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IN VIVO AND *IN VITRO* HEPATOPROTECTIVE POTENTIAL OF KAEMPFEROL, A FLAVONE GLYCOSIDE FROM *CAPPARIS SPINOSA*

P. Bigoniya[1]*, C. S. Singh[1] and B. Shrivastava[2]

[1]Radharaman College of Pharmacy, Ratibad, Bhopal- 462 002, Madhya Pradesh, India.

[2]School of Pharmaceutical Sciences, Jaipur National University, Jaipur- 302 025, Rajasthan, India.

*Corresponding Author Email: p_bigoniya2@hotmail.com

ABSTRACT

This study was designed to investigate in-vivo and in-vitro hepatoprotective activity of kaempferol isolated from Capparis spinosa L. in CCl_4 intoxicated rat. Kaempferol is a bioflavonoid reported with antioxidant, anti-inflammatory and antiapototic effects which can prevent CCl_4 induced hepatic lipid peroxidative damage mediated by oxidative free radical. Kaempferol was isolated from C. spinosa stem, and authenticated by HPTLC and HPLC techniques. Wistar albino rats were treated with vehicle, silymarin (20 mg/kg) and kaempferol at 25, 50 and 100 mg/kg, p.o. continuously for 14 days. CCl_4 was administered on 7^{th} day to every alternate day for a week. Pre and post treatment of kaempferol resulted in the significant decrease (P<0.01 and 0.001) in relative weight of liver. Kaempferol showed extremely significant (P<0.001) decrease in thiopental induced sleeping and 76.13% hepatoprotection in bromosulphathalein (BSP) uptake test. Treatment with kaempferol (50 and 100 mg/kg) resulted in significant normalization (P<0.01 to 0.001) of biochemical parameters except protein and albumin. Kaempferol showed dose dependent elevation of glutathione and reduction in lipidperoxidase enzymes in liver tissue. Kaempferol effectively reversed CCl_4 induced damage and showed normal architecture of hepatocytes, portal tracts and central veins with mild necrosis and lymphocyte infiltration. The results revealed the protective and preventive effect of kaempferol on CCl_4 induced hepatotoxicity by ameliotaing oxidative stress and hepatocyte damege, which may be mediated through its antioxidant, anti-inflammatory and antiapototic property.

KEY WORDS

Antioxidant, Bromosulphathalein, Capparis spinosa L., Hepatoprotective, Kaempferol, Thiopental

INTRODUCTION

Flavonoids are a group of plant secondary metabolites with variable phenolic structures. They are widely distributed in the plant kingdom and are common constituents of fruits, vegetables, grains, barks, roots, stems, flowers, tea and some beverages. Flavonoids may play a role in decreasing risk of chronic diseases associated with a diet rich in plant derived foods. A positive relationship between the ingestion of foods containing flavonoids and a reduced risk of developing cancer and cardiovascular diseases has indeed been observed in some epidemiological studies (Hertog et al, 1993; Neuhouser, 2004). In vitro and in vivo investigations have shown plausible mechanisms by which flavonoids may confer cancer and cardiovascular protection (Middleton et al, 2000). Evidence also suggests that certain flavonoids may be useful in the treatment of several diseases with wide range of pathologies, which has revealed that flavonoids are common bioactive constituents of plants.

The flavonoid kaempferol (3,5,7-trihydroxy-2-(4-hydroxyphenyl)-4H-1-benzopyran-4-one) is a yellow compound, commonly found in plant derived foods (Calderon-Montano et al, 2011). Pharmacological studies revealed that kaempferol suppressed

eosionphil infiltration and inflammation in airway epithelial cells of mice with allergic asthma (Gong et al, 2012). Xiao et al, (2012) reported that kaempferol protects rats from doxorubicin induced cardiotoxicity in vivo and in vitro. Zhang and Liu, (2011) assessed that kaempferol flavone improves chronic hyperglycemia impaired pancreatic beta-cell viability and insulin secretory function. Li and Pu, (2011) investigated neuroprotective effect of kaempferol on mouse model of Parkinson's disease. Cellular level interaction studies showed antioxidant (Choi, 2011), antiapoptotic (Siegelin et al, 2008), free radical scavenging (Singh et al, 2008) and anti-inflammatory effects of kaempferol.

The present study aims at the isolation and standardization of kaempferol from *C. spinosa* stems followed by its characterization with the aid of chromatographic techniques. Evaluation of hepatoprotective and antioxidant potential of kaempferol against CCl_4 induced hepatotoxicity was performed in order to ascertain the hepatoprotective effect of kaempferol.

MATERIALS AND METHODS

Sample collection and preparation: The stems of *Capparis spinosa* L. were procured from Department of Agriculture Botany, Mahatma Phule Krishi Vidyapeeth, Ahmednagar, Maharashtra, India and taxonomically identified by Dr. Suresh Dhodake, Officer Incharge, Medicinal and Aromatic Plants Project, MPKV, Ahmednagar, Maharashtra, India (Ref. no. MAPP/certificate/27). The collected plant parts were shade dried, pulverized with mechanical pulverizer for size reduction and put in transparent polythene bags.

Chemicals: Kaempferol standard was purchased from Sigma Aldrich, USA. Bromosulphathalein was procured from HiMedia Lab. Ltd., Mumbai and thiopental was obtained as gift sample from Neon Lab. Ltd., Mumbai. Other chemical used were of analytical grade and were procured locally. Biochemical kits were obtained from Aspen Diagnostic Pvt. Ltd., India.

Isolation of kaempferol: Dry powdered stems of *C. spinosa* (2.00 kg) were extracted three times with methanol containing 1% concentrated HCl at room temperature following the cold maceration. The marcs were combined and filtered through medical gauze and concentrated using rotary vacuum evaporator. The weight of crude extract was 18.8 gm and the yield was found to be 0.94%. The whole crude extract was hydrolyzed with HCl (1.8 N, 5 ml for each 150 mg of crude extract) under nitrogen atmosphere and the resulting solution was extracted with amyl alcohol (Hadizadeh et a, 2003; Strack and Way, 1989).

The organic layer was washed with distilled water until the pH of aqueous phase remained constant. The organic layer was separated out, evaporated in rotary vacuum evaporator and obtained as solid mass (3.94 gm). The percentage yield of solid mass was found to be 0.19%. This acid hydrolyzed product was subjected to thin layer chromatography and HPTLC on Merck silica gel 60 F_{254} plates using benzene: acetic acid: water in the ratio of 125:72:3 to confirm the isolation of kaempferol (Agarwal and Kamal, 2007).

Authentication of isolated kaempferol by analytical HPTLC technique: HPTLC analysis was performed on a CAMAG system equipped with high pressure auto sample injector of 100 µl capacity, scanner, UV chamber and CAMAG-Linomat-5 software system for data analysis. One mg of standard kaempferol and two mg of isolated kaempferol was dissolved in 5 ml of ethanol and filtered through the Whatman filter paper. Two µl of standard and sample solution was spotted individually by auto injector as a band onto a 10 × 10 cm^2 precoated Merck silica gel 60 F_{254} plate. The plate was run upto a height of 8 cm in saturated mobile phase of benzene: acetic acid: water in the ratio of 125:72:3. After development, plate was removed, air dried and finally dried with aid of mechanical drier. The scanning of plate was performed at 254 nm and data was recorded.

Authentication of isolated kaempferol by analytical HPLC technique: The chromatography analysis was performed on Shimadzu liquid chromatography

system, equipped with prominence (LC-20AD) pump, SPD-M20A photodiode array UV-visible detector working in the range 190-800 nm, a quaternary solvent delivery system, degasser (DGU-20A5) and a rheodyne injection valve fitted with a 20 µl injection loop. The chromatographic data was recorded and processed with LC solution integrated software. Baseline resolution of kaempferol was obtained at 25 ± 2°C using stainless steel column (Thermo, 250 mm × 4.6 mm), packed with octadecylsilane bonded to porous silica (5 µm). An isocratic solvent system consisting of solution A (water: orthophosphoric acid, pH 3.0) and solution B (acetonitrile: methanol in ratio of 75:25) in the ratio of 50:50 was used. The mobile phase was passed through 0.45 Polyvinylidene fluoride filter (PVDF) and degassed before use. The flow rate was kept constant at 1 ml/min and effluents were monitored at 370 nm (Maheshwari et al, 2011) following injection 20 µl sample.

Standard and sample kaempferol solutions were prepared individually by dissolving 0.5 mg/ml in methanol. From this, 2 ml of each were diluted to 10 ml with methanol to get a final concentration of 100 µg/ml. The solutions were filtered through a 0.45 µm membrane filter prior to HPLC analysis.

Experimental animals: Laboratory bred Wistar albino rats of either sexes weighing between 150-250 gm were maintained under standard laboratory conditions at 25 ± 2°C, relative humidity 60 ± 10% and photoperiod of 12 hr-dark and light. Commercial pellet diet and water were provided *ad-libitum*. Animals were allowed for free access to water and food during the experiment but no water and food were allowed before and after 1 hr of dosing. In order to avoid diurnal variation, all the experiments were carried out at same time of day i.e. between 10:00 am to 05:00 pm. Ethical Committee approvals was obtained from Institutional Animal Ethical Committee of Radharaman College of Pharmacy, Bhopal before carrying out the experiments (IAEC/RCP/Oct-2010/01).

Assessment of hepatoprotective activity of kaempferol

Experimental design: Animals were randomly divided into 6 groups with 12 rats in each. Group I and III-VI were treated as vehicle control, positive control (silymarin, p.o.) and three different doses of kaempferol (25, 50 and 100 mg/kg, p.o.) continuously for 14 days. On 7th day, groups III-VI including group II (CCl_4 control) were treated with CCl_4 (1 ml/kg), 2 hr after drug treatment and afterwards on alternate days for a week. On the 14th day, 2 hr after drug treatment, 6 animals from each group were used to determine thiopental induced sleeping time and remaining 6 animals from each group were used to access serum biochemical parameters for serum marker enzymes, bromosulphathalein (BSP) uptake test, free radical scavenging ability of liver and histopathological study of liver. The oral LD_{50} value of kaempferol in rat was reported to be 980 mg/kg (Anonymous, 2011). Therefore, an oral dose of 25, 50 and 100 mg/kg of kaempferol has chosen for this study, which is relatively safe and can achieve the maximal protective effect after CCl_4 administration. Vehicle control animals were treated with normal saline (0.2 ml/100 gm). Standard drug silymarin (20 mg/kg) and test drug kaempferol were prepared freshly in 1% gum acacia in normal saline.

Evaluation parameters: Body weights of all the animals were recorded on 1st, 7th and on 14th day before sacrifice. On the 14th day, 2 hr after drug treatment six animals of each group were given thiopental sodium (40 mg/kg) intraperitoneally and the effects of drug on CCl_4 induced prolongation of thiopental sodium sleeping time were studied (Singh et al, 2001). Remaining six animals of each group were anaesthetized by light ether anaesthesia and blood was withdrawn by intracardiac puncture. Blood was allowed to coagulate for 30 min at room temperature and serum was separated by centrifugation at 3000 rpm for 5 min (Remi Centrifuge, Model RM 12 C). The serum was used to estimate serum SGOT, SGPT, ALP, cholesterol, HDL, LDL, tri-glyceride, total and direct bilirubin, protein

and albumin as per method described in diagnostic kits (Aspen Diagnostic Pvt. Ltd., India). The liver, kidney, spleen and heart were harvested, washed in normal saline, blotted in filter paper and weighed. Each liver was cut into three parts. From part one, three slices of 60 mg were weighted and used for BSP uptake. Percentage hepatoprotection was calculated with the method described by Ranjan and Subramanyan, (1965). Second part of the liver was used for estimation glutathione, lipid peroxidase and superoxide dismutase (Ellman, 1959; Jeyanthi and Subramanian, 2010; Mohanty et al, 2004). Third part of the liver was preserved in 10% formalin solution for histopathological assessment of liver damage. Hematoxylene and Eosin Staining of liver tissues and permanent tissue slides was prepared by following the method described by Nanji et al, (2001)

Statistical analysis

The results were expressed in term of Mean ± SEM. Experimental data of various physical and biochemical parameters were analyzed using one way ANOVA followed by Turkey-Kramer multiple comparisons using InStat-3 graph pad version. Differences between compared groups were considered significant at $P<0.05$.

RESULTS

Kaempferol was obtained as a yellow powder (m.p. 278°C). The isolated kaempferol and standard kaempferol was subjected to TLC and HPTLC analysis on Merck silica gel 60 F_{254} plates using solvent system benzene: acetic acid: water (125:72:3). Isolated kaempferol showed two spots having R_f value 0.32 (82.34% area) and 0.70 (17.66% area). The R_f of isolated and standard kaempferol was found to be identical (0.32) and HPTLC chromatograms were represented in **Figure 1 a and b**. The isolated kaempferol was further authenticated by HPLC fingerprinting and chromatograms were represented in **Figure 2 a and b**. The retention time of isolated kaempferol (14.967 min) was well comparable with that of standard kaempferol (14.965 min).

Vehicle control group showed 6.05 and 8.93% increase in body weight on 7^{th} and 14^{th} day respectively. CCl_4 treated group showed an increase of 6.38% in body weight on 7^{th} day followed by a drastic decrement of 5.63% with reduced food consumption on 14^{th} day. Standard drug silymarin and kaempferol (50 and 100 mg/kg) treatment showed 6.52, 4.65 and 5.24% increase in body weight on 7^{th} day followed by gain in body weight by 1.96, 1.71 and 2.13% respectively on 14^{th} day (**Table 1**).

Administration of kaempferol (25, 50 and 100 mg/kg) for 14 days resulted in the significant decrease ($P<0.01$ and 0.001) in relative weight of liver whereas, all the three doses of kaempferol showed non significant changes in relative weights of kidney, spleen and heart in comparison to negative control group..

CCl_4 intoxication significantly increased the thiopental induced sleeping time (213.68 ± 5.03 min) compared to vehicle control (69.13 ± 4.16 min). The treatment with silymarin and kaempferol (25, 50 and 100 mg/kg) extreme significantly ($P<0.001$) decreased thiopental induced sleeping time. Kaempferol (100 mg/kg) revealed a protection of 97.05% compared to 96.64% of silymarin (**Table 1**).

In vitro BSP uptake study of kaempferol (25, 50 and 100 mg/kg) treated rats showed dose dependent hepatoprotection of 62.50, 73.86 and 76.13% at 30 min respectively whereas, silymarin showed a hepatoprotection of 79.54% (**Table 2**).

Biochemical parameters revealed that the levels of marker enzymes SGOT, SGPT, ALP, cholesterol, triglyceride, LDL, total and direct bilirubin in plasma was severely increased whereas, the level of HDL, protein and albumin decreased in CCl_4 intoxicated animals. In contrast to that, kaempferol (50 and 100 mg/kg) showed significant ($P<0.01$ and 0.001) decrease in SGOT, SGPT, ALP, cholesterol, triglyceride, LDL, and total and direct bilirubin level in comparison to CCl_4 treated animals. Whereas, serum HDL level was significantly increased ($P<0.01$) at 100 mg/kg dose of kaempferol in comparison to CCl_4 toxicated animals. Results revealed that

kaempferol showed non significant change in the protein and albumin levels when compared to the CCl_4 toxicated animals. Silymarin treatment extremely significantly (P<0.001) normalized all the serum biochemical parameters except albumin and protein (**Table 3**).

Results of free radical scavenging ability showed that kaempferol treatment caused dose dependent elevation of glutathione (P<0.05 to 0.001) at 25, 50 and 100 mg/kg doses in comparison to the negative control group whereas, a significant (P<0.05 and 0.001) decrease in the lipid peroxidase level was observed at 50 and 100 mg/kg doses. Kaempferol treatment significantly enhanced (P<0.05) superoxide dismutase level at 100 mg/kg dose (**Table 4**).

The histopathological examination of kaempferol (50 mg/kg) showed normal lobular pattern with a mild necrosis and lymphocyte infiltration. Kaempferol (100 mg/kg) showed normal parenchymal architecture, portal tracts and central veins with few zonal necrosis (10×) (**Figure 3**).

Table 1: Effect of kaempferol on body weight and thiopental induced sleeping time of CCl_4 treated rats.

Treatment (mg/kg, p.o.)	Body weight in gm		% Change in body weight	% Hepatoprotection in thiopental induced sleeping time
	0 day	14th day	14th day	
Vehicle control	170.16 ± 7.52	185.36 ± 8.06	8.93	-
CCl_4 (1 ml/kg)	157.50 ± 5.62	148.62 ± 12.64	-5.63	-
Silymarin (20)	150.46 ± 7.18	153.40 ± 8.64	1.96	96.64
Kaempferol (25)	175.62 ± 9.27	170.50 ± 7.43	-2.91	63.23
Kaempferol (50)	218.75 ± 8.72	222.50 ± 10.37	1.71	93.47
Kaempferol (100)	175.30 ± 6.03	178.75 ± 5.64	2.13	97.05

Values are expressed as mean ± SEM for six animals

Table 2: Hepatoprotective effect of kaempferol on BSP uptake of CCl_4 treated rat liver slices.

Treatment (mg/kg, p.o.)	% Hepatoprotection		
	10 min	20 min	30 min
Vehicle control	-	-	-
CCl_4 (1 ml/kg)	-	-	-
Silymarin (20)	24.70	53.26	79.54
Kaempferol (25)	17.64	32.60	62.50
Kaempferol (50)	20.00	41.30	73.86
Kaempferol (100)	23.52	47.82	76.13

Values are expressed as mean ± SEM for six animals

Table 3: Hepatoprotective effect of kaempferol on serum biochemical parameters of CCl_4 treated rats.

Treatment (mg/kg, p.o.)	SGOT IU/l	SGPT IU/l	ALP U/l	Cholesterol mg/dl	Triglyceride mg/dl	HDL mg/dl	LDL mg/dl	Bilirubin mg/dl		Protein gm/dl	Albumin gm/dl
								Total	Direct		
Vehicle control	32.62 ± 3.06	37.62 ± 4.51	51.07 ± 3.94	98.29 ± 5.18	121.05 ± 4.57	39.54 ± 2.73	34.54 ± 2.27	1.16 ± 0.02	0.46 ± 0.05	7.45 ± 1.21	5.26 ± 1.29
CCl_4 (1 ml/kg)	82.53 ± 4.69	86.23 ± 5.92	127.12 ± 9.78	170.65 ± 10.26	191.27 ± 9.43	19.53 ± 1.16	112.86 ± 5.24	2.24 ± 0.03	1.35 ± 0.11	4.08 ± 0.29	2.17 ± 0.22
Silymarin (20)	45.66 ± 4.12***	41.82 ± 3.34***	58.19 ± 5.03***	110.85 ± 6.73***	128.38 ± 9.15***	43.28 ± 2.04***	41.89 ± 2.81***	1.56 ± 0.03***	0.64 ± 0.03***	6.52 ± 0.98^{ns}	4.02 ± 0.79^{ns}
Kaempferol (25)	62.81 ± 5.46*	56.93 ± 2.55***	108.45 ± 5.37^{ns}	145.53 ± 6.02^{ns}	165.09 ± 5.20^{ns}	21.74 ± 1.65^{ns}	90.78 ± 6.38*	1.28 ± 0.01***	0.59 ± 0.03***	6.86 ± 0.92^{ns}	3.33 ± 0.98^{ns}
Kaempferol (50)	57.91 ± 2.67**	49.73 ± 3.04***	82.81 ± 4.92***	126.26 ± 6.28***	144.81 ± 6.34***	23.08 ± 1.98^{ns}	74.21 ± 5.93***	1.17 ± 0.02***	0.58 ± 0.09***	6.92 ± 1.10^{ns}	3.51 ± 0.64^{ns}
Kaempferol (100)	49.85 ± 2.57***	38.31 ± 2.96***	65.48 ± 2.78***	105.95 ± 5.24***	129.99 ± 4.58***	30.34 ± 1.54**	49.66 ± 4.26***	1.14 ± 0.01***	0.52 ± 0.07***	6.95 ± 1.13^{ns}	3.57 ± 0.39^{ns}

Values are expressed as mean ± SEM for six animals

Table 4: Effect of kaempferol on free radical scavenging ability of CCl_4 treated rat liver

Treatment (mg/kg, p.o.)	Glutathione µg/gm of liver	Lipid peroxidase nmol/gm of protein	Superoxide dismutase Unit/mg of protein
Vehicle control	25.37 ± 1.93	4.61 ± 1.04	7.11 ± 0.97
CCl_4 (1 ml/kg)	6.62 ± 1.69	19.15 ± 1.50	3.04 ± 0.39
Silymarin (20)	20.15 ± 2.44***	6.22 ± 2.37***	7.13 ± 1.09**
Kaempferol (25)	15.12 ± 1.09*	16.27 ± 1.32^{ns}	3.37 ± 0.22^{ns}
Kaempferol (50)	16.07 ± 1.58**	13.84 ± 0.83*	4.56 ± 0.39^{ns}
Kaempferol (100)	18.92 ± 1.52***	10.16 ± 1.28***	6.02 ± 0.34*

Values are expressed as mean ± SEM for six animals

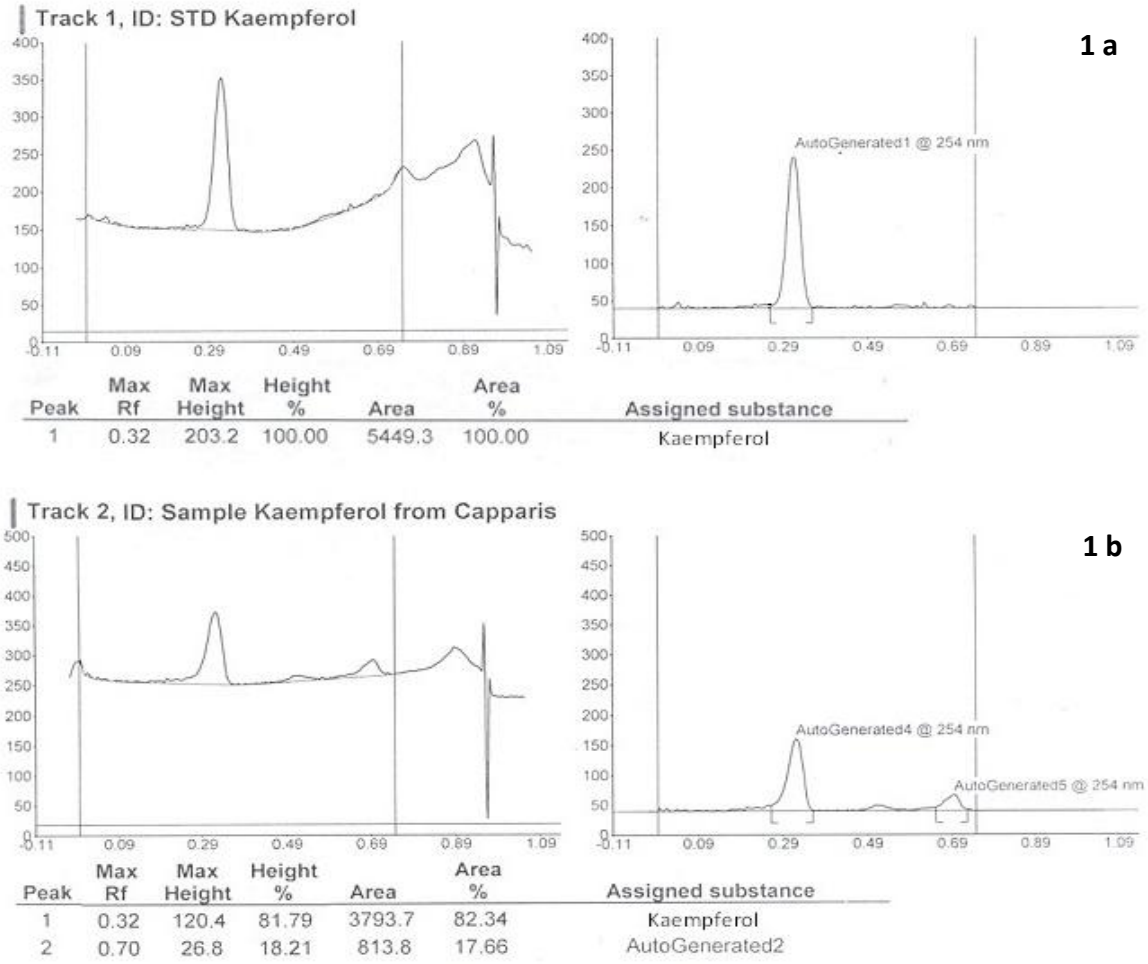
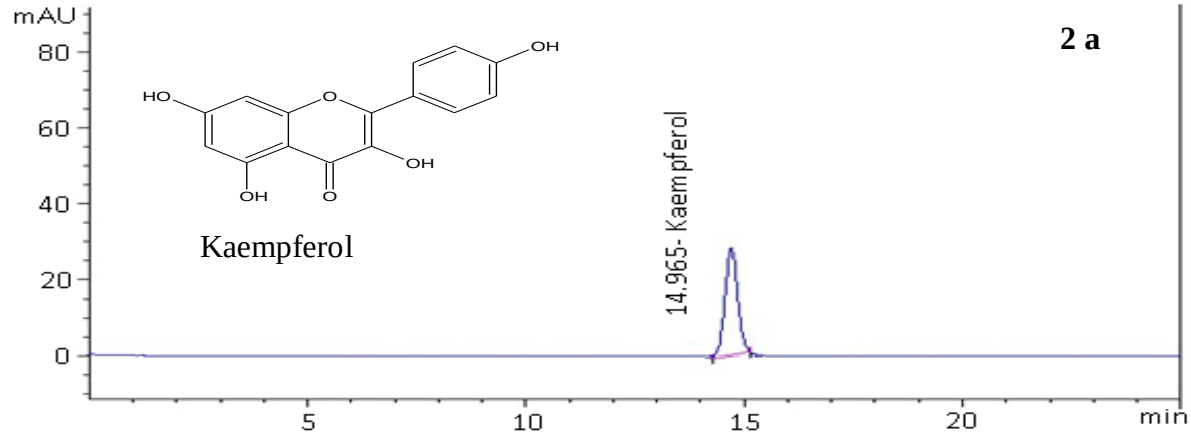


Figure 1: HPTLC chromatogram of (a) Std. kaempferol and (b) Isolated kaempferol in solvent system benzene: acetic acid: water (125:72:3) scanned at 254 nm

Figure. 1 a: Standard kaempferol showed R_f 0.32

Figure. 1 b: Isolated kaempferol showed R_f 0.32 and 0.70 having identical R_f 0.32 as that of standard kaempferol with area percent 82.34



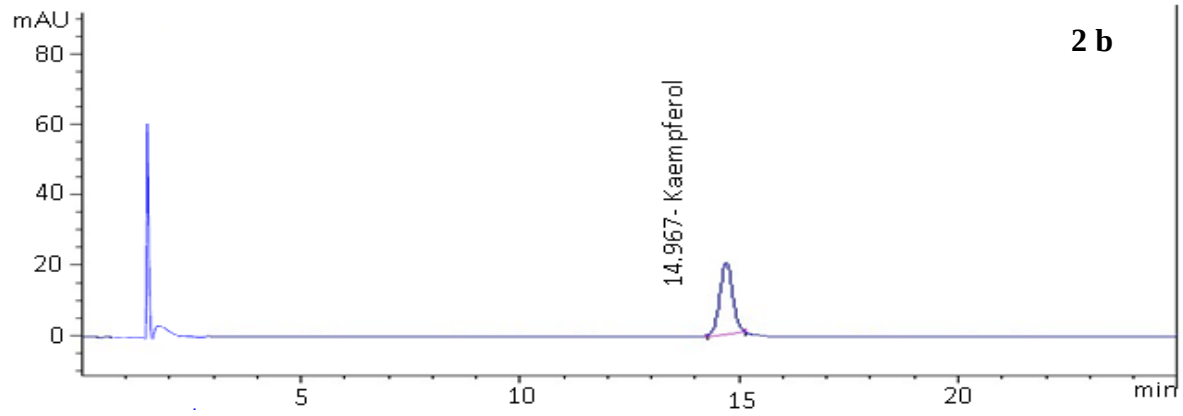


Figure 2: HPLC chromatogram of (a) Std. kaempferol and (b) Isolated kaempferol in mobile phase solution A (water: orthophosphoric acid, pH 3.0) and solution B (acetonitrile: methanol in ratio of 75:25) (50:50) scanned at 370 nm

Figure. 2 a: Standard kaempferol showed retention time at 14.965 min

Figure. 2 b: Isolated kaempferol showed retention time at 14.967 min identical with standard kaempferol

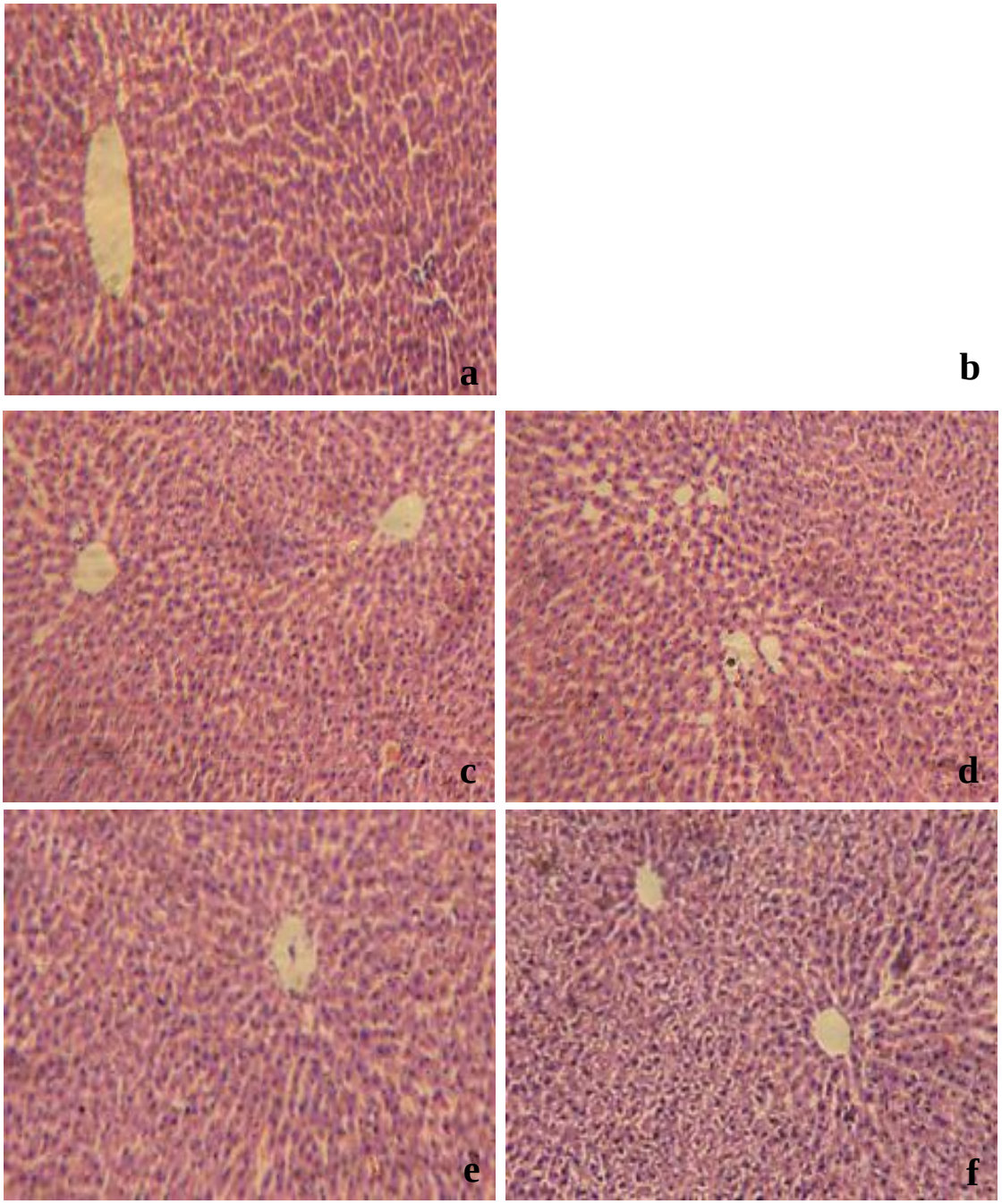


Figure 3: Assessment of CCl_4 induced hepatotoxicity by histopathology in haematoxylin-eosin stained liver sections. (a) Control group showed normal hepatic cell with well preserved cytoplasm; (b) CCl_4 intoxicated liver tissue showed patches of liver cell necrosis with inflammatory collections and the loss of cellular boundaries; (c) Silymarin treated animal showed minimal fatty changes and focal necrosis with slightly altered hepatocytes; (d) Kaempferol (25 mg/kg) showed focal coagulative, lobular and centrilobular necrosis and mild fatty vacuolation; (e) Kaempferol (50 mg/kg) showed normal lobular pattern with a mild necrosis and lymphocyte infiltration; (f) Kaempferol (100 mg/kg) showed normal parenchymal architecture with cords of hepatocytes, portal tracts and central veins (10×).

DISCUSSION

Epidemiological studies revealed that a diet rich in plant derived foods has a protective effect on human health. Identifying bioactive dietary constituents is an active area of scientific investigation that may lead to new drug discovery. Kaempferol is a flavonoid found in many edible plants (e.g. tea, broccoli, cabbage, kale, beans, endive, leek, tomato, strawberries and grapes) and in plants or botanical products. Numerous preclinical studies have shown that kaempferol and some glycosides of kaempferol have a wide range of pharmacological activities including anti-inflammatory (Rho et al, 2011), antimicrobial, anticancer (Huang et al, 2010), cardioprotective (Matei and Hillebrand, 2010), antidiabetic (de Sousa et al, 2004), analgesic and antiallergic activities (Hirose et al, 2009).

Considering the antioxidant, antiapototic, anti-inflammatory and antialergic effect of kaempferol, this study protocol was developed to explore hepatoprotective potential covering all the aspects *viz* effect on functional integrity of hepatic microsomal enzyme following CCl_4 induced hepatotoxicity on rats, assessing in-vitro BSP uptake, barbiturate induced sleeping time, serum biochemical parameters, antioxidation capability and cellular regenerating potency to have an inside view on mechanism of action. Kaempferol belongs to a group of naturally occurring compounds called flavonoids that are found widely in the plant kingdom. Kaempferol might be a rational candidate for the prevention of liver injury among individuals exposed to hepatotoxic agents.

CCl_4 is a well known hepatotoxin which is widely used to induce toxic liver injury and to study the cellular mechanisms behind oxidative damage in laboratory animals. CCl_4 needs metabolic activation by mixed function oxidase to produce the hepatotoxic trichloromethyl radical ($CCl_3^{\cdot}$). This toxic free radical reacts with various biologically important substances such as amino acids, nucleotides and fatty acids as well as proteins, nucleic acids and lipids. The radical can also react with oxygen to form $CCl_3OO^{\cdot}$, a highly reactive species. $CCl_3OO^{\cdot}$ initiates a chain reaction of lipid peroxidation which attacks and destroys poly-unsaturated fatty acid; in particular those associated with phospholipids which results in steatosis. This affects the permeability of mitochondrial endoplasmic reticulum and plasma membrane resulting in the loss of Ca^{2+} sequestration and homeostasis heavily contributing to the cellular damage (Rajesh and Latha, 2004).

The outcome of the present study showed that oral administration of kaempferol in rats does not have any adverse effect on the body weight gain. Kaempferol treatment resulted in reversal of body weight loss caused by CCl_4 treatment in rats. Higher liver to body weight ratio was observed in CCl_4 treated rats. Measurement of liver body weight ratio is a good approach to determine the changes in liver histopathology signifying hepatic lesions and liver injury associated with the toxicological effects of CCl_4. Liver enlargement was significantly reduced in rats administered with kaempferol and this was comparable to the effects of silymarin.

CCl_4 induced hepatic injury decreases the activity of cytochrome P_{450} enzymes and thereby the metabolic functional ability of the hepatocytes. This causes delay in the metabolism of barbiturate and slowed down the excretion of thiopental, thereby prolonging the sleeping time induced by these drugs. Pretreatment with kaempferol restored the thiopental induced sleeping time indicating normalization of cytochrome P_{450} working capacity and other related hepatic mixed function oxidase enzyme systems (Girish et al, 2009).

The liver injury due to toxins results in defective excretion of serum transaminases and bile by hepatocytes intern increasing their levels in serum (Rajesh and Latha, 2004). Current study findings revealed that pre and post treatment of kaempferol restored serum transaminase and ALP values indicating hepatoprotective activity. The protective effects may be a result of stabilization of plasma membrane and preserving the structural integrity of cells as well as repair of hepatic tissue damage caused by CCl_4 (Pari and Murugan, 2004).

Increase in lipid concentration results in liberation of lysosome and trigger cells degeneration. Major

component of total cholesterol is LDL which is directly related to coronary artery disease (Peterson et al, 2004). Rats treated with CCl_4 alone showed significant increase in cholesterol, triglyceride and LDL level, confirming that oral administration of CCl_4 induced hepatotoxicity. In the present study, significant decrease in the cholesterol, triglycerides and LDL was observed in kaempferol treated rats which may be due to reduction in the absorption of cholesterol (Bobek et al, 1991). Drugs like statins administration results in reduction of LDL cholesterol by inhibiting HMG-CoA reductase in the liver (Pichandi et al, 2011). In this study, reduction in the LDL cholesterol in CCl_4 treated rats after kaempferol administration was observed may be due to the HMG-CoA reductase inhibitory action of kaempferol.

The values of serum total bilirubin and direct bilirubin indicated the presence of hyperbilirubinemia in CCl_4 treated rats corelating liver dysfunction. Presence of increased direct bilirubin is probably an indication of acute hepatitis induced by CCl_4 (Alhassan et al, 2012). Kaempferol treatment significantly prevented severity of liver damage caused by CCl_4 as evidenced by the low level of total and direct bilirubin in the serum. This revealed the hepatoprotective potential of kaempferol and the recovery of liver damage was at a significant level.

The reduction in serum total protein in CCl_4 treated group was also recorded which was elevated on treatment with kaempferol, indicating its curative effect against liver cell damage. Clawson, (1989) reported that the decline in protein content may be due to defect in protein biosynthesis as well as disruption and disassociation of polyribosome from endoplasmic reticulum following administration of CCl_4. According to Rajesh and Latha, (2004), improvements in the level of total protein after treatment with the natural products may be due to promotion of ribosome assembly on endoplasmic reticulum which facilitates uninterrupted protein biosynthesis.

Albumin is the most abundant protein in human plasma, representing 55-56% of total protein synthesized in liver at a rate that is dependent on protein intake subject to feedback regulation by the plasma level. Albumin is filtered through the kidney glomeruli and most of that is reabsorbed by proximal tubule cells and degraded by their lysosomal enzymes into fragment that are returned to the circulation (Al-Hashem et al, 2009). In this study, there was a decrease in serum albumin after treatment with CCl_4 indicating poor liver functions or impaired synthesis, either primary due to liver cells damage or secondary to diminished protein intake and reduced absorption of amino acids (Shibutani et al, 2001). On the other hand, kaempferol treatment increased albumin concentration in CCl_4 treated rats indicating that kaempferol may decrease lipid peroxidation as well as increase the activities of plasma protein thiols (Al-Hashem et al, 2009).

In vivo antioxidant defense mechanism fight against free radicals and reactive oxygen species induced damage, in which the endogenous enzymatic and non-enzymatic antioxidants such as glutathione, lipid peroxidase and superoxide dismutase play an important role (Chow, 1979; Blokhina et al, 2003). Glutathione is as an essential intracellular reducing substance for the maintenance of thiol groups on intracellular proteins and antioxidant molecules in living organisms (Davis et al, 2001). Perturbation of glutathione status in a biological system has been reported to lead serious consequences (Bandyopadhyay et al, 1999). Glutathione conjugate with free radicals directly, marking them ready for renal excretion, which is especially important for dealing with the products of hepatic cytochrome P_{450} enzyme activity. The sulfhydryl portion of the glutathione can be used to reduce a variety of free radicals in a reaction catalyzed by the antioxidant enzyme, glutathione peroxidase (Webb and Twedt, 2008). Lipid peroxidase, superoxide dismutase and other antioxidant enzymes constitute a mutually supportive team of defense against reactive oxygen species. Superoxide dismutase is a metalloproteinase to detoxify superoxide anions as an efficient dismutative mechanism and is the first enzyme involved in the antioxidant defense (Salvemini et al, 2002). However, once the balance between reactive

oxygen species production and antioxidant defenses is lost, oxidative stress consequently occurs, which through a series of biological events deregulates the cellular functions leading to various pathological conditions (Bandyopadhyay et al, 1999).

The results of the study revealed that pre and post treatment of kaempferol effectively blocked the CCl_4 reduced abnormal changes in the level of glutathione, indicated that kaempferol has a potent antioxidant property towards chemical induced hepatic injury (Sahu and Gray, 1996). Elevated level of MDA in CCl_4 treated rats indicates excessive formation of free radicals and activation of lipid peroxidation system resulting in hepatic damage. The significant decline in the concentration of MDA in the rat's liver tissue treated with both CCl_4 and kaempferol indicates anti-lipid peroxidative effect of kaempferol (Chen et al, 2011).

CONCLUSION

The results of this study demonstrate that kaempferol isolated form *C. spinosa* has a potent hepatoprotective action upon CCl_4 induced oxidative stress and liver toxicity in rat. The hepatoprotective effect of kaempferol can be correlated with its efficiency to normalize the levels of serum marker enzymes and enhance antioxidant defiance status. The findings of this study suggest that kaempferol can be used as a safe and effective alternative chemopreventive agent in the management of liver disorders.

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***Corresponding Author:**
Dr. (Mrs.) Papiya Bigoniya,
Principal,
Radharaman College of Pharmacy,
Fatehpur Dobra,
Bhadbada Road, Ratibad,
Bhopal- 462 002, M.P., India.
E -mail: p_bigoniya2@hotmail.com