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ORIGINAL ARTICLE

Quantitative Assessment of Tactile Allodynia and Protective Effects of flavonoids of *Ficus carica* Lam. Leaves in Diabetic Neuropathy

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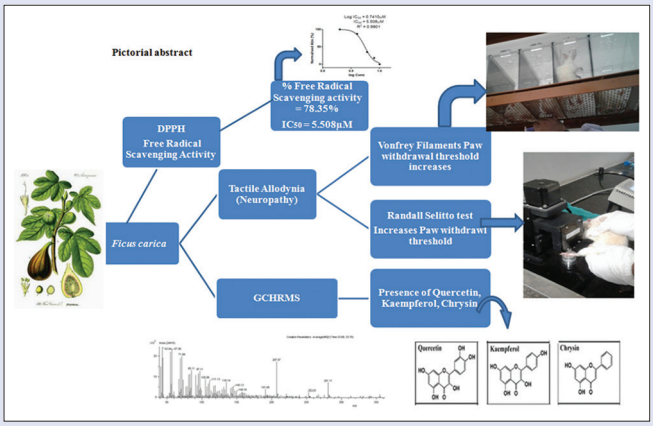
ABSTRACT

Background: Flavonoids, a group of polyphenols responsible for protective role against many diseased conditions, provide antioxidant activity which is the reason for their medicinal properties. Tactile allodynia is a behavioral biomarker of neuropathy that is well estimated by von Frey filaments and Randall–Selitto test. **Objective:** *Ficus carica* Lam. leaves were studied for the conformation of flavonoids in ethyl acetate fraction of methanolic extract (FCEA) using GC-HRMS for the identification of flavonoids. It was analyzed for antioxidant activity by *in vitro* free radical scavenging activity, performed using 2,2-diphenyl-1-picrylhydrazyl (DPPH) followed by blood glucose-level estimation, evaluation of neuropathic pain, and kidney and liver function tests in diabetic rats. **Materials and Methods:** The shade-dried leaves of *F. carica* Lam. were extracted with methanol and after that fractionated using ethyl acetate (FCEA). The characterization of FCEA was established using GC-HRMS. *In vitro* free radical scavenging activity was performed using DPPH assay. Diabetes was induced using streptozotocin (40 mg/kg/intraperitoneally), and effects of FCEA were studied on blood glucose level, neuropathy markers, and liver and kidney functions of diabetic rats. **Results:** GC-HRMS results highlighted the presence of quercetin, kaempferol, and chrysin in FCEA with free radical scavenging activity of 78.35% and IC_{50} value of 5.508 µM. FCEA reduces glucose levels and also shows protective effects in case of diabetic neuropathy as it increases the threshold of withdrawal latency in tactile allodynia and also decreases the serum glutamic-oxaloacetic transaminase, serum glutamic-pyruvic transaminase, blood urea nitrogen, and creatinine levels. **Conclusion:** The protective effects of FCEA against diabetic neuropathy, hepatoprotective and nephroprotective effects might be due to strong antioxidant property of important flavonoids present which is confirmed in the study.

Key words: Chrysin, diabetic neuropathy, kaempferol, quercetin, Randall Selitto, von Frey

SUMMARY

- The research work shows the presence of quercetin, kaempferol, and chrysin in *Ficus carica* Lam. leaves; along with this, it has depicted *in vitro* free radical scavenging activity by 2,2-diphenyl-1-picrylhydrazyl assay method. After quantitative assessment of tactile allodynia, this plant sample has proven protective effects in diabetic neuropathy, and these effects were compared with surgical model of neuropathy by von Frey filaments and Randall–Selitto test.



Abbreviations used: BSTFA: N, O-Bis (trimethylsilyl) trifluoroacetamide; DPPH: 2,2-diphenyl-1-picrylhydrazyl; FCEA: Ethyl acetate fraction from methanolic extract of leaves of *Ficus carica* Lam.; GC-HRMS: Gas chromatography–high-resolution mass spectrometry.

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INTRODUCTION

Polyphenolic substances are ubiquitously found chemical constituents in a variety of plants having medicinal properties.[1-4] A large number of plants contain flavonoids; they further consist of flavones, flavonols, isoflavonoids, anthocyanidins, and chalcones.[2,3] It has a protective role in carcinogenesis,[4,5] inflammation,[4,5] atherosclerosis,[4] thrombosis,[4] diabetes, and cardiovascular diseases[5] and has activities such as antiviral,[4,5] antimicrobial,[4] antihepatotoxic,[4] antiosteoporotic,[4,6] antiulcer,[4] immunomodulatory,[4] antiproliferative,[4,6] and apoptotic[4] as a result of their antioxidant actions.[4-6] In the last few years, gas chromatography–mass spectrometry (GC-MS) has established as a firm platform for analysis of plant's secondary metabolites and phytoconstituents. It is a hyphenated system, a compatible technique

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for the identification and quantification purpose. The unknown organic compounds in a complex mixture can be determined by interpretation and also by matching the spectra with reference spectra.[7-9] *Ficus carica* Lam. belongs to order *Urticales* and family *Moraceae*. It is cultivated worldwide for its edible fruit. Fruits are a rich source of phenolic acids, vitamins, minerals, and organic acids. Conventionally, it has medicinal benefits for ailments of cardiovascular, respiratory, spasmodic, and inflammatory problems. Leaf extract has shown significant hypoglycemic effect.[10,11]

The present study was designed to analyze the flavonoids present in the leaves of *F. carica* Lam. using gas chromatography–high-resolution mass spectrometry (GC-HRMS) technique and evaluate their *in vitro* free radical scavenging activity. As there is no report regarding usefulness of ethyl acetate fraction of methanolic extract of *F. carica* Lam. (FCEA) in neuropathic pain, this study also aimed to assess its effects on diabetes-induced neuropathic pain followed by liver function and kidney function marker analysis as diabetes leads to multiple disturbances including liver and kidney functions, to study its medicinal effects. For estimation of action in diabetic neuropathy, behavioral biomarker of neuropathy like tactile allodynia was used as the animal model, and von Frey filaments and Randall–Selitto methods were preferred for quantitative assessment.[12-16]

MATERIALS AND METHODS

Drugs and chemicals

Streptozotocin (STZ, Spectrochem Pvt. Ltd.) was dissolved in cold 0.01 M citrate buffer and pH 4.5 and always prepared freshly for immediate use within 30 min;[6] metformin (Metmin tablet), Jenburkt Pharmaceuticals Ltd.; gabapentin (Gabapin capsule), Intas Pharmaceuticals; quercetin (Deepa Chemicals); and all other chemicals used in this study were of analytical grade.

Plant material

The leaves of *F. carica* were collected from Daulatabad area of Aurangabad, Maharashtra, India. They were authenticated by the Department of Botany, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad, Maharashtra, India, and a voucher specimen 0588 has been deposited in the herbarium of Dr. Babasaheb Ambedkar Marathwada University, Aurangabad, Maharashtra, India.

Experimental animals

Sprague Dawley rats of either sex weighing between 180 and 250 g were used. The rats were acclimatized to the animal house condition for 1 week before carrying out any experimental work. The rats were fed *ad libitum* with normal pellet diet and water. They were housed at standard conditions of temperature (23°C ± 12°C), humidity (45 ± 5%), and 12-h light and dark cycle. The experimental protocol for animal experiments was approved by the Institutional Animal Ethics Committee (IAEC) of Y.B. Chavan College of Pharmacy, Aurangabad (Approval no. CPCSEA/IAEC/P'col-52/115).

Separation and phytochemical screening of ethyl acetate fraction from methanolic extract of leaves of *Ficus carica*

The leaves were shade dried. The coarse powder was subjected to extraction with petroleum ether followed by methanol in Soxhlet apparatus, and the extract was fractionated to obtain flavonoid-rich fraction using separating funnel.[17] The extract was screened for the presence of phytoconstituents such as alkaloids, glycosides, tannins, steroids, triterpenoids, saponins, and flavonoids.[18]

In vitro free radical scavenging activity

2,2-diphenyl-1-picrylhydrazyl radical scavenging assay

2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay is based on the measurement of the scavenging ability of drug (antioxidant substances) toward the stable radical. 1.0 mL of FCEA (10 mg/mL) was mixed with 1.0 mL of 0.8-mmol/L DPPH solution. The mixture was shaken vigorously and allows standing for 30 min, and the absorbance was measured at 517 nm against a reagent blank. The standard used was ascorbic acid. The percentage inhibition for scavenging DPPH radical was calculated according to the equation:

$$\% \text{ scavenging activity} = \left[1 - \left(\frac{\text{A sample}}{\text{A control}}\right) \times 100\right]$$

Where A sample and A control stand for absorbance of sample and control, respectively. The FCEA concentration providing 50% inhibition (IC_{50}) was calculated from graph of normalized absorbance (%) plotted against log concentration.[19,20] FCEA and standard ascorbic acid were used in concentrations 2 μgm/ml, 4 μgm/ml, 6 μgm/ml, 8 μgm/ml, and 10 μgm/ml.

Gas chromatography–high-resolution mass spectrometry

GC-HRMS analysis was performed by splitless injection of 1.0 μL of the sample on a Hewlett-Packard 6890 (USA) and gas chromatograph fitted with a cross-linked 5% phenyl methyl siloxane HP-5 MS capillary column (30 m × 0.32 mm × 0.25 μm coating thickness) and it coupled with a mass detector. GC-MS operating conditions were as follows: injector temperature 200°C, transfer line 230°C, oven temperature program 60°C–280°C with ramping 5°C min^{-1}, carrier gas: helium at 1.5 mL min^{-1}, mass spectra: EI+, and ion source temperature: 280°C. The individual components were identified by NIST MS 2.0 f Structural Library. Electron impact (EI) MS spectrum was scanned at 70 eV with instrument details as follows:

- Make of MS: Jeol
- Model: Accu TOF GCV
- Specification: EI/CI source
  - Time of flight analyzer
  - Mass range-10–2000 amu
  - Mass resolution-6000
- Make of GC: Agilent
  - 7890
  - FID detector
  - Head Space injector
  - CombiPAL autosampler

Identification of constituents

Mass spectra comparison was used to identify constituents with those of authentic compounds or with reference spectra in the computer library (NIST MS 2.0 f Structural Library), and confirmation is done by comparison of their retention indices with authentic compounds or with data in the literature.[4,21-23]

Induction of experimental diabetes

Experimental rats were fasted overnight and injected with STZ at a multiple dose of 40 mg/kg body weight for 3 consecutive days. The solution was injected intraperitoneally within 5 min after dissolving in citrate buffer pH 4.5.[17] The rats in group NC were injected with distilled water as a vehicle control. The animals were allowed to drink 5% glucose solution *ad libitum* overnight to overcome hypoglycemia. Random blood glucose was estimated at the time of induction of diabetes and was checked regularly until stable hyperglycemia was achieved. The mice with moderate diabetes having glycosuria and hyperglycemia (blood glucose levels of 250 mg/dl) were included in the study as stable hyperglycemic animals.[17]

Surgical model of neuropathy

To obtain the surgical control group, partial sciatic nerve ligation technique was followed. Skin incision was made dorsal to pelvis. The common sciatic nerve was first exposed by separating the muscles and then loosely ligated with a chromic gut suture. Fascia, muscle, and skin incision were closed using silk suture. After the completion of surgery and recovery of animals, the group was used for comparative study to assess tactile allodynia.[12,24,25]

Experimental design

- Group NC: Normal control, vehicle treated
- Group DC: Diabetic control, vehicle treated
- Group SC: Surgical control, vehicle treated
- Group DM*: Diabetic, metformin treated (120 mg/kg, po)
- Group DG*: Diabetic, gabapentin treated (120 mg/kg, po)
- Group DQ: Diabetic, quercetin treated (40 mg/kg, po)
- Group $FCEA_{25}$: Diabetic, FCEA treated (25 mg/kg, po)
- Group $FCEA_{50}$: Diabetic, FCEA treated (50 mg/kg, po)
- Group $FCEA_{100}$: Diabetic, FCEA treated (100 mg/kg, po)

(*Two different standards are used in the study as till date, no allopathic treatment is found to be clinically useful against diabetic neuropathy.[24,26] Metformin is an oral hypoglycemic while gabapentin helps against neuropathic pain.)

Collection of blood and determination of blood glucose

Blood samples from the experimental groups were collected from tail vein. The samples so collected were analyzed for glucose estimation using Flavin Adenine Dinucleotide-Glucose Dehydrogenase (FAD-GDH) method by Contour TS glucometer.

Diabetic neuropathy

Rats may develop hyperglycemia and other clinical diabetic symptoms within 3 days of STZ injection. After 1 week, rats showing blood glucose above 250 mg/dl were selected for study. After 3 weeks of stable diabetes, drug treatment was performed, and pretreatment (pre-T/T) studies of parameters of neuropathy were performed. On completion of dosing period, rats were again tested for different parameters of neuropathy.

Tactile allodynia

Von Frey filaments model

Stimulus presentation and testing paradigms as described by Chaplan *et al.* were followed to obtain 50% threshold calculations and estimate tactile allodynia in rats. Animals were placed in acrylic chamber with wire mesh at bottom which gives full access to the paws. Animals were allowed to get acclimatized in the cage for about 15 min. The paw was touched with von Frey filaments following up-and-down method to calculate 50% withdrawal threshold. Von Frey hairs were subjected to the paw perpendicularly with sufficient force to cause buckling response against paw and held for about 6–8 s. Sharp withdrawal of paw was considered to be positive response.[13,27]

About 50% g threshold was calculated by formula:

$$(10^{[xf-k\delta]})/10{,}000$$

Where x_f – value (in log units) of final von Frey hair used.

k – tabular value for the pattern of positive/negative responses (as provided in the table[13]).

δ – mean difference (in log units) between stimuli (here, 0.224).

Randall–Selitto model

Nociceptive withdrawal threshold was estimated on Randall–Selitto test apparatus on the paws of experimental animals. Hold animals with soft cotton cloth to immobilize it. Place gently the paw on the apparatus and allow the tip of device to apply on paw with application of increasing mechanical force and withdrawal latency to the pressure supported was noted down.[28]

Biochemical parameters

Blood urea nitrogen and serum creatinine were assessed as a measure of kidney function markers. Aspartate transaminase and alanine transaminase were assayed as a measure of liver function markers.[15]

Statistical analysis

All the data expressed by two-way ANOVA, followed by Bonferroni test using Prism Graphpad version 5 (Graphpad Software Inc., California, USA) with $P < 0.001$, <0.05, and <0.01 statistical significance.

RESULTS

Phytochemical screening

The preliminary phytochemical screening of FCEA revealed the presence of alkaloids, tannins, steroids, triterpenoids, saponins, and flavonoids. This shows high possibility of its medicinal value. The results are shown in Table 1.

In vitro free radical scavenging activity

The DPPH free radical scavenging activity of FCEA obtained was 78.35%. The percentage inhibition (IC_{50}) obtained in different concentrations of FCEA was compared to that of standard ascorbic acid and is shown in Table 2 and Figure 1.

Constituents of ethyl acetate fraction from methanolic extract of leaves of *Ficus carica* Lam.

The results pertaining to GC-MS analysis of the FCEA lead to the identification of a number of compounds. These compounds were identified through mass spectrometry attached with GC; the gas chromatogram is depicted in Figures 2, 3 and the various constituents present are listed in Table 3.

Determination of effects on blood glucose

Blood glucose was randomly estimated for 5 consecutive weeks. Detailed observations are given in Table 4. It was observed that animals showing >250 mg/dl blood glucose after 3 weeks were continued for diabetic neuropathic studies, and FCEA and standard treatments were followed. FCEA shows dose-dependent improvement in the blood

Table 1: Preliminary phytochemical screening

Phytoconstituent	Inference
Alkaloids	+
Glycosides	-
Flavonoids	++
Tannins	+
Saponins	+
Terpenoids	+
Steroids	+
Resins	-

++: Active constituents in high amount; +: Active constituents in low amount; -: The absence of active constituents

Table 2: Free radical scavenging activity

Sample	IC_{50} value (μM)
Ascorbic acid	4.867
FCEA	5.508

IC_{50} values were calculated using Prism GraphPad version 5. FCEA: Ethyl acetate fraction from methanolic extract of leaves of *Ficus carica* Lam.

glucose levels in the 4th and 5th weeks with $P < 0.001$ significance in comparison to diabetic control.

Diabetic Neuropathy

Tactile Allodynia

Von Frey filaments model

Nociceptive withdrawal threshold expressed in terms of 50% threshold of diabetic control and surgical control was significantly ($P < 0.001$) decreased as compared to normal control. The FCEA-treated groups show dose-dependent significant ($P < 0.05$, 0.001) increase in paw withdrawal threshold as compared to diabetic and surgical control Table 5.

Randall–Selitto test model

Nociceptive withdrawal threshold expressed in terms of supported pressure resulting from application of Randall–Selitto probe of diabetic and surgical control has found to decrease significantly ($P < 0.001$) as compared to normal control while FCEA has shown dose-dependent increase in supported pressure as compared to diabetic and surgical control significantly ($P < 0.001$) [Table 6].

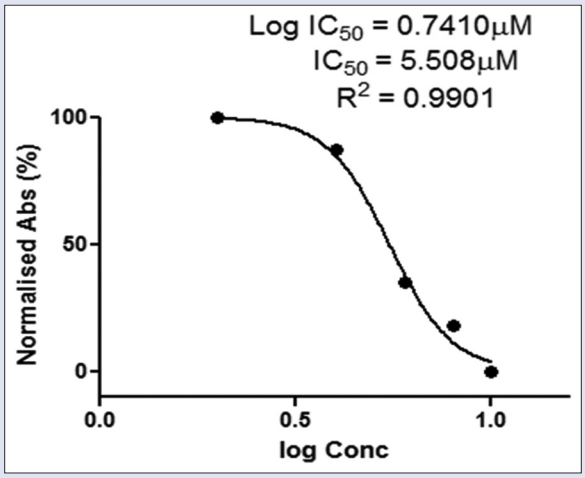

Figure 1: Percentage inhibition in different concentrations of ethyl acetate fraction from methanolic extract of leaves of *Ficus carica* Lam., IC_{50} value and R^2 value

Table 3: Constituents present in fractionated using ethyl acetate

Retention time	Name of constituents	Molecular formula	Molecular weight
5.28	Benzenemethanol, α, α, 4-trimethyl	$C_{10}H_{14}O$	150
5.64	2,4,-cycloheptadien-1-one, 2,6,6, trimethyl (Eucarvone)	$C_{10}H_{14}O$	150
11.16	1-Butanone, 2-hydroxy-1-phenyl	$C_{10}H_{12}O_2$	164
15.06	2-Pentadecanone, 6,10,14 trimethyl	$C_{18}H_{36}O$	268
15.49	1,2-Benzenedicarboxylic acid, bis (2 methylpropyl) ester	$C_{16}H_{22}O_4$	278
16.34	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270
17.22	n-Hexadecanoic acid (Palmitic acid)	$C_{16}H_{32}O_2$	256
19.67	9,12,15-octadecatrienoic acid, methyl ester	$C_{19}H_{32}O_2$	292
20.09	Octadecanoic acid, methyl ester	$C_{19}H_{32}O_2$	298
20.51	Bis (2-ethylhexyl) maleate	$C_{20}H_{36}O_4$	340
23.89	Octadecane, 3-ethyl-5-(2-ethylbutyl)	$C_{26}H_{54}$	366
25.03	Tert-hexadecanethiol	$C_{16}H_{34}S$	258
31.69	ethyl iso-allocholate	$C_{26}H_{44}O_5$	436

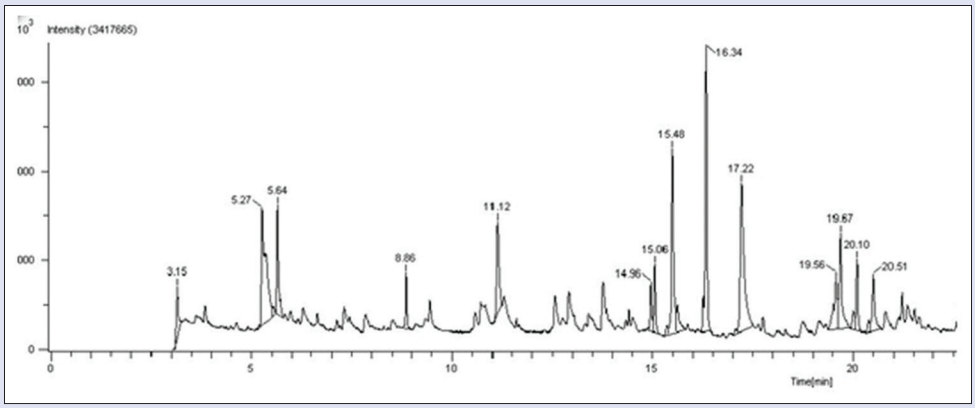

Figure 2: Gas chromatogram of ethyl acetate fraction from methanolic extract of leaves of *Ficus carica* Lam.

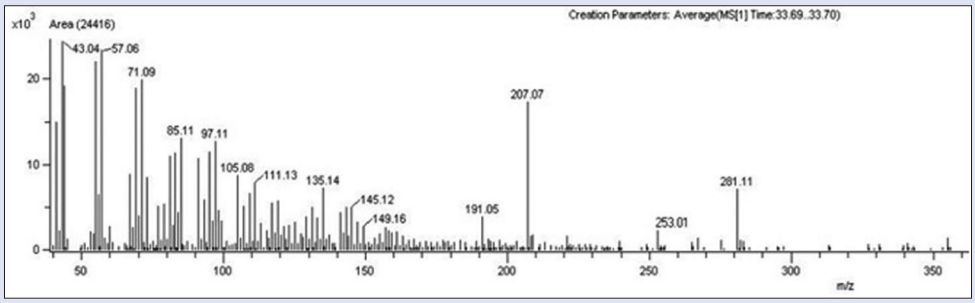

Figure 3: Mass spectra of ethyl acetate fraction from methanolic extract of leaves of *Ficus carica* Lam.

Table 4: Effect of fractionated using ethyl acetate on blood glucose level

Groups	Initial	1 week	2 weeks	3 weeks	4 weeks	5 weeks
NC	84.5±3.0	83.8±2.9	82±3.7	84.8±3.7	84.5±3.1	82.8±3.1
DC	88±2.1	158.8±16.9*	362.1±27.5***	454.3±29.2***	501.6±19.7***	475.6±84.5***
DM	82±3.2	192.5±28.3	323±24.1	392.5±25.8	180.5±18.3***	102±2.8
DQ	84.1±2.3	266.5±21.9	338.5±14.2	451.8±11.2	157.3±12.3***	107.1±2.1***
$FCEA_{25}$	83.6±1.9	178±12.5	312.8±19.5	412.5±14.4	324.1±7.4***	201.8±9.8***
$FCEA_{50}$	84.6±2.4	166.3±9.4	301.5±19.7	470±7.8	251.6±10.9***	133.8±2.6***
$FCEA_{100}$	85.5±3.2	244.6±13.7	377.5±20.3	471.1±11.5	146.3±2.0***	101.3±2.0***

Data are represented in the form of mean±SEM (n=6), Two-way ANOVA followed by Bonferroni test with *P<0.05 and ***P<0.001 significance, all groups are compared with DC and DC is compared with NC. NC: Normal control; DC: Diabetic control; DM: Diabetic, metformin; DQ: Diabetic, Quercetin; FCEA: Ethyl acetate fraction from methanolic extract of leaves of *Ficus carica* Lam.; SEM: Standard error of mean

Table 5: Effect of fractionated using ethyl acetate; in tactile allodynia using von Frey filaments

Groups	50% threshold (g)
NC	8.81±0.7
DC	1.37±0.09***
SC	1.72±0.1***
DM	1.49±0.1
DG	8.53±0.6***
DQ	6.09±0.08***
$FCEA_{25}$	3.29±0.1**,*
$FCEA_{50}$	4.02±0.1***
$FCEA_{100}$	5.26±0.1***

Data are represented in the form of mean±SEM (n=6), One-way ANOVA followed by Bonferroni test with *P<0.05 and ***P<0.001 significance, all groups are compared with DC, SC and DC, SC are compared with NC. NC: Normal control; DC: Diabetic control; DM: Diabetic, metformin; DQ: Diabetic, Quercetin; FCEA: Ethyl acetate fraction from methanolic extract of leaves of *Ficus carica* Lam.; SEM: Standard error of mean

Table 6: Effect of fractionated using ethyl acetate in mechanical allodynia using Randall–Selitto apparatus

Groups	Supported pressure (g)
NC	318.06±18.9
DC	81.16±3.4***
SC	82.54±3.2***
DM	76.43±4.6
DG	305.85±24.8***
DQ	220.45±18.3***
$FCEA_{25}$	113.37±3.2
$FCEA_{50}$	134.49±2.7
$FCEA_{100}$	210.67±18.4***

Data are represented in the form of mean±SEM (n=6), One-way ANOVA followed by Bonferroni test with ***P<0.001 significance, all groups are compared with DC, SC and DC, SC are compared with NC. NC: Normal control; DC: Diabetic control; DM: Diabetic, metformin; DQ: Diabetic, Quercetin; FCEA: Ethyl acetate fraction from methanolic extract of leaves of *Ficus carica* Lam.; SEM: Standard error of mean

Biochemical parameters

The markers of liver and kidney function tests assessed, and FCEA dose dependently improved the liver and kidney functions as compared to diabetic control group [Table 7].

DISCUSSION

Results of phytochemical screening put forward that our fractions are rich in bioactive compounds. These phytoconstituents are confirmed with the help of sophisticated technique of GC-HRMS. According to the report of Tokusoglu *et al.* 2003, N,O-Bis (trimethylsilyl) trifluoroacetamide (BSTFA) derivative of quercetin and kaempferol shows peaks at m/z 281 and 207, respectively. The mass spectra of FCEA show peaks at m/z 281.11 and 207.07 [Figure 3]. Apart from molecular peak of 281.11, FCEA shows a strong peak at 207.07 fragment. By referring the literature, it can be stated that along with the 13 components tabulated in Table 3, strong peaks of quercetin and kaempferol are also obtained from FCEA.[21] Spectrum of FCEA also shows peak at m/z 253.01, and this fragment depicts the presence of one more flavonoid chrysin in the fraction.[23] This predicts that FCEA is having high medicinal importance and its activity may be justified due to the presence of these flavonoids which are well known for their pharmacological values.[23,29-33]

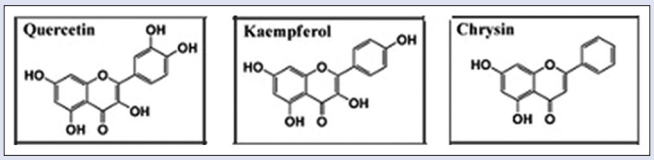

Figure 4: Chemical structure of quercetin, kaempferol, and chrysin

Fragmentation pattern of phytoconstituents

HRMS of standard quercetin as depicted in Figure 3 shows molecular peaks at m/z 281.08, and strong peaks are also noted at m/z 207.06 and 253.02. Quercetin with molecular weight 303 shows peak at 281, and kaempferol with molecular weight 285 can also show at 207; the work explained in this research is justified by BSTFA derivatization of these flavonoids.[21] This explains fragmentation obtained in the current study is due to silylation that produces silyl derivatives which are more volatile, less stable, and more thermally stable. Silylation occurs through nucleophilic attack and has reaction affinity for the functional groups in the following order:

Alcohol hydroxyl > Phenol hydroxyl > carboxyl > Amine > Amide.

Quercetin, kaempferol, and chrysin in their structures have the presence of phenolic hydroxyl group [Figure 4], and this justifies the affinity for fragmentation pattern in these spectra obtained from standard and FCEA, respectively. The hydroxyl groups present on these bioactive compounds are even responsible for their pharmacological activities such as antioxidant capacity as the presence of the hydroxyl group is depicted to enhance the antioxidant activity.[34]

DPPH is a stable nitrogen-centered free radical used in testing *in vitro* free radical scavenging activity. When the stable DPPH radical accepts an electron from the antioxidant compound, the violet color of the DPPH radical gets reduced to yellow-colored diphenylpicrylhydrazine radical which is measured colorimetrically. Substances which show this reaction can be considered as antioxidants and hence radical scavengers.[20,29] The DPPH-free radical scavenging activity of FCEA was 78.35%, and IC_{50} value was found to be 5.508 µM with R^2 value that depicts the fit to linearity was obtained as 0.9901. This assay provides an easy and rapid way to determine *in vitro* antioxidant activity, and also, the low IC_{50} value provides an evidence for its higher radical scavenging and antioxidant activity.

Table 7: Effects of fractionated using ethyl acetate on markers of liver and kidney functions

Group	SGOT	SGPT	BUN	Creatinine
NC	38.28±1.08	35.44±0.73	12.90±0.19	0.54±0.02
DC	63.23±1.54***	57.88±0.70***	35.60±1.05***	1.10±0.06***
DM	39.45±0.64***	40.67±0.34***	21.77±0.95***	0.61±0.01***
DQ	38.33±0.69***	35.70±0.89***	14.05±0.21***	0.57±0.004***
$FCEA_{25}$	60.97±0.5**	56.73±0.6	30.7±0.2**	0.9±0.07
$FCEA_{50}$	52.30±0.8***	49.76±1.1***	23.9±1.3***	0.6±0.02***
$FCEA_{100}$	40.51±1.26***	36.97±0.91***	14.52±0.51***	0.60±0.008***

Data are represented in the form of mean±SEM (n=6), Two-way ANOVA followed by Bonferroni test with **P<0.01 and ***P<0.001 significance, all groups are compared with DC, and DC is compared with NC. SGOT: Serum glutamic oxaloacetic transaminase; SGPT: Serum glutamic pyruvic transaminase; BUN: Blood urea nitrogen; NC: Normal control; DC: Diabetic control; DM: Diabetic, metformin; DQ: Diabetic, Quercetin; FCEA: Ethyl acetate fraction from methanolic extract of leaves of *Ficus carica* Lam.; SEM: Standard error of mean

Diabetic neuropathy is associated with decrease in paw withdrawal latency which is a behavioral biomarker of neuropathy, and STZ-induced diabetic animal model is widely accepted for study of neuropathy.[12,35] In tactile allodynia, quantitative assessment is estimated by von Frey filaments and Randall–Selitto test, and 50% threshold for nociceptive withdrawal latency provides quantitative assessment of tactile allodynia expressed by von Frey filaments. Randall Selitto is another adequate and sensitive method to quantify tactile allodynia in neuropathy; threshold for withdrawal latency is measured in the form of supported pressure in Randall–Selitto test.[13,27] Thus, in the present study, tactile allodynia has been successfully quantified and proved to be useful and versatile techniques for the assessment of appearance of neuropathic pain.

The abnormal high concentration of glucose due to diabetes causes severe derangement in protein metabolism and results in the development of negative nitrogen balance. This in turn increases urea and creatinine levels which serve biochemical diagnostic markers for assessing renal impairment and drug-induced toxicity.[15] The observed alteration identified, in the levels of blood urea and serum creatinine in group of diabetic rats reverted to near control by treatment with FCEA, indicating renal protective nature of the extracts during diabetes.[27] It is expressed by the results that FCEA attenuates and helps against diabetic neuropathy.

In the present study, the activities of serum glutamic-oxaloacetic transaminase (SGOT) and serum glutamic-pyruvic transaminase (SGPT) in serum were altered in diabetes. In diabetic animals, the change in the levels of SGOT and SGPT indicates change in metabolism where the enzymes are involved. Transaminases are active in the absence of insulin due to availability of amino acids in blood of diabetics, and thus increased activities of transaminases cause gluconeogenesis and ketogenesis.[15] SGOT and SGPT levels also act as an indicator of liver function, and hence restoration of normal level of these enzymes indicates restoration of normal functioning of liver. Overall results show that FCEA has nephroprotective and hepatoprotective action in diabetes. This amiolerative action is due to flavonoids such as quercetin, kaempferol, and chrysin reported in the fraction by GC-HRMS.

CONCLUSION

The current research reveals attenuating effects of ethyl acetate fraction of methanolic extract of *F. carica* Lam. on diabetic neuropathy and GC-MS characterization of the same clarifies that flavonoids quercetin, kaempferol, and chrysin are responsible for its protective action. Thus, FCEA can be a potential herb for patients suffering from diabetic neuropathy and associated complications of liver and kidney functions. It has DPPH-free radical scavenging activity of 78.35% showing hepatoprotective and nephroprotective effects in diabetic rats. This study provides a platform for the study of mechanism behind its action against neuropathy and can be studied by profiling oxidative stress and damage to nerve tissue.

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Conflicts of interest

There are no conflicts of interest.

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