



Hepatoprotective and antioxidant efficacy of ethanolic extract of *Curcuma amada* rhizome against paracetamol induced hepatic toxicity in experimental animals

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ABSTRACT

The modern medicine gets up a massive hike in the treatment of various ailments but scarcely any drugs having to stimulate liver activity, offering tutelage to the liver from any harm or promote the palingenesis of hepatic cells. However, a variety of herbal drugs applied in the conventional system of medicine for liver efficiency. Therefore, rhizome of *Curcuma amada* (Family: Zingiberaceae) are selected to allocate the hepatoprotective activity in scientifically approbate experimental models. This study is an effort to explore the 50% ethanolic extract of rhizome of *Curcuma amada* (CAE) in a different experimental model for hepatoprotective activities and *in vitro* antioxidant. 50% ethanolic extract of *Curcuma amada* (CAE) (100 and 200mg/kg) hepatoprotective efficiency was examined against paracetamol (1000 mg/kg) induced hepatotoxicity, elevated hepatic enzymes alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP), total bilirubin direct bilirubin content in the serum. Moreover, CAE induced antioxidant protection against hepatotoxic disservice of paracetamol was estimated by evaluating several antioxidative biomarkers such as reduced glutathione (GSH), superoxide dismutase (SOD), and catalase (CAT) in the blood serum by using spectrophotometric analysis. It could be concluded that the rhizome of *C. amada* possesses an intense antioxidant property. Its active principle wipeout free radicals and strive a tutelary impact against oxidative harm induced to cellular macromolecules. 50% ethanolic extract of *C. amada* (CAE) is tenable to amplify and maintain the working of hepatic enzymes implicated in detention of ROS.



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INTRODUCTION

Curcuma Amada is a herbaceous plant and belongs to family *Zingiberaceae* and commonly called as Amahaldi in Hindi, Mango ginger in English, Kathumachal in Sanskrit, and Manghainchi in Malayalam. This is widely cultivated in Gujrat and grows as a wild plant in West Bangal, Uttar Pradesh, Tamil Nadu and Karnataka (Samant, 2012). Ayurveda is a traditional Indian system of medicine and utilized the plant as an anti-inflammatory, blood purifier, antioxidants, anti-asthmatic, stomachic, carmi-

native and also used as regenerator of liver tissue. The rhizome is significant in the cachectic condition of pitta, dyspepsia, flatulence, colic ulcer, colic wound, pruritis, cough, bronchitis, anorexia, fever, sprain, skin disease and gout. *Curcuma Amada* widely applied for culinary purposes to enhance the colour and flavour of food along with this is having a convention to use in Chinese folk system of therapy, especially in the cure of jaundice, menstrual ailments, haemorrhage, hematuria and as an anti-inflammatory (Joshi and Chauhan, 2013). The acetone extract of rhizome of *C. Amada* consists the chief chemical constituents are curcumin, dimethoxy curcumin and bis-methoxy curcumin (Gupta and Singh, 2013). Car-3-ene and cis-ocimene contribute the characteristic mango odour of the rhizome. The rhizome of *Curcuma amada* is having 1% essential oil encompass 47.2% ocimene, 18% α -pinene, 11.2% linalool, 9.3% safrole and 9.1% linalyl acetate (Chopra et al., 1956). The phenolic acid derivatives included 26% caffeic acid (195mg/g), 24% gentisic acid (180mg/g), 20% ferulic acid (150mg/g), 10% gallic acid (75mg/g), 7% cinnamic acid (52.5mg/g), 7% protocatechuic acid (52.2mg/g) and very little quantity 4% of syringic acid (75mg/g), and 2% coumaric acid (30mg/g) are found in *Curcuma Amada* (Siddaraju and Dharmesh, 2007). The rhizome of *Curcuma amada* Roxb, having 45% starch and showed their functional activities (Policegoudra et al., 2007b). The characteristic mango odour of *Curcuma amada* is due to car-3-ene and cis-ocimene. Microwave-assisted ethanolic extraction of *Curcuma amada* rhizome isolated mangiferin (Padmapriya et al., 2012). The extraction of *Curcuma Amada* rhizome by using chloroform as a solvent, three terpenoids were isolated, namely amadannulen, amadaldehyde and difurocumenolol (Policegoudra et al., 2007a). The aqueous and ethanolic extract exhibited significant analgesic, antipyretic and anti-anthelmintic activity (Rakh et al., 2014). The extract of *Curcuma amada* rhizomes showed anti-inflammatory activity in the albino rat. The methanolic extract of both the leaves and rhizome of *Curcuma Amada* showed the anticancerous activity (Sivaprabha et al., 2015). *Curcuma amada* has outstanding action against allergic rhinitis (Amresh et al., 2004).

MATERIALS AND METHODS

Plant collection and identification

The rhizome of *Curcuma amada* Linn. (Family: Zingiberaceae) was collected. The rhizome was taxonomically identified and authenticated at the National Botanical Research Institute (NBRI), Luc-

know. A herbarium specimen (NBRI-SOP-202) was conserved in the departmental museum for upcoming reference.

Extract Preparation

The rhizome of *Curcuma amada* (CAE) separated and dried under shade. The coarse powder of rhizome was subjected to petroleum ether to secede the fatty material and followed the marc was extracted by using 50%v/v ethanol. The ethanolic extract was decanted, cooled and concentrated by using a rotary evaporator, and resulting viscous extract was freeze-dried under reduced pressure.

Phytochemical test

Phytochemical screening of 50% ethanolic extract rhizome of *curcuma amada* was performed (Trease and Evans).

Animal

Sprague-Dawley rats (140±20 g) and mice (30±10 g) of both genders were obtained from the animal house. The animals were placed for a week before and during the experiments in a cross-ventilated departmental animal house at 22±2°C, which facilitated for light and dark cycle of 12 hrs. Standard rodent pellet diet was provided to the animal (Bharat Ansh Scientific). The study protocol was approved by Institutional Animal Ethical Committee MGIP, Lucknow, registration no: (1957/PO/Re/S/17CPCSEA).

Acute toxicity

Acute toxicity was studied on mice (30±10 g). The acute toxicity of ethanolic extracts of *Curcuma amada* (CAE) was figured out at doses of 5, 50, 300, 500 and 2000 mg/kg, conceding the OECD 423 guidelines and dose of 2000 mg/kg illustrated lethal manifestations. Therefore, in agreement with OECD guideline 423, it is represented as an LD₅₀ cut-off value. Doses 100 and 200 mg/kg body weight were preferred for pharmacological investigation by fixed-dose methods.

Hepatoprotective activity

Paracetamol induced hepatotoxicity

The experiments were carried out on Sprague-Dawley rats of weighing (140±20 g), which were divided into five groups and each having six animals. Group first designated as normal control which was administered orally 10ml/kg body weight of water as vehicle once a day for 28 days. Group second served as toxic and administered paracetamol 1000 mg/kg. Rats of Groups third, fourth and fifth received paracetamol at a dose of 1000mg/kg body weight *p.o.* for the same duration. Group third and fourth rats were simultaneously getting 50%

Table 1: Effect of 50% ethanolic extract of *C. amada* (CAE) on hepatic biomarkers against Paracetamol-induced hepatotoxic rats

Group	Treatment Dose (mg/kg)	SGOT (U/L)	SGPT/(U/L)	Total bilirubin (mg/dl)	Direct bilirubin (mg/dl)	ALP level (U/L)
I	Control	125.42±10.32	76.43±6.31	0.915±0.020	0.195±0.016	132.13±12.18
II	Paracetamol 1000 mg/kg	551.87±21.48***	351.20±23.63***	1.71±0.11***	1.520±0.140	332.21±22.40***
III	CA 100+ Paracetamol	202.32±18.21***	164.32±17.32***	1.07±0.09**	0.823±0.034***	200.21±19.17**
IV	CA 200+ Paracetamol	147.55±11.80***	101.80±13.85***	0.91±0.03***	0.675±0.027***	161.46±17.55***
V	Silymarin 100mg/kg	138.32±10.64***	92.42±11.22***	0.90±0.03***	0.386±0.025***	148.21±17.08***

Values are mean of ± S.E.M. n=6

P values: †<0.001 compared with respective control group (Group-I)

P value: *<0.05, **<0.01, ***<0.001 compared with controlgroup (Group-1)

Table 2: Effect of 50% ethanolic extract of *C. amada* (CAE) on serum lipid profile against Paracetamol induced hepatotoxic rats

Group	Treatment & dose (mg/kg)	TC (mg/dl)	TG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
I	Control	113.4±4.7	121.7±3.379	39.66±1.015	49.44±4.674
II	Paracetamol 1000 mg/kg	181.4±3.753	175.4±3.916	28.23±1.153	118.1±3.192
III	CA 100 + Paracetamol	163.7±3.784*	155.0±2.902**	31.67±1.137*	101.1±4.403
IV	CA 200 + Paracetamol	138.3±2.404***	131.3±2.404***	33.34 ±1.32**	73.17±4.090***
V	Silymarin 100 mg/kg	119.6 ± 2.87	126.0±2.128***	37.56±1.282***	56.40±4.125***

Values are mean of ± S.E.M. n=6

P values: †<0.001 compared with respective control group (Group-I)

P value: *<0.05, **<0.01, ***<0.001 compared with controlgroup (Group-1)

Table 3: Effect of 50% ethanolic extract of *C.amada* on antioxidant parameters against Paracetamol induced hepatotoxic rats

Group	Treatment (mg/kg)	GSH	LPO	CAT	SOD
I	Control	42.33±3.13	185.44±11.13	73.73±9.31	9.51±1.20
II	Paracetamol 1000 mg/kg	20.01±3.03***	480.37±20.23***	38.23±4.65**	4.10±1.30***
III	CA 100 + Paracetamol	32.28±4.21*	232.02±15.86***	58.11±6.14*	7.16±1.30*
IV	CA 200 + Paracetamol	37.02±3.21**	192.52±12.11***	63.55±7.11**	9.12±1.10**
V	Silymarin 100mg/kg	39.38±2.71***	191.51±11.01***	69.11±7.31**	9.12±1.20**

Values are mean of ± S.E.M. n=6

P values: †<0.001 compared with respective control group (Group-I)

P value: *<0.05, **<0.01, ***<0.001 compared with controlgroup (Group-1)

ethanolic extract of *Curcuma amada* (CAE) at a dosing of 100 and 200 mg/kg body weight p.o. and group fifth administered silymarin 100 mg/kg body weight for the same duration. On day 29th, the overnight fasted animals were euthanized by decapitation, under ether anaesthesia (Rao et al., 2006). The liver specimen was obliterated, and the blood sample was gathered. The gathered blood sample was permitted to clot, and serum was isolated at 2500 rpm for fifteen minutes. The biochemical criterion like serum catalyst: aspartate aminotransferase (AST, U/L), alanine amino transaminase (ALT, U/L), (Reitman and Frankel, 1957), alkaline phosphatase (ALP, U/L) (King, 1965), and total direct bilirubin (TBL, mg/dl) (Malloy and Evelyn, 1937) were assayed by using kits. Lipid peroxidation (LPO) (Jamall and Smith, 1985) catalase (CAT) (Aebi, 1984) and reduced glutathione (GSH) (Anderson, 1985) were estimated in the liver homogenate.

RESULTS AND DISCUSSION

Phytochemical screening

The 50% ethanolic extract of *Curcuma amada* rhizome was exposed to a qualitative test. It showed the presence of carbohydrate, protein, steroid, glycoside, flavonoid, tannin, saponin and alkaloid.

Acute toxicity study

The extract did not give any indication of mortality up to a dose of 200mg/kg p.o. in experimental animals. Hence, at a dosing of 100mg/kg and 200mg/kg body weight were utilized for hepatoprotective investigations.

Effect of *C. amada*(CAE) on hepatic biomarkers against paracetamol-induced hepatopathy

The hepatoprotective property of 50 % ethanolic extract of *Curcuma amada* (CAE) was measured by evaluating the levels of ALT, AST, ALP, total bilirubin and direct bilirubin in experimental rodents. Results showed that the ingestion of paracetamol in Sprague Dawley rats caused a significant ($p < 0.05$) growth in the level of ALT, AST, ALP, total bilirubin and direct bilirubin level in the serum specimen contrast to the control group animals (Table 1). After the treatment with extract, the level of all the hepatic biomarkers was reduced in a dose-dependent manner. The decline in the level of AST, ALT, totals bilirubin and direct bilirubin were more pronounced than the hepatoprotective activity of CAE particularly at a dose of 200mg/kg was comparable to Silymarin potent hepatoprotective agent.

Effect of *C. amada* on serum lipid profile levels

The effect of 50% ethanolic extract of *Curcuma*

amada (CAE) in different dosing of 100mg/kg and 200mg/kg were studied on serum lipid profile of animals. Results of serum lipid profile were depicted in Table 2. Paracetamol treated animals showed the increased levels of total serum cholesterol, serum triglyceride, serum low density lipoprotein however reduced in serum high density lipoprotein level. The results which show that exposure of animals to paracetamol cause significant ($p < 0.001$) elevation in the cholesterol levels. The administration of CAE caused a reduction in the measure of Serum TC, Serum TG, serum LDL level, although serum HDL level increased in a dose-dependent manner.

Effect of *Curcuma amada* on antioxidant enzymes

The hepatoprotective activity of CAE was also evaluated by measuring its antioxidant activity by determining by the levels of GSH, SOD, CAT, and lipid peroxidation in the liver homogenate. The data indicated that the level of GSH, CAT and SOD were significantly decreased in paracetamol-intoxicated rats in comparison to the untreated animals (Table 3). The administration of CAE retral the extent of GSH, CAT and SOD towards the normal level in a dose-dependent manner, which indicates its strong antioxidant efficacy of CAE extract. It exhibited that GSH level was significantly ($P < 0.05$) raised to ($32.28 \pm 4.21^*$) mg/dL (in animals that were treated with 100mg/kg) and ($37.02 \pm 3.21^{**}$) mg/dL (in animals that were treated with 200mg/kg) when treated with different concentrations of CAE compared to the paracetamol-intoxicated rats ($20.01 \pm 3.03^{***}$) mg/dL. However, administration of CAE increased serum level of CAT significantly are ($58.11 \pm 6.14^*$) and (63.55 ± 7.11) mmol/mol in animals treated with 100 and 200mg/kg, respectively. However, oral administration of CAE also restored LPO and SOD levels in a dose-dependent pattern.

Paracetamol is a benign antipyretic agent, which is innocuous at therapeutic doses but with toxic doses can cause fatal liver necrosis in humans, mice and rats. When taken in large doses, it occurs a potent hepatotoxin, elicit fulminant liver and renal tubular necrosis, and that can be fatal in both humans and animals (Friedman, 1993). Most incidence of paracetamol concerned hepatic dilapidation has measured from the single-dose taken in an attempt to commit suicide. In some cases, accidents occurred with large single or multiple doses received with therapeutic intent (Muriel et al., 1992). The probable course of action that may be accountable for safe-keeping against paracetamol inspired hepatocellu-

lar dilapidation by rhizome of *C. Amada* extract comprise the following mechanism (i) *C. Amada* behave in itself as a free radical scavenger that inhibits those radicals concerned in paracetamol metabolism by microsomal enzymes. Thus clenching the oxygen-related free radicals. *C. amada* could interrupt their interaction with polyunsaturated fatty acids and hence stanch the progress of lipid peroxidative activity leading to MDA production. (ii) Treatment with 50% ethanolic extract of rhizome of *C. Amada* manifested a tremendous impact on the glutathione status of liver cells. *C. amada* significantly increases the liver SOD and GSH. These measures suggest that an elevated amount of glutathione in the liver would provide the tissue a better conservancy contrary to oxidative stress, thus facilitating to the elimination of hepatotoxicity induced by different hepatotoxins as reported earlier (Moore *et al.*, 1985). The preliminary chemical observation of 50% ethanolic extract of *C. amada* (CAE) shows the presence of flavonoids. These flavonoids may offer hepatocellular protection at least in part, through its decadence of oxidative stress interruption of cytochrome P-450 and by bolstering the levels of antioxidants and antioxidant enzyme system such as SOD and GSH (Gilani *et al.*, 1998; Peres *et al.*, 2000). The mechanism of action exhibited by *C. amada* (CAE) also predicts that the hepatocellular protection may be due to cell membrane stabilization, hepatic cell palingenesis and inducement of antioxidative enzyme systems. The paracetamol (PCM) induced hepatotoxicity are relevant to other acute hepatic inflammation and promotion of liver ailment with an increment of AST, ALP, ALT, LDH, cholesterol, bilirubin (Davidson and Eastham, 1966). The extract of CAE at different dosing of 100 and 200mg/kg significantly ($p < 0.05$ to < 0.01) decreases the AST, ALP, ALT, total bilirubin, direct bilirubin and physical parameters, were raised in the PCM intoxicated rats and also produced maximum well-being at a dose of 200 mg/kg body weight in both studies.

CONCLUSIONS

The 50% ethanolic extract of *Curcuma amada* (CAE) at dose level 100 mg/kg and 200 mg/kg were administered orally to induced rats. In this present study, it has shown that *Curcuma Amada* demonstrates a strong hepatoprotective activity at a dose of 200 mg/kg. The acute toxicity of extract of *Curcuma Amada* is safe to be used in different formulations. The biochemical parameters induced by the administration of paracetamol were enhanced under the effect of *Curcuma Amada* at 100 mg/kg 200 mg/kg. Thus, the choice of suitable plant extract in case of hepatotoxicity induced by paracetamol is essential.

The present study shows the concept of traditional medicine has many benefits.

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Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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