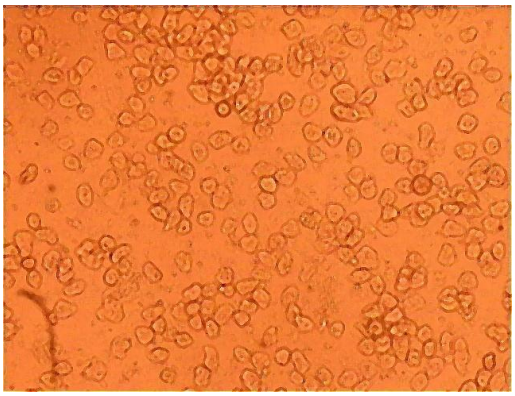
S.NO.	Treatment	Dose	ASAT U/L	ALAT U/L	ALP U/l	Total protein gm/dl	Albumin G/l	Total Bilirubin mg/dl	Direct Bilirubin mg/dl	TGL mg/dl
1	Normal	---------	65.91± 0.70	33.93± 1.32	440±1.08	7.13± 0.05	4.90± 0.04	0.318 ±0.06	0.166 ±0.06	77.40 ±0.77
2	D-GalN treated	400 mg/kg b.w	134.40± 1.72[a]	72.01 ±0.98[a]	905.1±4.17[a]	4.35 ±0.13[a]	3.643± 0.11[a]	2.12 ±0.02[a]	0.48 ±0.01[a]	27.6 ±1.13[a]
3	Std. silymarin	25 mg/kg b.w	80.48 ±1.63[a]	46.48± 0.929[a]	495.9±2.97[a]	6.95 ±0.01[a]	4.696 ±0.02[a]	0.460 ±0.09[a]	0.170 ±0.02[a]	55.09 ±0.90[a]
4	*A .galanga* Hydro-alco(hot)	200 mg/kg b.w	88.84± 2.38[a]	52.72 ±0.59[a]	522.4±1.77[a]	6.84 ±0.01[a]	4.545 ±0.09[a]	0.470 ±0.01[a]	0.174 ±0.03[a]	56.14 ±1.13[a]
5	*A .galanga* Hydro-alco(hot)	400 mg/kg b.w	85.11 ±3.44[a]	48.10± 0.75[a]	502.47±5.71[a]	6.55 ±0.02[a]	4.59 ±0.03[a]	0.465 ±0.04[a]	0.179 ±0.04[a]	58.72 ±0.61[a]
6	*A .galanga* Hydro-alco(cold)	200 mg/kg b.w	89.26± 2.27[a]	55.90 ±1.16[a]	562.72±1.18[a]	6.16 ±0.008[a]	4.24 ±0.02[a]	0.430 ±0.02[a]	0.181 ±0.03[a]	65.09 ±0.93[a]
7	*A .galanga* Hydro-alco(cold)	400 mg/kg b.w	88.02 ±1.27[a]	52.41± 0.877[a]	533±1.9[a]	6.58± 0.01[a]	4.57 ±0.04[a]	0.452 ±0.04[a]	0.186 ±0.05[a]	68.90 ±0.68[a]
8	*G.sylvestre* hydro-alco (hot)	200 mg/kg b.w	103.68±0.44[a]	63.57 ±0.87[a]	649.12±3.29[a]	5.73 ±0.01[a]	3.94 ±0.03[a]	0.632 ±0.01[a]	0.259 ±0.06[a]	70.46 ±0.72[a]
9	*G.sylvestre* hydro-alco (hot)	400 mg/kg b.w	91.07 ±3.32[a]	58.56± 0.17[a]	606.70±4.05[a]	5.96 ±0.01[a]	4.12 ±0.01[a]	0.644 ±0.01[a]	0.225 ±0.03[a]	72.15 ±1.26[a]

All values are expressed as Mean ± SEM (n=4), a= (P<0.001) when compared to D-GalN treated group. (U/L = Units/liter, mg/dl = milligram/deciliter, gm/dl = gram/deciliter, G /I = gram/liter)

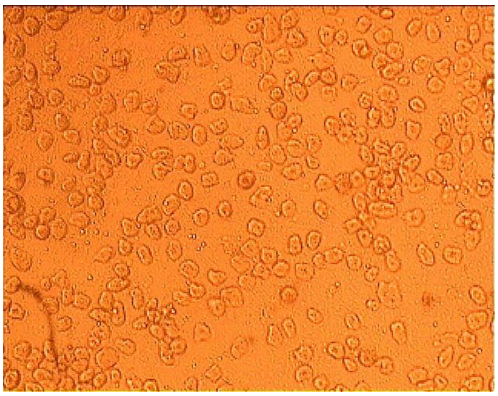
Table 9- *In vivo* antioxidant activity on liver and kidney homogenates

S. No.	Treatment	Dose	SOD unit/min/mg of tissue		CAT unit/min/mg of tissue		TBARS nM MDA/mg of tissue	
			liver	kidney	liver	kidney	liver	kidney
1	Normal	---------	1.155 ± 0.03	0.608 ±0.04	12.419 ± 0.43	5.37 ± 0.64	8.37 ±0.52	2.268 ±0.01
2	D-GalN treated	400 mg/kg b.w	0.838 ± 0.004[b]	0.405 ± 0.06[b]	7.380 ± 0.91[b]	2.08 ±0.82[b]	25.56 ±0.54[b]	6.812 ±0.04[b]
3	Std. silymarin	25 mg/kg b.w	1.095 ± 0.002[b]	0.605 ± 0.01[b]	11.275 ± 0.28[b]	3.61 ±0.02[b]	8.42 ±0.03[b]	2.544 ±0.02[b]
4	*A .galanga* Hydro-alco(hot)	200 mg/kg b.w	0.990 ± 0.001[b]	0.530 ± 0.12[b]	9.45 ± 1.23[b]	2.96 ±0.06[b]	12.82 ±0.08[b]	2.238 ±0.06[b]
5	*A .galanga* Hydro-alco(hot)	400 mg/kg b.w	1.078 ± 0.035[b]	0.598 ± 0.36[b]	10.07 ± 0.34[b]	3.71 ±0.07[b]	10.84 ±0.05[b]	2.668 ±0.04[b]
6	*A .galanga* Hydro-alco(cold)	200 mg/kg b.w	0.985 ± 0.002[b]	0.525 ± 0.008[b]	9.22 ± 0.45[b]	2.81 ±0.09[b]	12.12 ± 0.04[b]	2.672 ±0.02[b]
7	*A .galanga* Hydro-alco(cold)	400 mg/kg b.w	0.994 ± 0.001[b]	0.532 ± 0.17[b]	9.49 ± 0.63[b]	2.95 ±0.05[b]	12.98 ±0.64[b]	2.894 ±0.04[b]
8	*G.sylvestre* hydro-alco (hot)	200 mg/kg b.w	0.948 ± 0.005[b]	0.567 ± 0.21[b]	8.98 ± 0.73[b]	2.14 ±0.21[b]	13.92 ±0.72[b]	4.123 ±0.02[b]
9	*G.sylvestre* hydro-alco (hot)	400 mg/kg b.w	0.958 ± 0.002[b]	0.574 ± 0.36[b]	8.86 ± 0.52[b]	2.21 ±0.52[b]	14.57 ±0.09[b]	4.521 ±0.05[b]

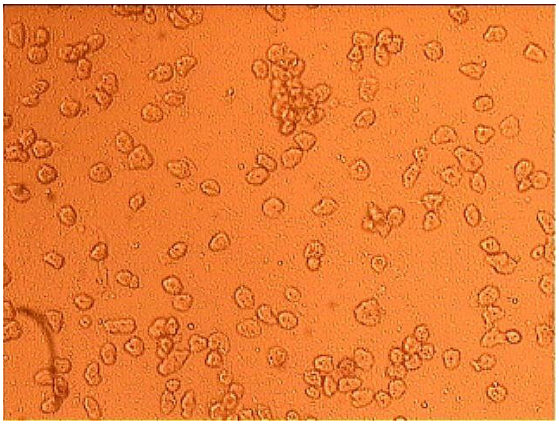
All values are expressed as Mean ± SEM (n=4), b= (P<0.001) when compared to D-GalN treated group.



Normal hepatocytes



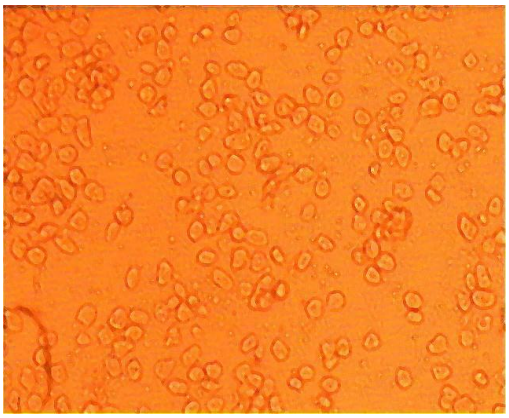
Silymarin treated 250µg/ml



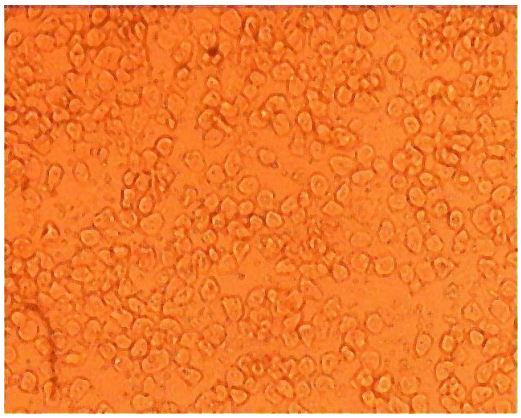
30 mM D-galactosamine induced

Effect of hot macerated hydro alcoholic (50%) extract of Alpinia galanga on rat hepatocytes

The hepatocytes were treated with different concentration of hydro alcoholic hot macerated extract are as follows,

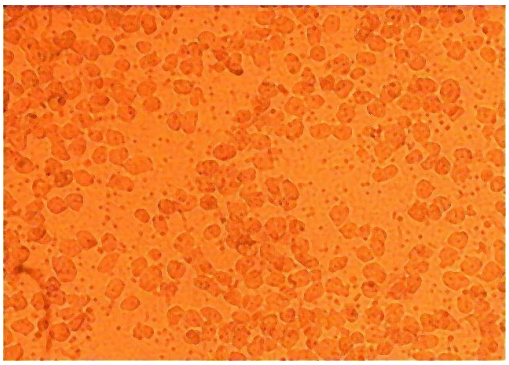


200 µg/ml concentration



400 µg/ml concentration

600 µg/ml concentration

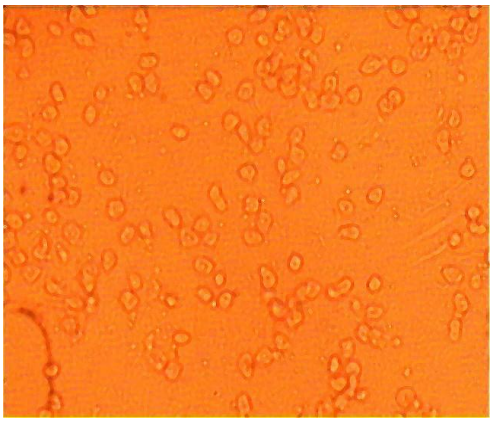


800 µg/ml concentration

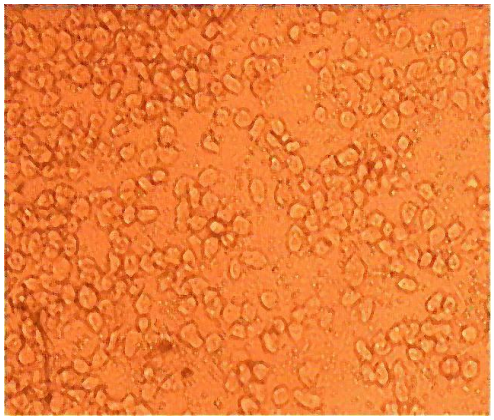
From the above study the antihepatotoxicity produced by extract at concentration of 200 and 400 µg/ml was effective against the D-galactosamine induced hepatotoxicity, where as at concentration of 600 and 800 µg/ml it is found cytotoxic.

Effect of cold macerated hydro alcoholic (50%) extract of Alpinia galanga on rat hepatocytes

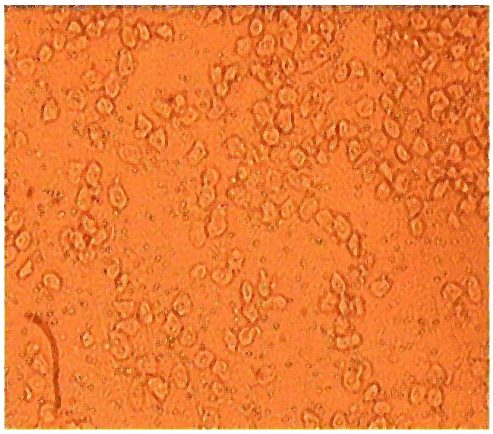
The hepatocytes were treated with different concentration of hydro alcoholic hot macerated extract are as follows,



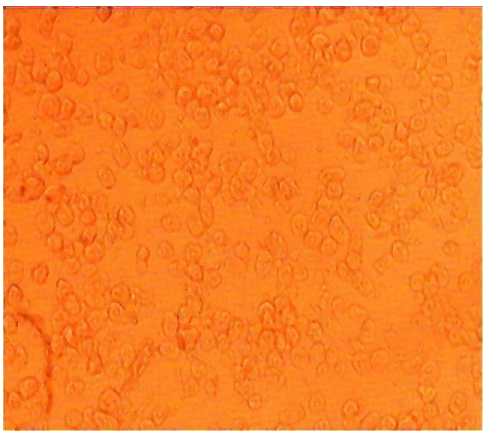
200µg/ml concentration



400 µg/ml concentration



600 µg/ml concentration



800 µg/ml concentration

From the above study the antihepatotoxicity produced by extract at concentration of 200 and 400 µg/ml was effective against the D-galactosamine induced hepatotoxicity, whereas at concentration of 600 and 800 µg/ml it is found to be cytotoxic.

The effects of the different extract of *Alpinia galanga* on freshly prepared isolated rat hepatocytes intoxicated with D-galactosamine are recorded in table 8. A significant increase in the levels of ASAT, ALAT, ALP, total bilirubin, direct bilirubin ($P < 0.001$) and a significant reduction in the levels of TGL, total proteins and albumin ($P < 0.001$) was observed in hepatocytes exposed to D-galactosamine when compared to normal hepatocytes. These cells when treated alone with the different extract of *Alpinia galanga* showed a significant restoration of the altered biochemical parameters towards the normal ($P < 0.001$, when compared to D-galactosamine treated group) and were dose dependent. A similar result was obtained when D-galactosamine intoxicated hepatocytes were treated with the Silymarin. However, the antihepatotoxic effect of extracts of the *Alpinia galanga* was observed at 200-400 µg/ml concentration.

***In vivo* antihepatotoxicity & antioxidant activity**

The effects of different extract of *Alpinia galanga* (rhizomes) on D-galactosamine intoxicated rats are recorded in table 9. Intoxication of rats treated with D-galactosamine significantly ($P < 0.001$) altered the biochemical parameters when compared with D-galactosamine induced rats. However, the Silymarin at 25 mg/kg body weight exhibited better results with no mortality.

Lipid peroxidation level of liver homogenates significantly increased ($p < 0.001$) in D-GalN treated rats when compared to control rats. Treatment with plant extract of 400 mg/kg dose of *Alpinia galanga* hot macerated, cold macerated extract and silymarin (25 mg/kg) showed significant ($p < 0.001$) decrease in LPO when compared with D-GalN treated rats are recorded in table 9.

The plant extract at 200 mg/kg dose also showed good significant ($p < 0.001$) decrease in LPO in liver homogenate when compared D-GalN treated rats as mentioned.

Administration of D-GalN caused a significant ($p < 0.001$) decrease SOD and CAT levels in rats when compared with normal animal. The plant extract at 400 mg/kg showed significant ($p < 0.001$) increase in SOD and CAT when compared to D-galactosamine treated rats. The

standard drug, silymarin treated rats also showed significant ($p < 0.001$) increase in SOD and CAT when compared to D-GalN treated rats.

CONCLUSION

Drug discovery and development consists of a series of processes starting with the demonstration of pharmacological effects in experimental cell and animal models and ending with drug safety and efficacy studies in patients. A main limitation is often the unacceptable level of toxicity with the liver as the primary target organ. The study of anti hepatotoxic effect of *Alpinia galangal* showed its activity that is equivalent to that of standard drug Silymarin. Further studies could be continued to isolate the active constituents which are responsible for the anti hepatotoxic activity.

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