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RESEARCH ARTICLE

In-Vitro and *In-Vivo* Anti-Hepatotoxic Evaluation of *Curcuma Aromatica* on D-Galactosamine Induced Toxicity

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ABSTRACT

Curcuma aromatica belongs to the family zingiberaceae. The dried rhizome of Curcuma aromatica was extracted with different solvents like petroleum ether, Toluene, Chloroform, Ethyl acetate, Acetone, Ethanol, Water,. The Phytochemical studies of extracts showed the presence of terpenoids, flavonoids, tannins, alkaloids, saponins and protein and amino acids. Toluene extract of *Curcuma aromatica* has shown high Total Phenol content, 265±1.08 mg/g which is expressed in terms of Gallic acid and high total flavonol content, 175±1.56 mg/g expressed in terms of rutin. Toluene extract of *Curcuma aromatica* has shown potent antioxidant activity with IC_{50} value of 50.62±0.998 µg/ml, with IC_{50} value of 75±0.87 with IC_{50} value of 43.75±1.24 µg/ml with IC_{50} value of 0.038±1.54µg/ml in DPPH, LPO method, in the Scavenging of Hydrogen Peroxide Radicals method and in the ABTS Radical Scavenging Method respectively. Toluene extract at concentration of 200 to 800 µg/ml showed a significant restoration of the altered biochemical parameters towards the normal and it was comparable with standard silymarin, using D- Galactosamine as toxicant. Toluene extract was found to have dose dependent increase in percentage viability of the cells. The 200 and 400 mg/kg b.w toluene extracts of *Curcuma aromatica* showed a significant restoration of enzyme levels in *in-vivo* studies. The results were encouraging to state that the hepatoprotective activity exhibited by the toluene extracts of *Curcuma aromatica* was found to be nearly equivalent with standard silymarin.

KEYWORDS

Curcuma Aromatica, Antihepatotoxic, D-Galactosamine, Antioxidant, Silymarin

INTRODUCTION

Hepatotoxicity (from hepatic toxicity) implies chemical-driven liver damage. The liver plays a central role in transforming and clearing chemicals and is susceptible to the toxicity from these agents. Certain medicinal agents when taken in overdoses and sometimes even when introduced within therapeutic ranges may injure the organ.

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Other chemical agents which are used in laboratories and industries, natural chemicals (Eg. microcystins) and herbal remedies can also induce hepatotoxicity. Chemicals that cause liver injury are called hepatotoxins.[1,2]

Due to the late discovery of hepatotoxicity, many drugs are continuesouly taken off from the market. Liver is susceptible to injury, due to unique metabolism of drugs and other substances and their close relationship with the gastrointestinal tract because 75 % of blood coming to the liver arrives directly from the gastrointestinal organ and spleen via portal

veins which bring drugs and xenobiotics in the concentrated form. For either inducing hepatic injury or worsening the damage proves, several mechanism were responsible because many chemicals damage mitochondria an intra-cellular organelle that produces the energy. The hepatic cells will get injured because of the excessive release of oxidants. Oxidative stress is produced by the activation of some enzymes in the cytochrome p-450 systems such as CYP2E1. Liver damage gets aggravated due to the accumulation of bile acid inside the liver due to injury of hepatocytes. Non- parenchyma cells such as kuffer cells, fat storing stellate cells and leucocytes also have role in mechanism[3,4]. Use of herbal drugs in the treatment of liver diseases has a long tradition, especially in Eastern medicine. This research paper deals with the evolution of anti-hepatotoxicity activity of Curcuma aromatica in vitro and in vivo methods in D- galactosamine induced hepatotoxicity. Curcuma aromatica belongs to the family Zingeberaceae it is commonly called as Kasturi manjal. Wild plant, cultivated throughout India, chiefly Bengal and Kerala. Historically, rhizomes are used as tonic, carminative, and externally in combinations with astringents, bitters and aromatics to brusises, in sprains and in snake- bite. They are also used for skin eruptions and infections and to improve complexion.

Essential oil contains *ar*-curcumene and *β*-curcumene, d- and p- methoxy cinnamic acid. The colouring matter is curcumin. Numerous sesquiterpenoids of germacrone-guaiane skeletons have been identified Rhizomes are used in combination with astringents and aromatics for bruises, sprains, hiccough, bronchitis, cough, leucoderma and skin eruptions, carminative and adjunctant to other medicines and has effect on respiration, Spasmolysis and antagonist in amphetamine hyperactivity, and anti-dote for snakebite.[5]

MATERIALS AND METHOD

Collection and Authentication

The plant ***curcuma aromatica*** were identified and authenticated by Mr. P.S.S. Ramachandran, Abirami Botanicals, Tuticorin, Tamilnadu. India.

Extraction[6,7]

The dried Rhizomes of *curcuma aromatica* were powdered and extracted with petroleum ether, toluene, chloroform, ethylacetate, acetone, ethanol, water by hot maceration. The extract was filtered and the filtrate was evaporated to dryness in a rotary evaporator to yield a dark brown semisolid. The extracts were stored in a refrigerator till use.

Qualitative Phytochemical Screening

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 are to be performed for establishing profiles of given extracts for their nature of chemical composition. The extracts obtained as above were tested for the following qualitative chemical tests for the identification of various phyto constituents.[8,9]

Qualitative Phytochemical Analysis

Estimation of Total Phenol Content

Total phenol content of the extracts was determined by using the Folin-Ciocalteu method. This test is based on the oxidation of phenolic groups with phosphomolybdic and phosphotungstic acids. After oxidation a green – blue complex formed is measured at 750 nm. Folin-Ciocalteu reagent.[10,11,12]

Estimation of Total Flavonol Content [13]

0.5 ml of the extract 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 Biorad Laboratories Inc. Model 550. Using the standard curve the total flavonol content of extracts was calculated. The total flavonol content was expressed as quercetin equivalent in mg/g or % w/w of the extracts.

In Vitro Antioxidant Evaluation

Diphenyl Picryl Hydrazyl (DPPH) Radical Scavenging Method [14,15]

The assay was carried out in a 96 well microtitre plate. To 200µl of DPPH solution, 10µl of each of the test sample or the standard solution was added separately in wells of the microtitre plate. The final concentration of the test and standard solutions used were 1000 µg/ml to 0.9765 µg/ml. The plates were incubated at 37ºC for 20 minutes and the absorbance of each solution was measured at 490 nm, using ELISA reader against the corresponding test and standard blanks and the remaining DPPH was calculated. IC_{50} (Inhibitory Concentration) is the concentration of the sample required to scavenge 50% of DPPH free radicals.

$$\% \text{ Inhibition} = \frac{\text{Absorbance of control- Absorbance of sample}}{\text{Absorbance of Control}}$$

ABTS Radical Scavenging Method[16]

To 0.2 ml of various concentrations of the extract or standards, 1 ml of distilled DMSO and 0.16 ml of ABTS solution were added to make a final volume of 1.36 ml. Absorbance was measured spectrophotometrically, after 20 min at 734 nm using ELISA reader. Blank is maintained without ABTS. IC_{50} value obtained is the concentration of the sample required to inhibit 50 % ABTS radical mono cation.

Lipid Peroxidation (LPO) Assay[17]

The test samples (100 µl) of different concentrations were added to 1 ml of egg lectin mixture, control was without test sample. Lipid peroxidation was induced by adding 10 µl $FeCl_3$ (400 mM) and 10 µl L-ascorbic acids (200 mM). After incubation for 1 hour at 37°C, the reaction was stopped by adding 2 ml of 0.25 N HCl containing 15% TCA and 0.375% TBA and the reaction mixture was boiled for 15 min then cooled, centrifuged and absorbance of the supernatant was measured at 532 nm.

Scavenging of *Hydrogen Peroxide Radicals*[10]

A solution of H_2O_2 (20 mM) was prepared in PBS, (pH 7.4). Various concentrations of 1 ml of the extracts or standards in methanol were added to 2 ml of H_2O_2 solutions in PBS. The absorbance was measured at 230 nm, after 10 min against a blank solution that contained extracts in PBS without H_2O_2.

Preparation of Freshly Isolated Rat Hepatocytes[18,19,20,21]

The HEPES buffer and collagenase solution were warmed in a water bath (38ºC-39ºC 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.

Determination of Hepatoprotective Activity on Freshly Isolated Rat Hepatocytes by Estimating the Bio-chemical Parameters[22]

The hepatocytes isolated were incubated for 30 minutes at 37ºC 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 37ºC. 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 levels were determined in supernatant using Ecoline diagnostic kits. Total Protein

In-vivo Hepatoprotective Studies[23,24, 25,26,27]

Preparation of the Formulation

The toluene extract of *Curcuma aromatica* was dissolved or suspended in 0.3% sodium carboxy methyl cellulose and stored at +4ºC until use.

Induction of Heptotoxicity

D- Galactosamine at a dose of 1 ml/kg b.wt. (1%) was administered intraperitonially on the 8th day to induce liver damage

Randomization Numbering and Grouping of Animals[4,27]

The experimental design of the investigation was carried out in five groups with six animals in each group and given the regiments described below

Group I

Served as solvent control which received double distilled water (1ml/kg.b.wt) and 0.3% sodium carboxy methyl cellulose (CMC)

Group II

Served as negative control which received (1ml/kg.b.wt) of double distilled water and 0.3% CMC orally once a day for 7 days. On the Eigth day a single dose of D- Galactosamine (1ml/kg b.wt.) was given.

Group III

Received a single dose of 400 mg/kg b.wt. of Silymarin for 5 days followed by treatment with the toxicant on the 6th and 7th day.

Group IV

Received a single dose of (200mg/kg b.wt.) of Toluene extract of *Curcuma aromatica* for 7 days followed by treatment with the toxicant on the 8th day.

Group V

Received a single dose of (400 mg/kg b.wt.) of Toluene extract of *Curcuma aromatica* for 7 days followed by treatment with the toxicant on the 8th day.

Blood samples from all the animals were withdrawn 24 hours after the final administration of the toxicant by Sino orbital puncture. Serum was separated from the blood and Biochemical tests were carried out to determine the enzyme levels in treated group, toxicant group and control group. D-Galactosamine was administered by intra peritoneal route and all the other drugs were administered by oral route. The separated serum was estimated for ASAT, ALAT, ALP, total protein and Total Billirubin levels as per the procedure given below.

Determination of Total Cell Protein Content by Sulphorhodamine B (SRB) Assay

The monolayer cell culture was trypsinized and the cell count was adjusted to $1.0x10^5$ cells/ml using medium containing 10% new born calf serum. To each well of the 96 well microtitre plates, 0.1 ml of the diluted cell suspension (approximately 10,000 cells) were added. After 24 hours, when a partial monolayer was formed, the supernatant was flicked off, washed the monolayer once and 100 µl of different drug concentrations (1000 to 15.6 µg/ml) were added to the cells in microtitre plates. The plates were then incubated at 37ºC for 3 days in 5% CO_2 atmosphere, and microscopic examination was carried out and observations recorded every 24 hours. After 72 hours, 25 µl of 50% trichloro acetic acid was added to the wells gently such that it forms a thin layer over the drug dilutions to form an overall concentration of 10%. The plates were incubated at 4º C for one hour. The plates were flicked and washed five times with tap water to remove traces of medium, drug and

serum, and were then air-dried. The air-dried plates were stained with SRB dye (0.4% prepared in 1% acetic acid, Sigma Chemicals) for 30 minutes. The unbound dye was then removed by rapidly washing four times with 1% acetic acid. The plates were then air-dried. 100 µl of 10 mM tris base was then added to the wells to solubilize the dye. The plates were shaken vigorously for 5 minutes. The absorbance was measured using Microplate reader (ELISA Reader, Bio-rad) at a wavelength of 540 nm.

The percentage growth inhibition was calculated using the formula below:

$$\% \text{ Growth Inhibition} = 100 - \frac{\text{Average O.D. of test Compound}}{\text{Average O. D. of Control Cells}} \times 100$$

In Vitro Cytotoxicity Studies [6]

Determination of Mitochondrial Synthesis by Micro Culture Tetrazolium (MTT) Assay

The monolayer cell culture was trypsinized using TPVG and the cell count was adjusted to $1.0x10^5$ cells/ml using medium containing 10% new born calf serum. To each well of the 96 well microtitre plate, 0.1 ml of the diluted cell suspension (approximately 10,000 cells) was added. After 24 hours, when a partial monolayer was formed, the supernatant was flicked off, washed the monolayer once and 100 µl of (1000 to 15.6 µg/ml) drug concentrations were added to the cells in microtitre plates. The plates were then incubated at 37^0C for 3 days in 5% CO_2 atmosphere, and microscopic examination was carried out and observations recorded every 24 hours. After 72 hours, the drug solutions in the wells were discarded and 50µl of MTT (MTT: prepared in Hank's Balanced Salt Solution without phenol red [(HBSS-PR), 2 mg/ml, Sigma Chemicals)] was added to each well. The plates were gently shaken and incubated for 3 hours at 37^oC in 5% CO_2 atmosphere. The supernatant was removed and 50 µl of propanol was added and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a Microplate reader (ELISA Reader, Bio-rad) at a wavelength of 540nm.

The percentage growth inhibition was calculated using the formula below:

$$\% \text{ Growth inhibition} = 100 - \frac{\text{Mean O.D. of Individual Test Group}}{\text{Mean O.D. of Control Group}} \times 100$$

Statistical Analysis

The statistical analysis was carried out using student's *t*-test. The results were judged significant if $p<0.05$.

RESULTS AND DISCUSSION

The Powdered Rhizomes of *Curcuma aromatica* were subjected to successive soxhlet extraction using solvent Petroleum ether, Toluene, Chloroform, Ethyl acetate, Acetone, Ethanol, Water.

The percentage yield obtained was tabulated in Table 1.

Table 1: Percentage yield of various extracts of Curcuma aromatica

S.No	Name of the solvent used in the preparation of the extract	Percentage yield of the extract
1	Petroleum ether	5
2	Toluene	9.6
3	Chloroform	4.9
4	Ethyl acetate	2
5	Acetone	5.1
6	Ethanol	7.9
7	Water	13

Qualitative Phytochemical Analysis

Qualitative Phytochemical analysis of extracts of *Curcuma aromatic* shows a majority of compounds including tannins, alkaloids, glycoside, flavonoids and saponins.

Table 2: Phytoconstituents analysis of various extracts of Curcuma aromatica

Name of the test	Different extracts of Curcuma aromatica						
	Petroleum ether	**Toluene**	**Chloroform**	**Ethyl acetate**	**Acetone**	**Ethanol**	**Water**
Alkaloids	–	–	–	–	–	+	+
Carbohydrates	–	–	–	+	+	+	+
Phytosterols	–	+	+	–	–	–	–
Fixed oils and Fats	+	+	–	+	–	–	–
Saponins	–	–	+	–	–	+	+
Tannins	–	–	–	–	–	+	+
Proteins and Amino acids	–	+	–	–	–	+	+
Glycosides	–	+		+	–	–	–
Flavonoids	+	+	+	+	+	+	+
Volatile oils	+	+	–	–	–	–	–
Steroids	–	+	–	–	–	–	–
Terpenoids	+	+	–	–	–	–	–

(+) presence (-) absence

Estimation of Total Phenol Content

Total phenol content of extracts of *Curcuma aromatic* expressed as Gallic acid equivalents. Among the seven extracts tested for both plant, Toluene extract of *curcuma aromatica* has shown high total phenol content, 265±1.08 mg/g of Gallic acid.

Table 3: Estimation of total phenol content

S.No	Various extracts of Curcuma aromatica	Total phenol content (%) mg/g gallic acid of Curcuma aromatica
1	Petroleum ether	90.8±1.67
2	Toluene	265±1.08
3	Chloroform	98.9±1.78
4	Ethyl acetate	231±1.46
5	Acetone	188±0.98
6	Ethanol	83.5±0.13
7	Water	77.3±1.69

Estimation of Total Flavonol Content

Total Flavonol estimation of extracts of *Curcuma aromatica* expressed as Rutin equivalent. Among the seven extracts tested for both plant, Toluene extract of *Curcuma aromatica* has shown high total flavonol content, 175±1.56 mg/g of rutin.

Table 4: Estimation of Total Flavonol content

S.No	Various extracts of Curcuma aromatica	Total flavonol content (%) mg/g Rutin of Curcuma aromatica
1	Petroleum ether	55±1.13
2	Toluene	175±1.56
3	Chloroform	45±0.98
4	Ethyl acetate	132±0.67
5	Acetone	110±1.09
6	Ethanol	103±1.05
7	Water	36±1.45

In Vitro Antioxidant Screening

Diphenyl Picryl Hydrazyl (DPPH) radical Scavenging Method

Among the seven extracts tested for Curcuma aromatica, Toluene extract of *Curcuma aromatica* has shown high potent antioxidant activity with IC_{50} value of 50.62±0.998 µg/ml.

Table 5: Diphenyl picryl Hydrazyl (DPPH) radical scavenging method

S.No	Various extracts of Curcuma aromatica	IC_{50} (µg/ml) *Curcuma aromatica*
1	Petroleum ether	229.5±.1.12
2	Toluene	50.62±0.998
3	Chloroform	235.56±0.634
4	Ethyl acetate	118.75±0.667
5	Acetone	150.55±1.345
6	Ethanol	132.5±1.876
7	Water	427.75±1.436
8	Ascorbic acid	2.75 ± 0.09

Lipid Peroxidation Method (LPO)

Among the seven extracts tested for both plant, Toluene extract of *Curcuma aromatica* has shown high potent antioxidant activity with IC_{50} value of 75±0.87 µg/ml.

Table 8: Lipid peroxidation method

S.No	Various extracts of Curcuma aromatica	IC_{50} (µg/ml) *Curcuma aromatica*
1	Petroleum ether	247±1.67
2	Toluene	75±0.87
3	Chloroform	265±1.43
4	Ethyl acetate	136±1.09
5	Acetone	172±0.98
6	Ethanol	153±0.67
7	Water	447±1.16

Scavenging of Hydrogen Peroxide Radicals

Among the seven extracts tested for both plant, Toluene extract of *Curcuma aromatica* has shown high potent antioxidant activity with IC_{50} value of 43.75±1.24 µg/ml.

Table 9: Scavenging of Hydrogen Peroxide Radicals

S.No	Various extracts of Curcuma aromatica	IC_{50} (µg/ml) *Curcuma aromatica*
1	Petroleum ether	137±1.78
2	Toluene	43.75±1.24
3	Chloroform	250±0.65
4	Ethyl acetate	69±1.08
5	Acetone	123.43 ± 0.95
6	Ethanol	72.50±1.90
7	Water	270±0.01
8	Rutin	36.16±0.90

ABTS Radical Scavenging Method

Among the seven extracts tested for Curcuma aromatica, Toluene extract of *Curcuma aromatica* has shown high potent antioxidant activity with IC_{50} value of 0.038±1.54µg/ml.

Table 10: ABTS radical scavenging method

S.No	Various extracts of Curcuma aromatica	IC_{50} (µg/ml) *Curcuma aromatica*
1	Petroleum ether	8.067±1.12
2	Toluene	0.038±1.54
3	Chloroform	9.485±0.76
4	Ethyl acetate	0.134±0.87
5	Acetone	6.896±1.65
6	Ethanol	0.244±1.86
7	Water	11.674±1.98
8	Ascorbic acid	11.25±1.43

Table 11: Effect of treatment of toluene, ethyl acetate and alcoholic extracts of C*urcuma aromatica* on the biochemical parameters of D-gal intoxicated freshly isolated rat hepatocytes

Treatment	Conc. µg/ml	ASAT U/L	ALAT U/L	ALP U/L	Total Protein gm/dl	Total bilirubin mg/dl
Control	-	15 ± 0.21	17 ± 0.11	26± 0.4	0.882 ± 0.05	0.308 ± 0.005
D-gal	1%	74 ± 0.51[a]	68 ± 0.4[a]	90 ± 2.3[a]	0.176 ± 0.06[a]	0.608 ± 0.01[a]
D-gal and Standard	250	20 ± 0.81[b]	23 ± 0.8[b]	28 ± 0.3[b]	0.72 ± 0.02[b]	0.332 ± 0.002[b]
D-gal and Toluene extract	800 600 400 200	18.6 ± 0.37[b] 21.2 ±0.68[b] 25.1±0.301[b] 31±0.561[b]	24.2 ± 0.59[b] 27.1±0.481[b] 31.4±1.28[b] 32.2 ±1.281[b]	27.3 ± 0.81[b] 28.1±1.02[b] 30.2±1.36[b] 32.6±1.48[b]	0.690±0.04[b] 0.641±0.01[b] 0.581±0.02[b] 0.422±0.01[b]	0.340±0.007[b] 0.363±0.009[b] 0.381±0.02[b] 0.385±0.01[b]
D-gal and Ethylacetate extract	800 600 400 200	28.2±0.45[b] 33.1±0.31[b] 35.4±1.07[b] 39.8 ±1.56[c]	30.4±0.62[b] 32.1±0.77[b] 36.4±1.05[b] 40.2±1.01[c]	31.1±0.9[b] 34.2±1.1[b] 35.6±1.12[b] 38.1±1.01[b]	0.623±0.02[b] 0.587±0.02[b] 0.493±0.04[c] 0.412±0.04[c]	0.492±0.02[b] 0.510±0.04[b] 0.521±0.02[b] 0.590±0.02[c]
D-gal and Alcoholic extract	800 600 400 200	26.8±0.509[b] 30.0±0.48[b] 34.2±0.321[b] 38.1±0.20[c]	28.1±0.68[b] 31.2±2.41[b] 35.6±3.01[c] 38.3±1.2[c]	28.1±0.02[b] 30.2±1.2[b] 33.6±1.36[b] 34.1±1.09[b]	0.610±0.02[b] 0.554±0.01[b] 0.432±0.03[c] 0.402±0.02[c]	0.480±0.009[b] 0.494±0.01[b] 0.526±0.02[b] 0.533±0.01[c]

a = p<0.001 when compared with normal group, b = p<0.001 when compared to D-gal group

c = p<0.01 U/L = Units per litre, mg/dl = milligram per decilitre, gm/dl = gram per deciliter

Table 12: *In-vitro* cytotoxicity activity of *Curcuma aromatica* by SRB method

S.No.	Extract	Concentration in µg/ml	CTC_{50} in µg/ml
1.	Toluene	1000 500 250 125 62.5	110
2.	Ethyl acetate	1000 500 250 125 62.5	440
3.	Alcoholic	1000 500 250 125 62.5	420

Table 13: Hepatoprotective activity of different extracts of *Curcuma aromatica* on D-gal intoxicated HEp-G2 cells by MTT assay

Sr. No.	Treatment	Concentration in µg/ml	% viability
1.	Control	-	100
2.	D-gal	1%	22.2 ± 3.1
3.	D-gal and standard	50	95
4.	D-gal and toluene extract	100 50 25 12.5	70.2 ± 1.61 48.3 ± 1.03 28.1 ± 1.27 20.1 ± 1.22
5.	D-gal and ethylacetate extract	400 200 100 50	36.6 ± 1.12 32.4 ± 1.52 30.1 ± 1.46 29.2 ± 1.59
6.	D-gal and alcoholic extract	400 200 100 50	39.1 ± 2.01 35.2 ± 1.56 33.1 ± 1.61 28.6 ± 1.99

Table 14: Effect of treatment with extracts on biochemical parameters of D-gal intoxicated rats

Treatment	Dose	ASAT (U/L)	ALAT (U/L)	ALP (U/L)	Total protein (gm/dl)	Total bilirubin (mg/dl)
Normal	-	69 ± 6.928	35.67±2.082	284 ± 38.43	5.857±1.319	0.421 ± 0.024
Toxicant	1 ml/kg bw	140.3 ± 8.38^{a}	72.00 ± 2.64^{a}	473.3 ± 17.90^{a}	4.177 ± 0.197^{a}	1.076 ± 0.107^{a}
Toxicant and standard	100 mg/kg bw	79 ± 3.60^{c}	48.33 ± 4.93^{c}	345.7 ± 25.81^{c}	5.577 ± 0.077^{a}	0.4 ± 0.017^{a,c}
Toxicant and toluene extract	200 mg/kg bw	123.3 ± 5.13^{a}	62.67 ± 11.59^{a}	421.7 ± 33.08^{a}	3.923 ± 0.638^{b}	0.793 ± 0.061^{c}
Toxicant and toluene extract	400 mg/kg bw	98.33 ± 5.85^{a,b}	56.00 ± 6.08^{b,c}	365.7 ± 4.04^{b,c}	5.203± 0.761^{b,c}	0.4303 ± 0.046^{b,c}

Number of animals (n=6) a = $p<0.001$ when compared to normal group, b = $p<0.001$ when compared to toxicant group; c = $p<0.01$ when compared to toxicant group bw = body weight, U/L = Units per litre, mg/dl = milligram per decilitre, gm/dl = gram per decilitre.

Hepatoprotective Activity

Effect of treatment of toluene, ethyl acetate, alcoholic extracts of Curcuma aromatica on the biochemical parameters of D-gal intoxicated freshly isolated rat hepatocytes

A significant increase in the levels of ASAT, ALAT, ALP and total bilirubin ($p < 0.001$) and a significant reduction in the level of total protein ($p<0.001$) was observed in hepatocytes exposed to D-gal when compared to normal hepatocytes. These cells when treated along with the toluene extract of the plant showed a significant restoration of the altered biochemical parameters towards the normal ($p<0.001$ when compared to D-gal treated group) and was dose dependent.

Hepatoprotective activity of different extracts of Curcuma aromatic on D-gal intoxicated HEp-G2 cells

The D-gal exposed HEp-G2 cells showed a percentage viability of 22.2%. These exposed cells when treated with different concentrations of the toluene extract of the plant showed a dose dependent increase in percentage viability.

Effect of treatment with extract on biochemical parameters of D-gal intoxicated rats

Intoxication of rats treated with D-gal significantly ($p<0.01$ and $p<0.001$) altered the biochemical parameters when compared with normal control rats. Treatment with toluene extract of the plant at 400 mg/kg body weight showed a significant ($p<0.01$ and $p<0.001$) decrease in ASAT, ALAT, ALP and total bilirubin and a significant ($p<0.01$ and $p<0.001$) elevation in total protein levels in serum when compared with D-gal treated rats.

CONCLUSION

The dried rhizomes of *Curcuma aromatica* was subjected successive extract by using solvent Petroleum ether, Toluene, Chloroform, Ethyl acetate, Acetone, Ethanol, Water (soxhlet extraction)..

Phytochemical studies of extracts showed the presence of terpenoids, flavonoids, tannins, alkaloids, saponins and protein & amino acids.

Estimation of Total Phenol content were done for all the extracts. Among the seven extracts of rhizomes, Toluene extract of *Curcuma aromatica* has shown high Total Phenol content, 265±1.08 mg/g which is expressed in terms of Gallic acid.

Estimation of Total flavonol content was done for all the extracts. Among the seven extracts, Toluene extract of *Curcuma aromatica* has shown high total flavonol content, 175±1.56 mg/g expressed in terms of rutin.

In the DPPH method, toluene extract of *Curcuma aromatica* has shown high potent antioxidant activity with IC_{50} value of 50.62±0.998 µg/ml.

In the LPO method, toluene extract of *Curcuma aromatica* has shown high potent antioxidant activity with IC_{50} value of 75±0.87 µg/ml.

In the Scavenging of Hydrogen Peroxide Radicals method, toluene extract of *Curcuma aromatica* has shown high potent antioxidant activity with IC_{50} value of 43.75±1.24 µg/ml.

In the ABTS Radical Scavenging Method, toluene extract of *Curcuma aromatica* has shown high potent antioxidant activity with IC_{50} value of 0.038±1.54µg/ml.

On the basis of the antioxidant study toluene extract of *curcuma aromatica* was found to be very potent among all extract and it was selected for the in vivo study.

In vitro studies were carried out using primary rat hepatocytes. Ethyl acetate, toluene and alcoholic extracts of *Curcuma aromatica* with concentrations ranging from 200 µg/ml – 800 µg/ml were studied. All the extracts showed considerable protectively against D-Galactosamine induced toxicity in primary hepatic cells. Toluene extract at concentration of 200 to 800 µg/ml showed a significant restoration of the altered biochemical parameters towards the normal and it was comparable with standard silymarin, using D-Galactosamine as toxicant. All the biochemical parameters were estimated and compared with that of the control. In this study standard silymarin has been used along with the tests

extracts (200, 400, 600, 800 µg/ml). This silymarin showed very good restoration of enzyme levels to normal.

In-vitro systems based on cultured immortalized hepatoma cell lines from man are widely used for studies on toxicity, xenobiotic metabolism and carcinogenesis. The use of cells from man rather than animals not only avoids the killing of animals, but also has further advantage that possible species differences in responses, both to hepatotoxins and to plant extracts are avoided. The ethyl acetate, toluene and alcoholic extracts of *Curcuma aromatica* were tested on HEp-G2 cells because HEp-G2 retains many of the morphological and biochemical characteristics of normal cells. First, cytotoxicity studies were carried out using SRB method and according to the CTC_{50} values the dose of the extract was decided for MTT assay for hepatoprotective activity of extracts on D-gal intoxicated HEp-G2 cells. Toluene extract was found to have dose dependent increase in percentage viability of the cells.

The studies performed using *in vivo* model, rat groups treated with D- Galactosamine alone showed a marked elevation of levels of ASAT, ALAT, ALP and total bilirubin and decrease in the levels of total Protein. Whereas in the animals with plant extracts along with D-Galactosamine, it was restored considerably towards the normal levels.

The 200 and 400 mg/kg b.w toluene extracts of *Curcuma aromatica* showed a significant restoration of enzyme levels in *in-vivo* studies. The results were encouraging to state that the hepatoprotective activity exhibited by the toluene extracts of *Curcuma aromatica* was found to be nearly equivalent with standard silymarin.

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