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Original Article

Comparative Evaluation of Effect of Extracting Solvents on Therapeutic Activities of *Curcuma Aromatica* Rhizomes

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Abstract

There is the need for new drugs with better action and lesser side effects. A lot of research is being undertaken to find out new plant drugs, their extraction and pharmacological evaluation. One of such plant is *Curcuma aromatica* which belongs to the genus Curcuma and reported to have high ethno-botanical values in traditional medicine. The present study evaluates comparative pharmacological activity of four different extracts of *Curcuma aromatica* in healthy Wistar strain albino rats.Extraction of active constituents from powdered plant rhizomes was done by cold maceration technique using water, ethanol, hydroalcohol and toluene as solvents separately to get respective extracts. Acute oral toxicity study was performed to determine toxicity or side effects associated with the prepared plant extracts. The extracts were evaluated for anti-diabetic, anti-oxidant, anti-inflammatory and analgesic activity. Anti-diabetic activity was comparatively evaluated in alloxan induced diabetic rat models. Toluene extract was found to have relatively higher anti-diabetic activity. In vitro antioxidant potential of different extracts of was evaluated by DPPH radical scavenging activity. The results show anti-oxidant potency in toluene extract slightly better than other extracts. Aqueous extract of *Curcuma aromatica* rhizomes was found to have more potency in treatment of carrageenan induced paw oedema and also in its anti-nociceptive action against heat induced pain by Eddy's hot plate method in rats.Though the anti-diabetic and anti-oxidant activities were slightly superior in toluene extract, the risk of residual amount of toluene remained in the final extract and its teratogenic property subdues its benefits of treatment. Findings of this research prove our hypothesis that oral administration of aqueous rhizome extract has potential anti-diabetic, anti-oxidant, anti-inflammatory and analgesic activities.

Keywords: Analgesic activity, Anti Diabetic, Anti-inflammatory, Anti-Oxidant, Cold maceration, Curcuma aromatic.

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1. Introduction

Plants of Curcuma species, belonging to family Zinziberaceae are reported to show various pharmacological activities, the prominent being *Curcuma longa*, reported to have diverse pharmacological and therapeutic activities [1]. *Curcuma aromatica* [2], belongs to the same genus and moreover is reported to contain more percentage of active constituents than *Curcuma longa* [3]. Extracts of *Curcuma aromatica* from different extracting solvents are reported for antioxidant and anti-bacterial activity [4], anti-inflammatory activity [5], wound healing activity [6] and Anti-diabetic activity [7] while oil shows inhibitory effect on proliferation of hepatoma in mice [8] and mosquito repellent activity [9]. The reason behind these activities of the extracts might be because of different chemical constituents in varying proportions depending upon extracting solvent. The present research work was designed to comparatively evaluate four distinguished activities i.e. anti-diabetic, anti-oxidant, anti-inflammatory and analgesic activities in the aqueous, ethanolic, hydroalcoholic, toluene extracts of the rhizomes obtained from the respective solvents. Since organic solvents are associated with disadvantages like volatility, flammability, biocompatibility problems and residual extent, the study focuses on comparative effectiveness of aqueous extract with organic solvent based extract.

2. Materials and Methods

2.1. Materials

2.1.1. Plant Material

Rhizomes of *Curcuma aromatica* were collected from forest area near R.R district, Hyderabad, India. The specimen was authetified from Horticultural Department, Acharya N G Ranga Agricultural University, Hyderabad, Andhra Pradesh, India.

2.1.2. Drug Substances and Devices

Metformin was received as a gift samples from Dr Reddy's laboratory, Hyderabad, India. Indomethacin and Diclofenac sodium were obtained as research samples from Lupin Pharmaceuticals, Goa, India. Alloxan, DPPH and all other chemicals were purchased from SD Fine Chemicals, Mumbai, India and were of AR grade.

2.1.3. Experimental Animals

Healthy wistar albino rats of either sex of approximately same average body weight of 120-130g, procured from National Institute of Nutrition, Hyderabad, India were selected for the study. Qualified personnel inspected each animal and judged them as healthy and suitable to be experimental subjects. Each animal was assigned a distinct identification number and immediately placed in quarantine for 2 days for acclimatization. Each animal was observed for changes in general appearance and behaviour to ensure minimum inter subject variability [10]. The study animals were housed in clean polypropylene cages in animal house at temperature of 18 ± 2^0c, %RH 35-55% and supplied with water *ad libitum* and standard rodent feed obtained from Nutrilabs, Hyderabad, India. A total of 246 rats were used for this research study. Grouping of rats according to different studies is depicted in table 1. The animal study was carried out according to the guidelines prescribed in the Guide for the Care and Use of Laboratory Animals (published by the National Academy of Science, National Academy Press, Washington, D.C.)

2.2. Methods

2.2.1. Preparation of Extracts

The collected rhizomes were shade dried and subjected for size reduction to get coarse powder using dry grinder and passed through sieve number 40. The powder was divided into four equal parts of 2.5 kg and cold macerated [11] with purified water, ethanol, water: ethanol (1:1) mixture and toluene overly for 7 days at room temperature with occasional stirring. The containers were closed with cap and aluminium foil to prevent evaporation and exposure to contamination. After 7 days, the contents were filtered by vacuum filtration and the filtrate was evaporated by rotary vacuum evaporator. The product concentrate was collected and stored in airtight container in refrigerator below 10^0C. The suspensions of the extracts were prepared freshly during treatment regimen using 1%w/v Sodium CMC for per oral administration in rats.

2.2.2. Preliminary Phytochemical Screening

Aqueous, ethanolic, hydroalcoholic and toluene extracts were evaluated using standard chemical tests [12] to find out the presence or absence of various phyto-constituents like alkaloids (Dragondroff), carbohydrates (Molisch), glycosides (Borntrager's test), saponins, phytosterols and steroids (Salkowski), flavonoids (Shinoda), tannins (Gelatin test), phenolic compounds (Ferric chloride), Proteins (Ninhydrin) and terpenoids.

2.2.3. Acute Oral Toxicity Study

Acute oral toxicity study was performed as per OECD-423 guidelines to determine the toxicity of different extracts in rats [13]. Dose levels of 5, 50, 300, 2000 and 5000mg/kg of body weight of prepared extracts were used and each dose level was administered to 3 rats progressively through oral route by oral feeding syringe. All rats after dosing were

Table 1. Grouping of experimental animals for different studies.

Study	Group										
	Normal control	Negative control	Standard drug	Aq. Ext		H.Alc. Ext		Eth. Ext		Tol. Ext	
				300	500	300	500	300	500	300	500
Anti diabetic activity	6	6	6	6	6	6	6	6	6	6	6
Anti inflammatory activity	-	6	6	6	6	6	6	6	6	6	6
Analgesic activity	6	-	6	6	6	6	6	6	6	6	6

In every study number of animals used are 6. '-' indicates no group in that study.

observed individually at the 1st, 2nd, 4th, 6th hours and once daily over 14 days for clinical signs of toxicity such as changes in rate and depth of breathing, colour of body surfaces, frequency and nature of movement, marked involuntary contraction or seizures, contraction of voluntary muscle, loss of reflex and daily examination of body weight. After 14 days, all rats that received highest dose 5000mg/kg b.wt of extracts were sacrificed and their liver tissue histopathology was examined.

2.2.4. Evaluation of Anti diabetic Activity in Alloxan Induced Diabetic Model

Diabetes was induced in rats by a single intra-peritoneal (i.p) injection 1ml of freshly prepared 150mg/kg body weight (b.wt) solution of Alloxan monohydrate [14]. Normal rats were placed in control group, only those rats which developed high blood glucose levels of more than 300mg/dl were randomly divided into treatment groups, see table 1. Treatment was given daily after 2 days of alloxan administration till 14 days. Potency of extracts was compared with that of standard drug metformin at dose 150mg/kg body weight [15].

2.2.4.1. Blood Sample Collection

Blood samples were collected from tail vein [16] at 1st, 2nd, 4th, 8th and 12th hours post treatment on day 1 and on 7th, 14th days thereafter to determine the fasting blood glucose levels of rats in the study using One Touch Select Glucometer-Johnson & Johnson.

Parameters like body weight and fluid intake were also evaluated as an indication of the potency of plant's extract in treatment of symptoms like weight gain (body weight), polydipsia (fluid intake) associated with diabetes [17] and results are tabulated in tables 5, 6 respectively.

2.2.4.2. Evaluation of Anti-Oxidant Activity

The evaluation of radical scavenging activity i.e., anti oxidant activity was

Table 2. Preliminary phytochemical screening of various extracts of *Curcuma aromatica* rhizomes.

Test for	Aqueous extract	Ethonolic extract	Hydro alcoholic extract	Toluene extract
Alkaloids	+	+	+	-
Anthraquinones	-	+	+	+
Carbohydrates	+	+	-	-
Fixed oils & fats	-	-	-	+
Flavonoids	+	+	+	+
Glycosides	-	-	-	+
Phytoserols	-	-	-	+
Proteins & amino acids	+	+	+	+
Saponins	-	+	+	-
Steroids	-	-	-	+
Tannins	+	+	+	-
Terpenoids	+	+	+	+
Volatile oils	-	+	+	+

conducted by DPPH i.e., 1,1- diphenyl-2-picrylhydrazyl radical scavenging method [18]. Solutions of the extracts having concentrations 5, 10, 25, 50, 100, 250, 500 mcg/ml were prepared. Each extract solution of 0.5 ml was mixed separately with 3.0ml of o.1mM DPPH, in 95% ethanol and allowed to stand at room temperature for 30min under light protection. The absorbance of this solution was measured at 517nm in UV spectrophotometer Shimadzu 1800. Difference in absorbance between test and control i.e., DPPH in ethanol was calculated and expressed as % scavenging of DPPH radical [19]. The anti oxidant activity of extracts was compared with standard natural antioxidant Ascorbic activity.

The scavenging activity of samples corresponded intensity of quenching DPPH. Lower the absorbance of reaction mixture, higher is the free radical scavenging activity.

2.2.4.3. Evaluation of Anti-Inflammatory Activity

Anti-inflammatory activity of *Curcuma aromatica* extracts [6] was assessed by carrageenan induced paw oedema method [20]. Wistar albino rats were randomly divided into control group I and treatment groups II to X, see table 1. The animals were starved overnight and were injected with 0.1 mL of 1% carrageenan in 0.9% normal saline, [21] under the plantar aponeurosis of the right hind paw. Treatment was given through oral route 30 min prior to carrageenan injection in all groups as 1ml suspensions in 0.5%w/v of tween 80, Indomethacin 10 mg/kg in 0.5% tween 80 was used as standard. The anti-inflammatory activity was determined as the percentage of inhibition of inflammation after it was induced by carrageenan by taking volume of inflammation in control group as 100%. The paw volume of all the rats was measured with Plethysmometer-Orchid scientific, after 15 min and 30 min and every hour thereafter upto four hours post carrageenan injection [22].

2.2.4.4. Evaluation of Analgesic Activity

Eddy's hot plate method [23] was used to study analgesic activity of different extracts of *Curcuma aromatica*. The animals were fasted

Table 3. Acute oral toxicity studies for different extracts of *Curcuma aromatic*a rhizomes.

Group	Dose (mg/kg b.wt)	Death/total in different extract groups				% Death			
		Aqueous. Ext	Ethanolic. Ext	Hydro alc. Ext	Toluene. Ext	Aq. Ext	Eth. Ext	H.alc. Ext	Tol. Ext
I	50	0/3	0/3	0/3	0/3	0	0	0	0
II	100	0/3	0/3	0/3	0/3	0	0	0	0
III	300	0/3	0/3	0/3	0/3	0	0	0	0
IV	2000	0/3	0/3	0/3	1/3	0	0	0	33.33%
V	5000	0/3	0/3	0/3	2/3	0	0	0	66.66%

overnight with free access to water. Heat stimulus of pain was applied to animals by using Eddy's hot plate-Medicraft, India, maintained at a temperature of 55±1°C. The animals which showed paw licking or jump response within 8 sec were selected for the study and were divided randomly in treatment and control groups as 6 in each, 3/sex. Group I as normal control, received 1ml 1%w/v CMC suspension p.o. Group II served as standard and were injected Diclofenac sodium [24] (10mg/kg) intraperitoneally (i.p). Treatment with extracts was given in oral route, see table 1.

All the animals were individually placed on the hot plate maintained at 55±0.5°C. The response time was noted as the time at which animals reacted to the pain stimulus by heat. The response was characterised by acute discomfort either by paw licking or jumping in an attempt to avoid pain. The cut off time for the application of stimulus was 20 seconds. The increase in mean reaction time in treated groups was determined and compared with that of control group.The animals were subjected to the same test procedure at 30, 60, 90 and 120min after the administration of test, standard, control to record the pain latency [25].

3. Results and Discussion

3.1. Preliminary Phytochemical Screening

The results of various Qualitative tests to find out the presence of various phyto-constituents in different extracts are shown in table 2.

The extracts show presence of alkaloids, carbohydrates, flavonoids, tannins, terpenoids, phytosterols, curcuminoids. Important constituents like flavonoids, terpenoids were found to be present in all the extracts and alkaloids in all extracts except toluene extract.

Table 4. Fasting blood glucose levels (mg/dl) (mean ± SEM) (n=6) in different treatment group.

Experimental Group	Fasting Blood glucose levels(mg/dl) in different treatment group rats expressed as (Mean ± SEM)						
	POST TREATMENT						
	Day 1					Day 7	Day 14
	1st hr	2nd hr	4th hr	8th hr	12th hr		
Vehicle control	95±12	92±10	93± 12	95±10	92±10	97±11	95±10
Diabetic control	382±20	380± 20	383± 20	389±25	381±20	395±25	413±15[a]
Metformin (150mg/kg b.wt)	407±23	395± 20	388± 20	383±23	380±20	98±25	94±25[b]
Aqueous Extract (300mg/kg b.wt)	395±10	394±15	393±15	390±15	387±15	144±20	118±20[b]
Aqueous Extract (500mg/kg b.wt)	421±15	418± 15	411± 13	408±10	403±13	130±20	104±20[b]
Ethanolic Extract (300mg/kg b.wt)	401±20	400± 20	397±20	392±20	390±20	140±20	116±25[b]
Ethanolic Extract (500mg/kg)	428±20	420± 25	410± 15	402± 20	396±20	122±30	98±20[b]
Hydro alcoholic (300mg/kg b.wt	400±21	398± 20	397± 17	394±18	391±17	142±20	117±25[b]
Hydro alcoholic (500mg/kg b.wt)	398±19	395± 18	387± 17	383±19	376±16	131±19	104±25[b]
Toluene (300 mg/kg b.wt)	399±18	397± 19	393± 18	389±17	385±18	136±18	110±20[b]
Toluene (500 mg/kg b.wt)	395±21	384± 20	372± 19	365±18	360±19	119±20	96±23[b]

Values are expressed as mean ± S.D. p <0.01, treated diabetic groups[b]Vs diabetic –ve control group[a]

3.2. Acute Oral Toxicity Study

Aqueous, hydroalcoholic and ethanolic extracts of *Curcuma aromatica* rhizomes did not exhibit any mortality or any profound clinical signs of toxicity such as changes in rate and depth of breathing, colour of body surfaces, frequency and nature of movement, marked involuntary contraction or seizures, contraction of voluntary muscle, and loss of reflex and abnormal changes in body weight even at a highest dose administered. The acute oral toxicity test results are summarised in table 3.

From the results of acute oral toxicity test, toluene extract caused death in 33.33% rats at dose of 2000mg/kg body weight, 66.66% death at 5000mg/kg body weight, whereas in case of other extracts, there were no signs of toxicity or death.

Table 5. Body weights of Alloxan induced diabetic rats prior and post treatment with 500mg/kg b.wt Aqueous and Ethanolic extracts of *Curcuma aromatica* rhizome.

Group	Initial body weight before experiment(g)	Body weight at 4th day (g)	Final body weight at 14th day
Normal control	121.2±0.41	125.3±0.45	138.4±0.40
-ve control Alloxan treated	120.5±0.43	193.5±0.52	220.2±0.32[a]
+ve control Metformin	121.2±0.35	194.3±0.42	182.3±0.42[b]
Aq. Extract 500mg/kg b.wt	121.3±0.42	192.5±0.54	181.4±0.21[b]
Eth. Extract 500mg/kg b.wt	120.4±0.40	193.3±0.36	176.4±0.34[b]
H.Alc Extract 500mg/kg b.wt	121.5±0.38	193.3±0.48	180.2±0.36[b]
Tol. Extract 500mg/kg b.wt	120.6±0.41	195.6±0.50	170.4±0.41[b]

Values are expressed as mean ± S.D. p <0.05, treated diabetic groups[b]Vs diabetic control group (-ve control)[a]

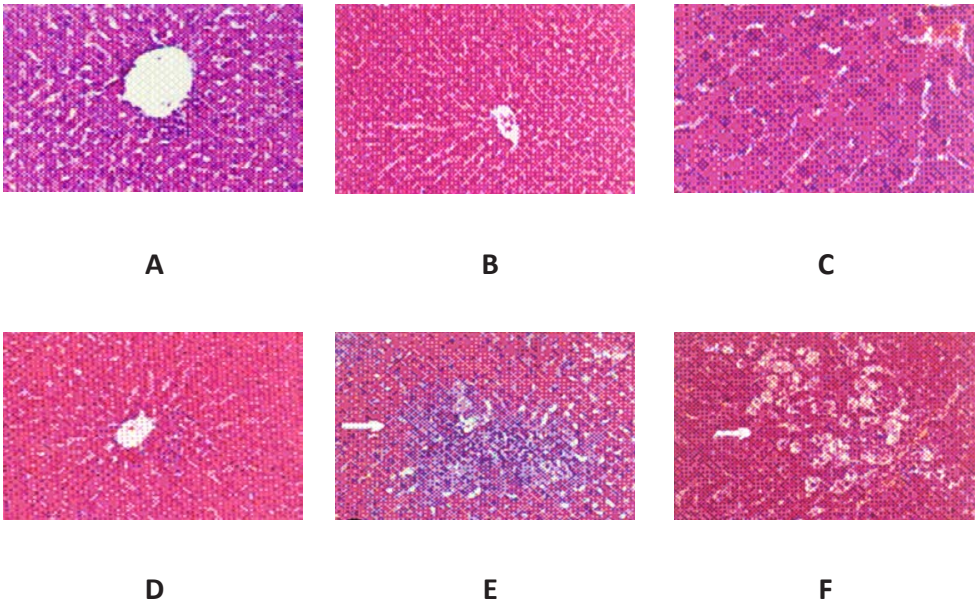

Figure 1. The representative hepatic histopathology stained with hematoxylin, eosin and magnified at 200x.

A. Normal control liver; **B**. Aqueous s extract 5000mg/kg treated liver; **C**. Hydroalcoholic extract 5000mg/kg treated liver;**D**. Ethanolic extract 5000mg/kg treated liver;**E**. Toluene extract 2000mg/kg treated liver;**F**. Toluene extract 5000mg/kg treated liver tissue specimen

healthy control rat shows no signs of pathological changes. No changes were observed in case of aqueous and hydroalcoholic extracts treated rats .The liver structure resembles to that of normal rat. The liver appears to have normal lobular architecture with central vein and radiating hepatic cords, see figures 1A, 1B, and 1C.

Bridging necrosis was present in ethanolic extract treated rat which exhibited areas of normal liver architecture and scattered patches of necrotic hepatocytes. see figure 1D.

The liver section of rats treated with toluene extracts shows necrosis of hepatocytes with liver architecture destruction and inflammatory infiltrate in portal tracts at dosage of 2000mg/kg and 5000mg/kg but with extensive hepatocyte degeneration at higher dosage, see figures 1E, 1F. This may be one of the reasons for observed mortality in toluene extract treated group. Apoptotic bodies were not detected in any of the slides. The liver histopathology raises the concerns of safety issues of organic solvents as extracting fluids for herbal drugs.

3.3. Anti-Diabetic Activity

Administration of alloxan 150mg/kg b.wt, i.p. led to the induction of diabetes with high blood glucose levels of more than 300mg/dl. The results of anti-hyperglycaemic effect of the extracts on the fasting blood glucose levels of diabetic rats are enumerated in table 4. Daily treatment of *Curcuma aromatica* aqueous and ethanolic extracts for 14 days led to a dose-dependent decrease in fasting blood glucose levels.

On 1st day of treatment i.e., 2 days after alloxan injection, fasting blood glucose levels in all the treatment groups there was a negligible decrease at 1st, 2nd, 4th, 8th and 12th hours.

On 7thday, fasting blood glucose level increased further in untreated diabetic negative control group, there was a significant reduction ($p<0.01$) in fasting blood glucose levels in standard metformin group. FBG levels in all animals treated with the extracts decreased in descending order toluene extract-ethanolic extract-Hydroalcoholic extract-Aqueous extract.

On 14th day, blood glucose levels of untreated diabetic negative control group II has shown further increase, whereas significant decrease ($p<0.01$) in fasting blood glucose levels was seen in all treatment groups in comparison with negative control. All the extracts at 500mg/kg b.wt lowered the abnormal fasting blood glucose levels to higher extent when compared to 300mg/kg b.wt extracts of the same. After14 days also toluene extract showed highest antidiabetic activity next to standard metformin following the earlier trend. But considering the risk benefit ratio, aqueous extract can also be considered as effective since residual amount of solvents remaining in the extract in this case have no toxicity concerns while in other cases, amount of residual solvents have to be estimated and controlled especially in case of toluene [26]. Table 4 shows Fasting blood glucose levels in all the animal groups.

Table6. Average Fluid intake by rats before and after treatment of diabetes.

Group	Initial fluid uptake (ml/day)	Fluid uptake on 4th day (ml/day)	Fluid uptake on 14th day(ml/day)
Normal control	21.25±0.20	21.40±0.25	21.25±0.40
-ve control Alloxan treated	21.50±0.15	87.25±0.25	98.50±0.25[a]
+ve control Metformin	21.35±0.30	73.30±0.35	43.35±0.40[b]
Aq. Extract 500mg/kg b.wt	21.45±0.40	82.55±0.20	41.45±0.20[b]
Eth. Extract 500mg/kg b.wt	22.15±0.40	78.35±0.35	39.40±0.35[b]
H.Alc Extract 500mg/kg b.wt	21.25±0.25	80.40±0.25	40.25±0.40[b]
Tol. Extract 500mg/kg b.wt	20.35±0.30	75.60±0.35	38.45±0.30[b]

Values are expressed as mean ± S.D. p <0.05, treated diabetic groups[b]Vs diabetic control group (-ve control)[a]

Table 7. Determination of 1, 1- diphenyl-2 picrylhydrazyl (dpph) radical scavenging activity.

Concentration mcg/ml	% Inhibition by standard ascorbic acid	% Inhibition by aqueous extract	% Inhibition by ethanolic extract	% Inhibition by hydroalcoholic extract	% Inhibition by toluene extract
5	22.08±0.12	16.11±0.15	17.12±0.11	14.06±0.10	18.03±0.13
10	39.08±0.11	34.12±0.12	35.11±0.14	30.12±0.13	36.13±0.10
25	53.16±0.14	47.17±0.11	49.12±0.12	41.13±0.12	51.15±.13
50	59.19±0.10	51.10±0.14	55.12±0.14	47.14±0.14	57.11±0.14
100	64.16±0.13	56.17±0.12	58.11±0.15	52.12±0.12	60.10±0.13
250	73.11±0.12	65.14±0.14	68.08±0.12	59.09±0.14	70.09±0.16
500	78.19±0.11	71.07±0.11	75.13±0.12	69.07±0.12	76.18±0.13

Values are expressed as mean ± S.D. p<0.05

Average changes in body weights of rats were determined after diabetes was induced, prior treatment and after completion of treatment regimen. Results obtained as shown in table 4, 5 support the hypothesis that, the proposed plant has antidiabetic activity i.e., plant extracts may be responsible to treat weight gain complication in diabetes. Fluid uptake was also observed and results were tabulated in table 6.

3.3.1. Statistical Analysis

The values are expressed as mean ± SEM. Statistical significance test for comparison was done by ANOVA and student t test, using Graph Pad Prism software program.

a- Group II was compared with Group I. $p<0.01$

b- Treatment groups were compared with group II. $p<0.01$

From the statistical analysis, the results show that anti diabetic activity of *Curcuma aromatica* Aqueous and Ethanolic extracts was found to be significant and potency of 500mg/kg b.wt dose of Group V and Group VII was found to be very much similar to standard drug metformin 150mg/kg b.wt of Group III.

After the induction of diabetes the average body weights in all the treatment groups was increased and so was the average fluid uptake. From the results of the changes in average body weights and average fluid uptake in rats

Table 8. Comparative evaluation of anti-inflammatory activity among different extracts of *Curcuma aromatica* rhizomes.

Treatment	Dose (mg/kg, p.o)	Mean paw volume (ml) (m±sem)						%Inhibition of oedema (4 hrs)
		15min	30min	1hr	2hr	3hr	4hr	
Carrageenan control	1ml	0.76 ±0.03	1.36±0.01	1.65±0.03	1.76± 0.02	1.79± 0.01	1.76 ±0.03[a]	-
Indomethacin	10	0.73± 0.02	1.33±0.02	0.81±0.03	0.60±0.02	0.42±0.02	0.25± 0.05[b]	85.79%
Aqueous	300	0.73±0.03	1.35±0.03	1.01±0.04	0.81±0.05	0.64±0.01	0.47± 0.03[b]	73.29%
Aqueous	500	0.74±0.02	1.36±0.02	0.84±0.02	0.63±0.03	0.45±0.02	0.32± 0.03[b]	81.81%
Ethanolic	300	0.71±0.02	1.39±0.02	1.11±0.03	0.95±0.01	0.78±0.01	0.62± 0.04[b]	64.77%
Ethanolic	500	0.74±0.01	1.38±0.02	0.90±0.01	0.73±0.02	0.57±0.03	0.42± 0.01[b]	76.13%
Hydro alcoholic	300	0.75± 0.01	1.37±0.01	0.93± 0.01	0.71± 0.03	0.58±0.02	0.43± 0.01[b]	75.56%
Hydro alcoholic	500	0.73±0.02	1.35±0.02	0.86±0.04	0.65±0.04	0.51±0.04	0.36± 0.03[b]	79.54%
Toluene	300	0.74 ±0.02	1.36±0.02	1.14± 0.01	0.96± 0.01	0.80±0.03	0.62± 0.04[b]	64.77%
Toluene	500	0.72±0.01	1.36±0.03	1.04±0.02	0.85±0.03	0.70±0.01	0.54± 0.03[b]	69.31%

Values are expressed as mean ± S.D. p <0.01, treatment groups[b]Vs Carrageenan control group (-ve control)[a]

before and after treatment indicated that gain in average body weight and increased fluid uptake in diabetic rats was affectively controlled by extracts. The results indicate potential of extracts in speedy recovery in diabetes associated symptoms apart from reduction in blood glucose.

3.4. Anti-oxidant Activity

Importance of anti oxidant activity is that many diseases like neuro-degeneration, gastric ulcer [18], diabetes can occur because of oxidative degeneration by free radicals. It was reported that the effectiveness in antioxidant activity of *Curcuma aromatica* was more compared to *Curcuma longa* [27], [28]. Anti oxidant study in this plant is carried out to alleviate free radical oxidation and from the results it was found that toluene extract of the plant rhizome has significant anti-oxidant activity ($p<0.05$) very much similar to standard antioxidant ascorbic acid. The other extracts of the plant rhizomes also showed significant antioxidant activity but slightly less than toluene extract, see table 7.

Decreasing order at 500mcg/ml: Ascorbic a cid (78.19±0.11)>Toluene extract (76.18±0.1) >Ethanolic extract (75.13±0.12) > Aqueous ex tract (71.07±0.11) > Hydroalcoholic extract (6 9.07 ±0.12). Since the antioxidant activity of all the other extracts lies in the close range we propose here that there may be some new active constituent responsible that has to be found out in st future udies.

3.5. Anti-Inflammatory Activity

It is found that there is no decrease in the paw oedema till 1hr after which there was significant reduction in oedema in all the treatment groups except carrageenan control group. There was a dose dependent activity with all the extracts. The %inhibition of paw oedema in all treatment groups at 4hr was significant ($P<0.01$) compared to carrageenan control group. The %inhibition in paw oedema after treatment was calculated, from the results shown in table 8 it is observed that there was a decreasing order of potency among the extracts in the following order standard indomethacin >aqueous extract > hydroalcoholic extract > ethanolic extract> toluene extract. From the results of anti inflammatory study, it can be inferred that aqueous extract has relatively more dose dependent anti-inflammatory activity compared to other extracts.

3.6. Analgesic Activity

The plant is reported for its wound healing activity, since wounds are associated with pain, the study of analgesic activity of plant was carried out to find out its potency to increase pain latency. All the extract of the *Curcuma aromatica* rhizomes showed significant ($p<0.01$) increase in the pain latency. In case of aqueous extract the activity was even better than standard drug Diclofenac sodium treated group. The effectiveness followed similar trend as of anti-inflammatory activity. It is found that the treatment groups

Table 9. Evaluation of analgesic activity among different extracts of *Curcuma aromatica* rhizomes.

Drug dose (mg/kg b.wt.)	Pain latency (seconds in m±sd) At different time periods after administration					%Increase in pain latency after 90min
	0 min.	30 min.	60 min.	90 min.	120 min.	
Control 1ml	4.5±0.10	4.3±0.11	4.1±0.15	4.6±0.15	4.5±0.14[a]	0%
Standard diclofenac sod.10mg/kg i.p	4.5±0.12	12.5±0.12	14.5±0.11	15.7±0.12	13.7±0.15[b]	71.33%
Aqueous 300	4.6±0.11	9.3±0.10	12.2±0.15	15.2±0.16	13.5±0.11[b]	69.73%
Aqueous 500	4.6±0.10	9.6±0.13	12.4±0.13	15.4±0.13	13.5±0.15[b]	70.12%
Ethanolic 300	4.3±0.15	6.8±0.14	9.3±0.16	11.2±0.15	9.3±0.14[b]	61.60%
Ethanolic 500	4.2±0.12	6.8±0.15	9.2±0.15	11.3±0.14	9.5±0.13[b]	62.83%
Hydroalcoholic 300	4.3±0.14	7.9±0.12	11.1±0.14	13.3±0.13	11.3±0.15[b]	67.66%
Hydroalcoholic 500	4.5±0.11	8.3±0.13	11.3±0.12	13.5±0.15	11.4±0.13[b]	67.66%
Toluene 300	4.0±0.13	6.5±13	9.4±0.11	10.6±0.14	9.2±0.10[b]	62.26%
Toluene 500	4.1±0.12	6.7±12	9.4±0.13	10.7±0.11	9.2±0.14[b]	62.26%

Values are expressed as mean ± S.D. p <0.01, treatment groups[b]Vs control group [a]

show maximum pain latency at 90min. The pain latency effect starts to decrease thereafter i.e. 120 min in all the cases including the standard drug. There was no dose dependent relation to the activity with all the extracts treated groups unlike observed in earlier activities. The increase in latency period at different time points significantly differed ($p<0.01$) compared to baseline values within the same treatment groups as shown in table 9.

4. Conclusion

The positive results for anti-diabetic, anti oxidant, anti inflammatory and analgesic potential of C*urcuma aromatica* rhizomes of aqueous, ethanolic, hydroalcoholic and toluene extracts confirms the use of the plant CA in treatment of various ailments and diseases in traditional medicine. The plant rhizomatic extracts showed dose dependent antidiabetic activity, anti inflammatory activity, in-vitro DPPH scavenging activity but the analgesic activity of plant extracts was not in dose dependent manner. From the results toluene extract was found to show relatively more potency in treating alloxan induced diabetes and anti oxidant activity, while aqueous extract showed more potency of anti inflammatory activity, analgesic activity. Considering the risk benefit ratio among the different extracts, we propose here that the plant *Curcuma aromatica* rhizomatic aqueous extract has the potent anti diabetic, anti oxidant, anti inflammatory and analgesic activity.

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