Pharmacognostical Evaluation of *Pyrus Pashia*

1. Morphological Characteristics

Fruit is a spherical berry. The size of the fruits varied from 1-2.5 cm in diameter. The surface of the fruit is dark greyish in color bearing numerous densely distributed white and yellow spots Figure 6. The fruit consists of fine wide radiating carpel chambers with one or two seeds attached in axile placentum; Figure 6 (Table 5).

2. Microscopical Characteristics

Microscopy of the fruit revealed that fruit had fleshy thick pericarp. The pericarp was differentiated into epicarp and thick mesocarp which formed the major part of the fruit. The epidermis consisted of thin layer of tangentially stretched thick walled cells. Inner to the epidermis was a thick zone of about 10 layers of tabular thick walled cells with darkly stained cell inclusions Figure 7(A). The epidermal layer and hypodermal layer of thick walled cells were 110 μm thick. Mesocarp, showed complex tissue system, and was differentiated into outer, middle and inner zones. The outer zone consisted of thick continuous mass of brachysclereids alternating with narrow bands of parenchyma cells; Figure 7(C, D). Sclereids were small polyhedral, isodiametric and compact. They had very thick lignified walls, narrow canal like simple pits and narrow lumen; Figure 7(D). Middle parenchymatous mesocarp, inner to the outer mesocarp included wide polygonal thin walled compact cells having dense dark tannin contents; Figure7 (E, F). The middle mesocarp showed vascular strands; Figure 7(D). The inner mesocarp formed the border of capillary chambers. The bordering zone consisted of thick layer of sclereids as well as parenchyma cells. The partition septum of the fruit was thick and parenchymatous in nature. The cells had dense accumulation of tannins; Figure 7(G). Seed of the fruit was flat and thin, dark in color having smooth texture. It was flat on the upper side, and slightly convex on the lower side Figure 8A. Seeds were 3 mm long and 1 mm thick; the

cotyledon was flat and occupied the interior of the seed Figure 3B. The seed coat exhibited complex structure. It showed outer continuous, compact layer of palisade cells Figure; 8(B). Palisade cells were vertically oblong, and had length and width of 70 and 25 μm respectively. The inner part of the seed coat was differentiated into outer thick walled parenchyma cells, and an inner zone which was also parenchymatous in nature. These two zones had fairly thick walled darkly stained cells. The middle zone comprised of thin walled polyhedral compact cells Figure 8 (C, D and E).

Parameters	**Observations**
Colour	Dark greyish, bearing numerous yellow and white dots on its skin surface.
Size (length/width)	2-5 cm/1-3 cm
Shape	Ovoid, oval
Taste	Sweet and astringent

Table.5 Macroscopical characterstics of *Pyrus pashia* fruit.

3. Powder Characterstics

The powder of *P. pashia* fruit exhibited following elements- thick masses of brachysclereids were seen scattered in the powder. Isodiametric, thick walled, lignified, solitary sclereides with several thin radiating pit canals were observed Figure 9(A). Parenchyma cells, Figure 9(B) which are thin walled along with dark inclusions are seen as compact masses. These parenchyma cells constitutes middle mesocarp region of *P. pashia* fruit. Some cells containing dark tannin component were also evident Figure 9(C). Thick circular plates of cells were often observed in powder. These circular plates are 110 μm in diameter, and are compact clusters of

brachysclereides attached on the seed surface. These sclereid masses are surrounded by radiating parenchyma cells of the epidermis Figure 9(D). Calcium oxalate crystals are visible as bright prismatic bodies when viewed under polarized light; Figure 9(E).

Figure. 6 Macroscopy of *Pyrus pashia* fruits; A: Fruits of *Pyrus pashia*, B: Whole fruit exhibiting apical and stalk part of the fruit, C: TS of fruit showing epicarp, mesocarp, carpel chambers and seeds. (St: stalk of the fruit, AP: Apical part, Ep: Epicarp, MC: mesocarp).

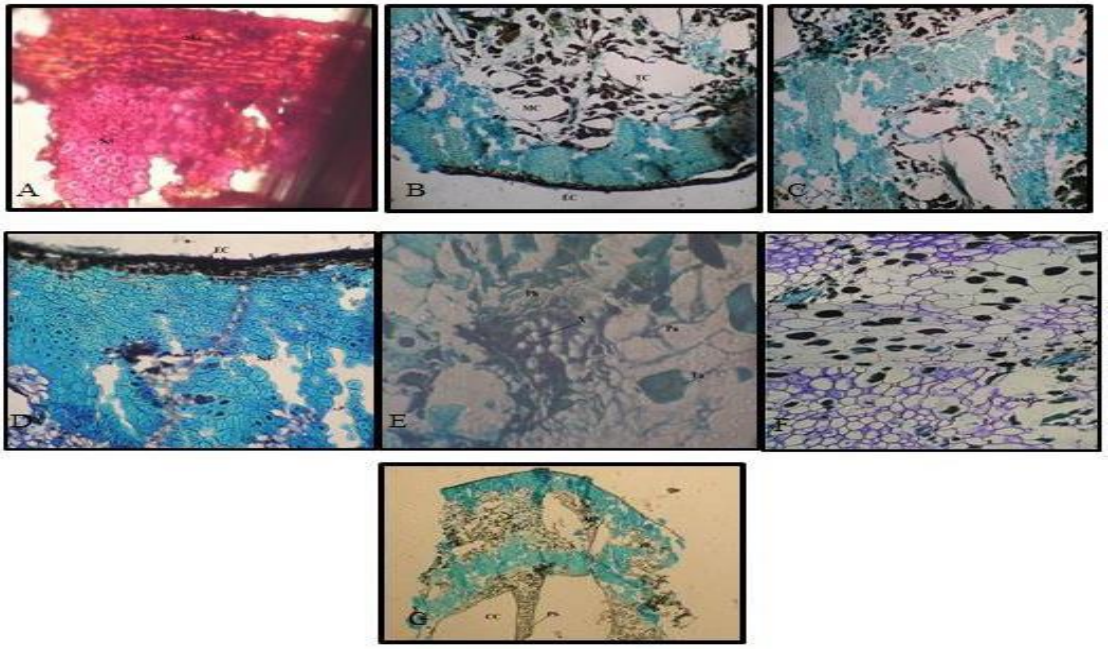


Figure. 7 Microscopy of fruit A: TS of the epicarp of fruit, B: TS of pericarp outer zone,C: TS of pericarp inner zone, D: Pericarp outer section, E: Vascular bundle in the pericarp. F: Parenchymatous region of the pericarp outer zone and inner zone. G: TS of the fruit showing outer pericarp and partition walls of carpel chamber. (Sec: sclerotic epicarp cells, Scl: sclereids, Tc: tannin cells, Mc: mesocarp cells, Ec: epidermal cells, Pa: parenchyma cells, Ph: phloem, X: xylem, OMC: outer mesocarp, IMC: inner mesocarp, CC: carpel chamber, PS: partition septum, MP: middle parenchyma.

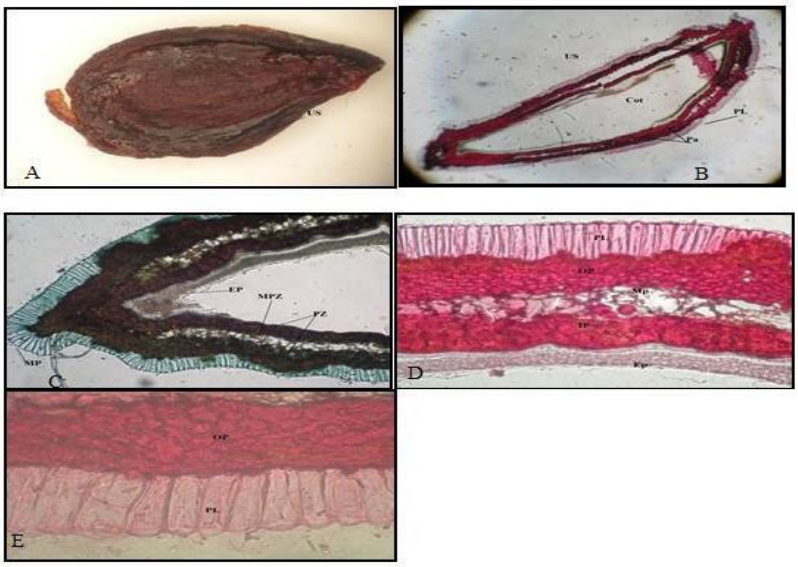


Figure. 8 Microscopy of seed: A: Seed surface feature, B: Sectional view of seed, C: Longitudinal section of seed micropylar end, D: Mid part of seed wall, E: Sectional view of seed coat layers. (US: Upper side, Cot: Cotyledon, Pa: Parenchyma cells, PL: Palisade layer, Ep: Epidemis, MP: Micropylar end, PZ: Parenchyma zone, MPZ: Middle parenchyma zone, OP: Outer parenchyma zones, IP: Inner parenchyma zone, Mp: Middle parenchyma.

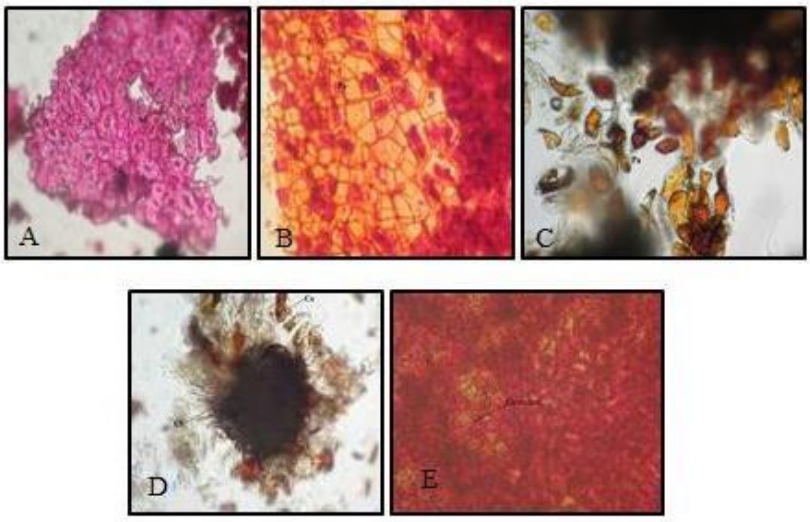


Figure.9 Powder characteristic of *P. pashia* fruit A: Brachysclereids B: Parenchyma cells C: Parenchyma cells with tannin content, D: Circular plate of brachyslcereides, E: Calcium oxalate crystals viewed under polarized light. (Scl: Brachysclereids, Pa: parenchyma cells, Cs: circular spots.

4. Physicochemical evaluation

Various physicochemical constants of the powdered crude drug *Pyrus pashia* were determined. Results are summarized in Table 6. Fluorescence characteristics of the powdered crude drug are depicted in Table 6. Analysis was carried out both in day light as well as under long U.V. (365nm) and short U.V (254 nm). Color identification was done using standard color index chart.

Parameters	**Results**
Foreign matter	1.40 ± 0.3 (%w/w)
Loss on drying	3.83 ± 1.2 (%w/w)
Total Ash	9.50 ± 0.50 (%w/w)
Acid insoluble ash	4.55 ± 0.20 (%w/w)
Water soluble ash	1.29 ± 0.15 (%w/w)
Alcohol soluble extractive	40.80 ± 1.02 (%w/w)
Water soluble extractive	28.40 ± 1.02 (%w/w)
Foaming index	Less than 100
Swelling index	4.5 ± 0.81
Volatile oil	Present
Crude fibre content	12.860 (% w/w) of plant material
Haemolytic index	79.47 ± 4.5 unit/gm

Results are reported as Mean ± S.E.M.

Table.6 Physicochemical parameters of *P pashia* fruit powder.

5. Fluorescence powdered drug analysis of *P. pashia* fruit

Powder+Reagent	**Fluorescence in daylight**	**Fluorescence (254 nm)**	**Fluorescence (365 nm)**
Powder as such	Brown	NF	NF
Powder+1N NaOH in methanol	Dark red	NF	NF
Powder+1N HCl in methanol	Maroon	NF	Spring green
Powder+1N HCl in water	Gold	Wheat	Sea green
Powder+1N HNO_3 in methanol	Dark red	NF	Yellow green
Powder+1N HNO_3 in water	Orange	Golden rod	Lime green
Powder+Iodine (5%)	Orange red	NF	NF
Powder+FeCl3 (5%)	Olive	NF	NF
Powder+KOH (50%)	Dark red	Golden rod	Sea green
Powder+Saturated Picric acid	Yellow	NF	NF
Powder+Acetic acid	Orange	Aquamarine	Lime green

Table.7 Fluorescence analysis of *Pyrus pashia* fruit powder.

6. Pesticide content analysis

Pesticide residue chlorinated as well as phosphated are summarised in Table.8.The results obtained revealed the presence of chlorinated and phosphated pesticide residue which were found within permissible limits as prescribed by WHO.

Pesticide	**First elute**	**Second elute**	**Third elute**
Chlorinated	Not more than 0.021 ± 0.002 mg/kg	Not more than 0.010 ± 0.003 mg/kg	Absent
Phosphated	Not more than 0.024 ± 0.001 mg/kg	Not more than 0.015 ± 0.005 mg/kg	Absent

Results are reported as Mean ± S.E.M.
Table. 8 Pesticide content analysis of *P. pashia*

7. Heavy Metal Analysis

Concentration of various heavy metals such as lead, cadmium, zinc and mercury are summarized in Table. 9 Results indicated that the values estimated were found to be within the limits as prescribed by the WHO guidelines.

Heavy metals (conc in ppm)	**Concentration (ppm)**
Lead (Pb)	Not more than 0.0065 ppm
Cadmium (Cd)	Not more than 0.00016 ppm
Zinc (Zn)	Not more than 0.1110 ppm
Mercury (Hg)	Not more than 0.1443 ppm

Table.9 Heavy Metal Analysis of *P. pashia.*

8. Extraction, Qualitatative and Quantitatative Estimation & Standardization of extract

8.1 Preparation of fruit extract and successive fractions

Yield of lyophilized ethanolic extract was found to be (EPP; 9.33%, w/w). The subfractions of EPP obtained successively by fractionation showed the yield as follows n-hexane (6.8%, w/w), chloroform (3.9% w/w), ethyl acetate (14.6% w/w), and butanol (13.9% w/w) fraction. The results for the preliminary phytochemical screening of ethanolic extract of *P.pashia* and its subfractions are represented in Table. 10

Phytoconstituents	Ethanolic extract (EPP)	Fractions				
		Hexane fraction	Chloroform fraction	Ethyl acetate fraction	Butanol fraction	Aqueous fraction
Alkaloid	+	+	+	+	+	-
Glycoside	+	–	–	+	+	+
Flavonoid	+	–	+	+	+	+
Steroid	+	+	+	+	+	–
Phenolic & tannin	+	–	+	+	+	+
Saponin	+	–	–	–	–	+
Amino acids	+	-	-	-	-	+
Proteins	+	-	-	-	+	+
Mucilage	+	–	–	–	–	+

Table.10 Phytochemical screening of EPP.

The phytochemical screening of *P.pashia* and its sub-fractions indicated the presence of polyphenolics. Other secondary metabolites found in EPP were alkaloids, steroids, saponins and mucilage. Total phenolic content quantified was 174.0 mg of total polyphenols/gm of EPP measured as gallic acid equivalent. The total flavonoid content of the EPP was measured spectrophotometrically against standard curve obtained by quercetin. EPP contained 81.82±0.86 mg of total flavonoids/gm of EPP measured as quercetin equivalent. The amount of alkaloid present in plant material was 1.19±0.13% w/w. Total saponin content in the plant material was found to be 44.48±1.42 mg/g diosgenin equivalent, while total carbohydrate was found to be 87.67±3.21 mg/g d-fructose equivalent .(Table.11)

Phytoconstituents	**EPP extract**	**EPP fractions**			
		PPC	PPEA	PPBU	PPAQ
Total phenolic (mg/g GAE)	**174±0.34**	-	87.54±0.50	128.63±0.53	76.12±1.61
Total alkaloid (% w/w)	1.19±0.13	0.38±0.10	0.21±0.10	0.52±0.12	-
Total flavonoid (mg/g QE)	**81.82±0.86**	3.39±1.26	48.18±4.46	49.72±2.02	40.05±3.78
Total saponin (mg/g DE)	44.48±1.42	-	-	-	16.43±3.27
Total carbohydrate (mg/g FE)	87.67±3.21	-	-	21.99±1.21	35.38±3.38

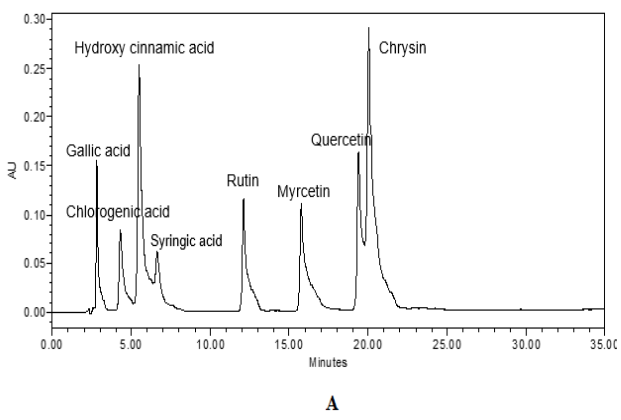
GAE: Gallic acid equivalent; QE: Quercetin equivalent; DE: Diosgenin equivalent; FE: Fructose equivalent. (Results are reported as Mean ± S.E.M.)

Table.11 Quantitative analysis of Ethanolic extract of *P.pashia* fruit.

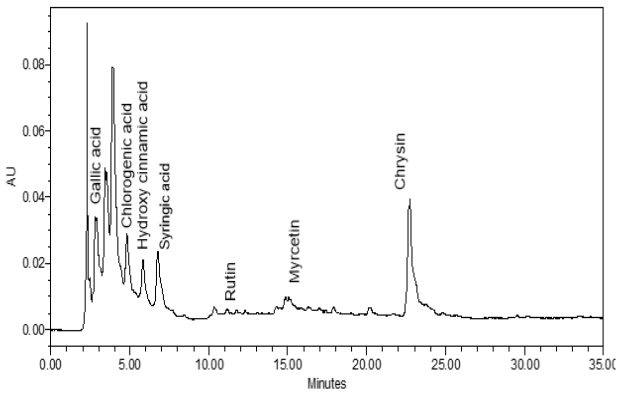
9. Characterization of polyphenolics and amino acids in *Pyrus pashia* fruits

9.1. Characterization of polyphenolics in EPP

Polyphenol content of fresh EPP extract were characterized by HPLC (Fig.10). The bioactive components of EPP were Gallic acid, Chlorogenic acid, Hydroxycinnamic acid, Syringic acid, rutin, myrcetin and chrysin (Table 12). We found that chrysin was the major polyphenol, 26.06±0.21mg/gm in EPP.



A



B

Figure.10 Characterization of polyphenolics in EPP; A- HPLC chromatogram of standard polyphenol; B- HPLC chromatogram of EPP.

Name	**mg/gm**
Gallic acid	2.76±0.05
Chlorogenic acid	21.8±0.12
Hydroxycinnamic acid	8.16±0.014
Syringic acid	8.94±0.24
Rutin	5.17±0.145
Myrcetin	6.38±0.047
Chrysin	26.06±0.21

Results are reported as Mean ± S.E.M.
Table. 12 Quantitative values of the polyphenols in EPP.

9.2 Amino acid profile of Pyrus pashia

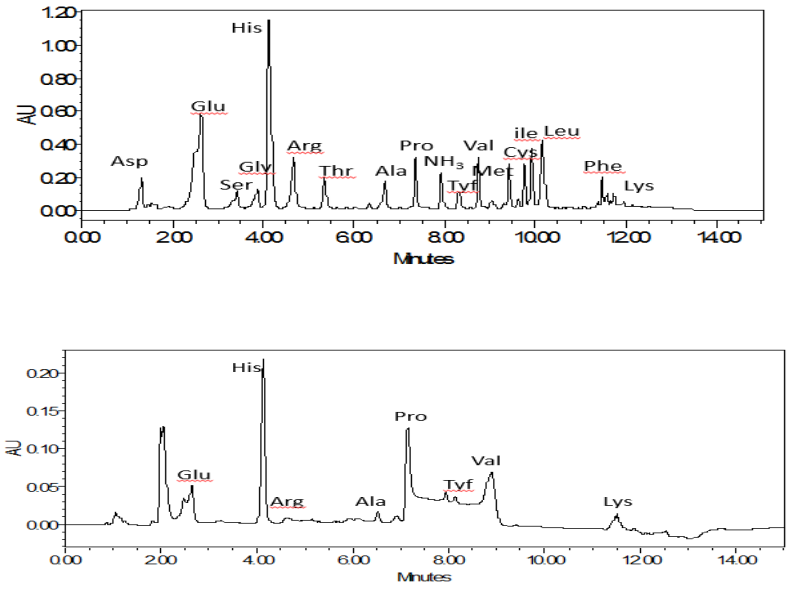


Figure.11 Amino acid profiling of EPP; A. Standard HPLC amino acid chromatogram B- HPLC chromatogram of EPP.

To our knowledge, there are no published reports regarding the amino acid analysis of the fruit extract of *P. pashia*. Figure. 11 showed the chromatographic representation of the amino acid content of extract with their standard amino acid (A) expressed as 100 mg/g. Extract showed the presence of charged amino acids such as glutamine and histidine Table.13.

Name	**mg/gm**
Aspargine	0.36±0.047
Glutamine	6.81±0.142
Glycine	3.41±0.014
Histidine	0.27±0.085
Tryptophan	2.57±0.125
cystine	0.055±0.36
Lysine	0.274±0.125

Table.13 Quantitative values of the amino acids in EPP

10. Characterization of isolated chrysin

Yellow powder obtained from DCM:Ethylacetate 50:50 as eluent was confirmed as flavone, and identified as chrysin after using TLC, U.V., FTIR,NMR and HRMS techniques. Spectral results confirmed that isolated compound (C1) was chrysin. Moreover, the purity of the isolated compound was checked using ultra performance liquid chromatography, quantified in the ethanolic extract and was found to be 1.73% w/w.

10.1 Physical properties and spectral data of isolated C1 (Chrysin)

Physical Properties

Colour: Yellow crystalline powder

Melting point: 285-286ºC

TLC profile

TLC analysis showed a single spot with R_f value of 0.75

(Dichloromethane: Ethylacetate; 30:70). Fig. 12

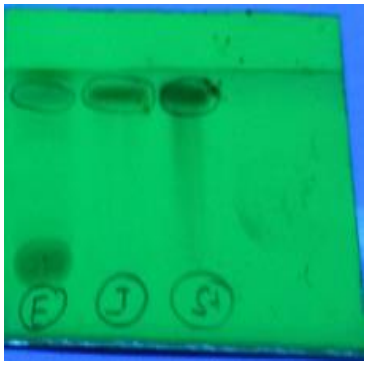


Figure.12 TLC analysis of EPP and isolated chrysin.
E= Extract; I= Isolated chrysin; St= Standard chrysin; DCM: Ethyl acetate (30:70)

U.V. analysis of C1 (Chrysin)

λ max of C1(Chrysin) was found to be 268.40 nm. Fig.13

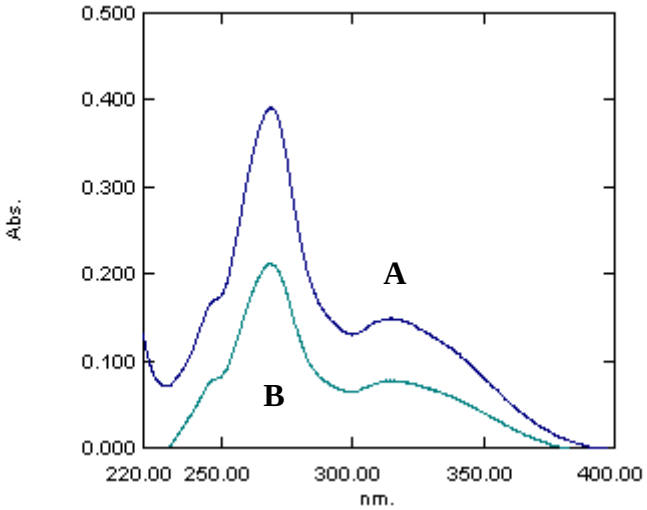


Figure.13 U.V. spectral analysis of A. Isolated Chrysin B. Standard Chrysin.

FTIR analysis of C1 (Chrysin)

(KBr, υ cm^{-1}): An intense band in the region of 3500– 3000 cm^{-1} appeared, which can be attributed to the symmetrical and asymmetrical stretching modes of O–H. A strong vibrational stretch of the carbonyl group C = O) was noted at 1610.61 cm^{-1}. All of the C–H stretching frequencies were in the range 2918.34 cm^{-1}. Transmittance at 1246.06 cm^{-1} corresponds to the

C–C bending vibration. Based on the IR spectral data, the isolated sample was confirmed to be chrysin (5, 7-dihydroxy flavone).Figure.14

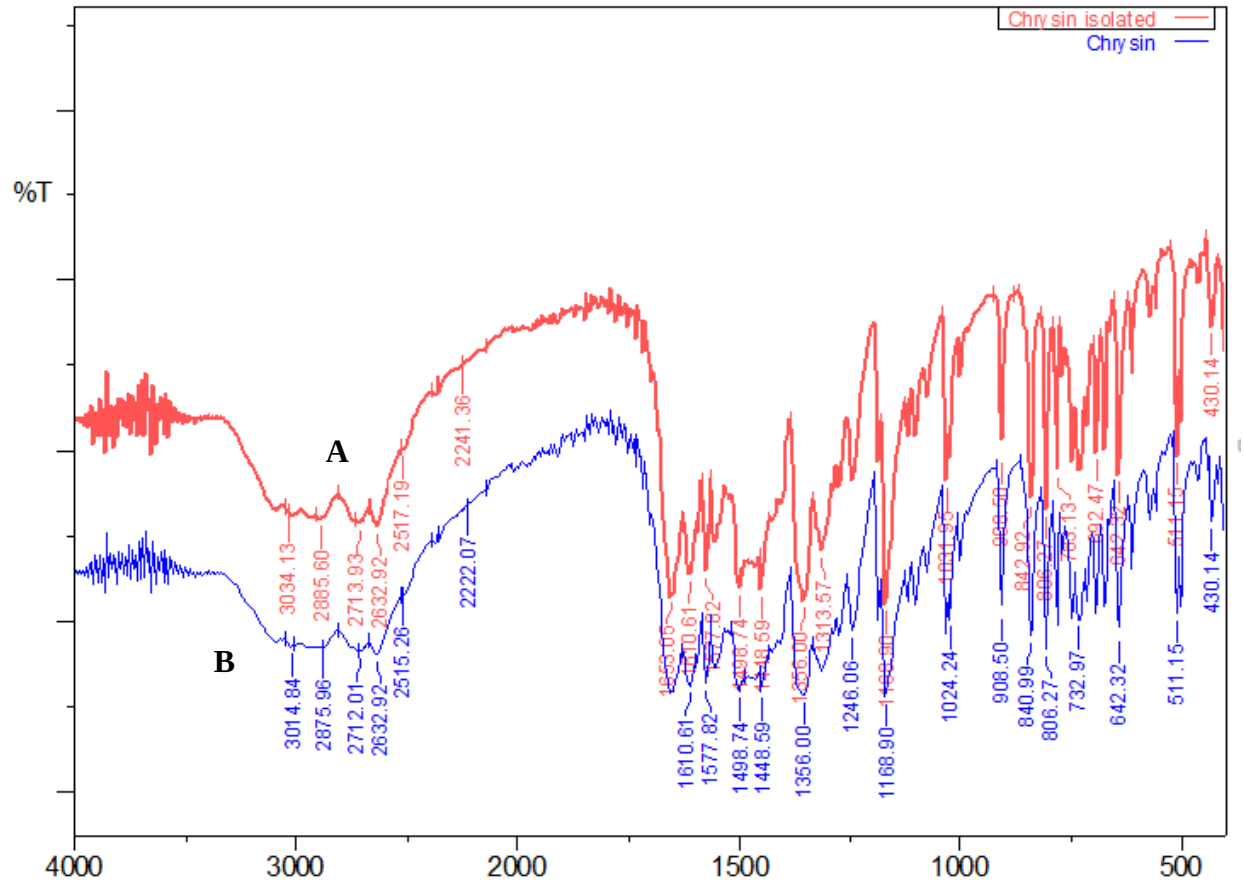


Figure. 14. FTIR analysis of A. Isolated chrysin B. Standard Chrysin.

NMR analysis of C1 (Chrysin)

^{1}HNMR (600 MHz, DMSO-*d*^{6})

Chemical Shift (ppm)	Multiplicity	Integration Ration	Assignment	Coupling Constant (Hz)
12.81	S	1	5-OH	-
10.92	S	1	7-OH	-
8.06- 8.04	M	2	H-2', H-6'	-
7.57- 7.56	M	3	H-3', H-4', H-5'	-
6.96	S	1	H-3	-
6.51	S	1	H-6	*J*= 1.8
6.20	S	1	H-8	*J*= 2.4

Table. 14 Detailed description of ^{1}HNMR spectra of isolated Chrysin.

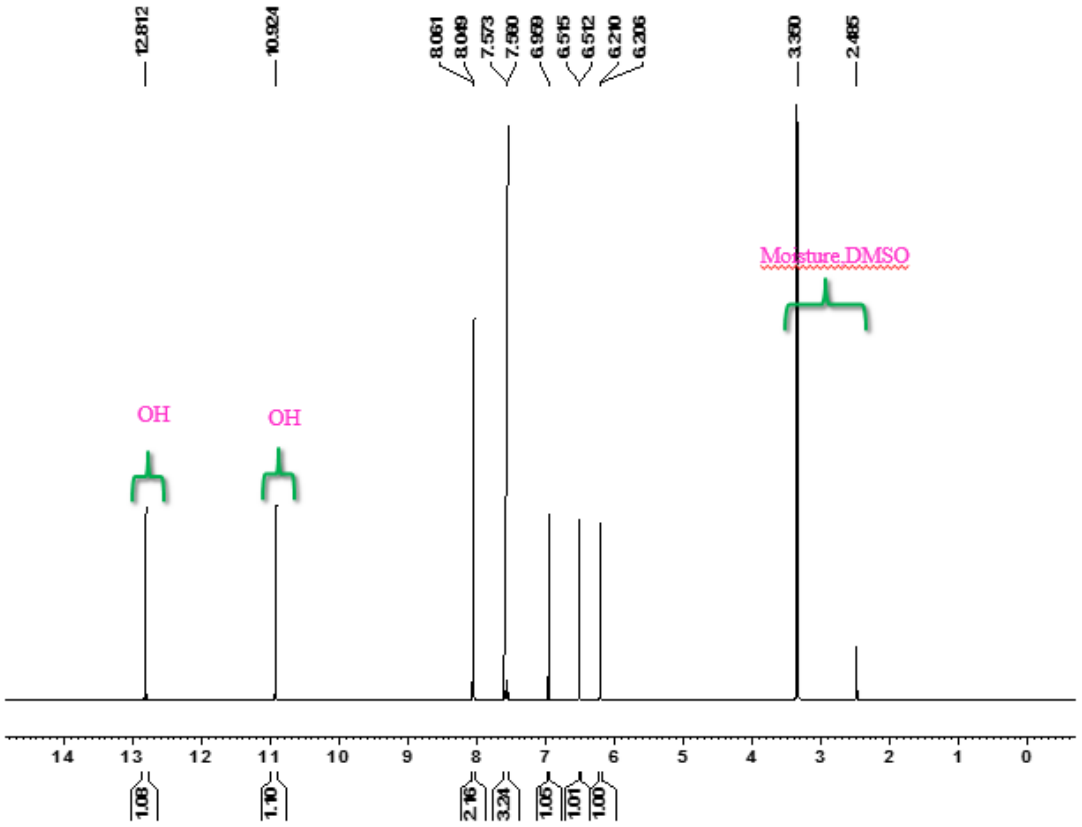


Figure.15. [1]HNMR of isolated Chrysin in DMSO-d_6.

[13]CNMR (150 MHz, DMSO-*d*[6]): δ 161.95(C-2), δ 104.47(C-3), δ 182.34(C-4), δ 163.69(C-5), δ 99.51(C-6), δ 164.91(C-7), δ 99.51(C-8), δ 157.96(C-9), δ 105.68(C-10), δ 132.48(C-1′) δ 126.89(C-2′) ,δ 129.61(C-3′), δ 131.21(C-4′), δ 129.61(C-5′), δ 126.89(C-6′).

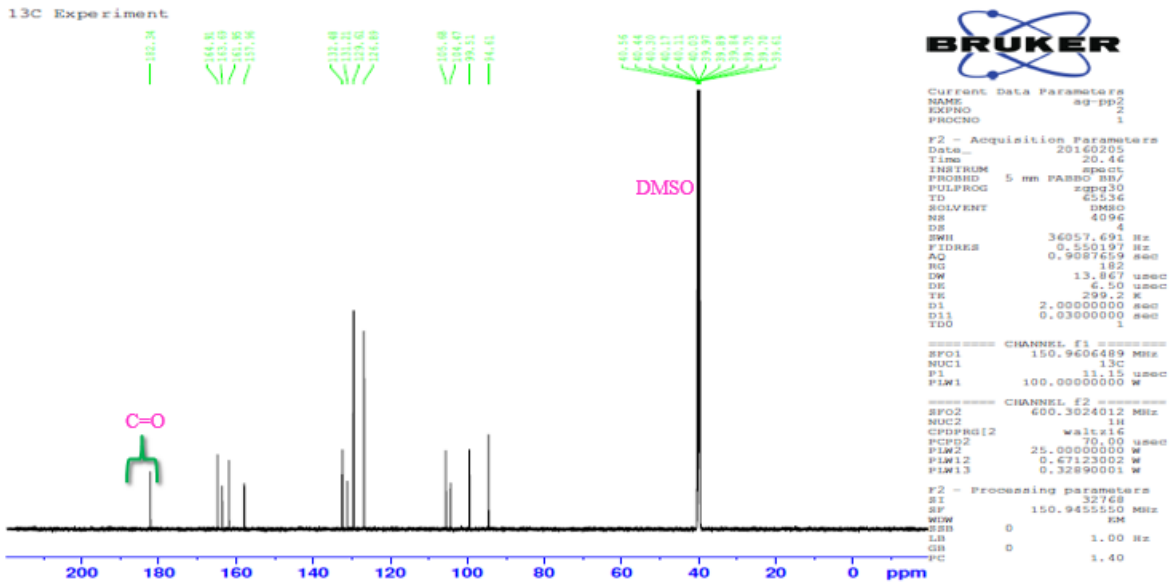


Figure. 16 ^{13}C NMR of isolated Chrysin.

DEPT 135 (Distortionless enhancement of polarization transfer) DEPT 135 spectra of isolated chrysin showed aromatic CH peaks. Moreover, no CH_2 peaks were observed. Furthermore there was disappearance of quaternary carbon and carbonyl peaks. Figure.17

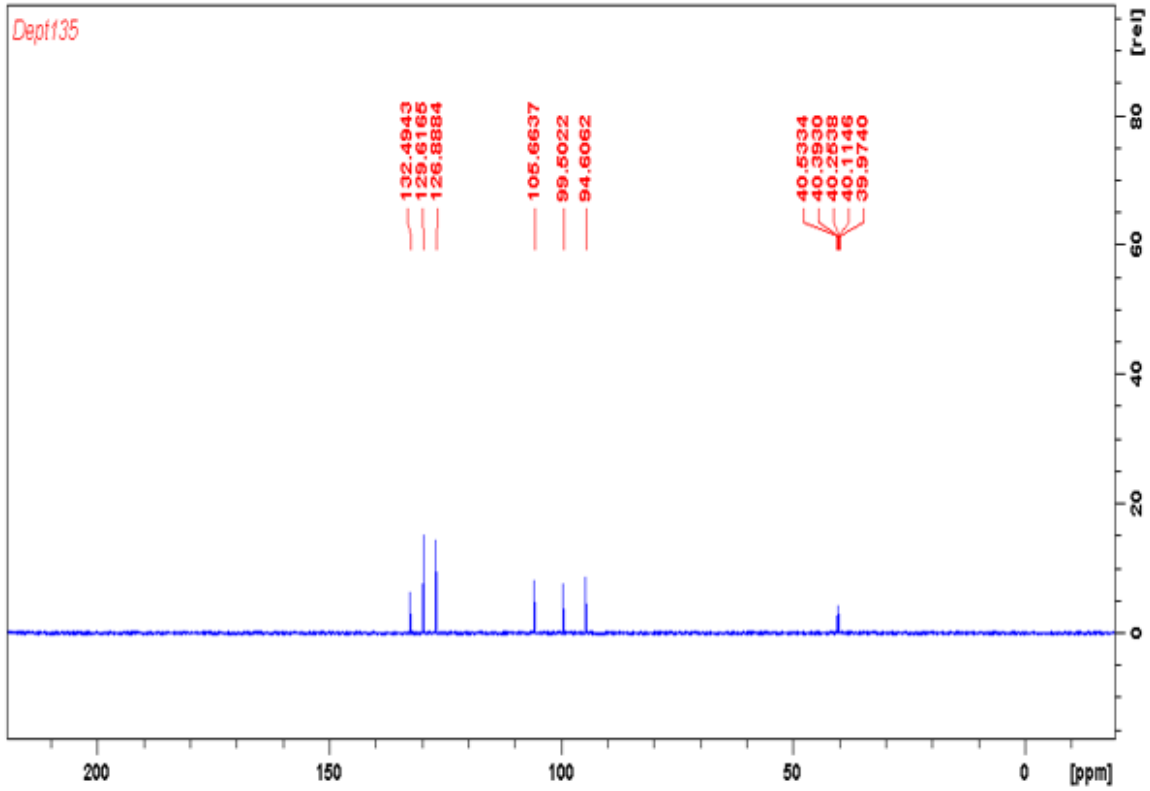


Figure.17 DEPT135 of isolated Chrysin

HSQC and HMBC (Heteronuclear Single Quantum Coherence) and (Heteronuclear Multiple Bond Correlation)

Comparison between ^{1}HNMR and ^{13}C NMR NMR spectra was performed using HSQC and HMBC experiments. Based on the cross peaks, protons attached to the carbon atoms were observed. Observation of the spectra confirmed that isolated compound was chrysin. Figure. 18-21.

HSQC

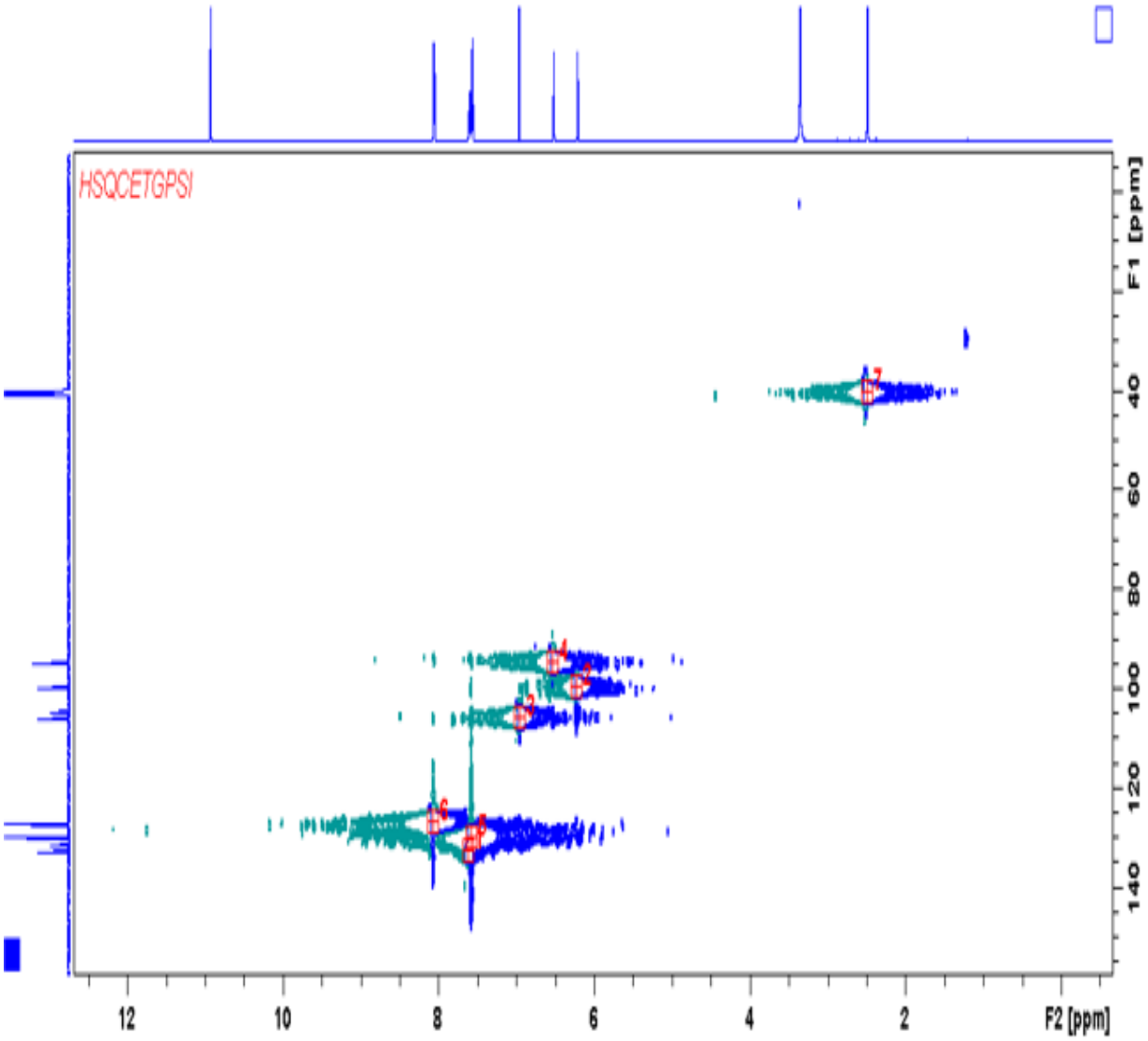


Figure. 18 HSQC spectra of isolated Chrysin.

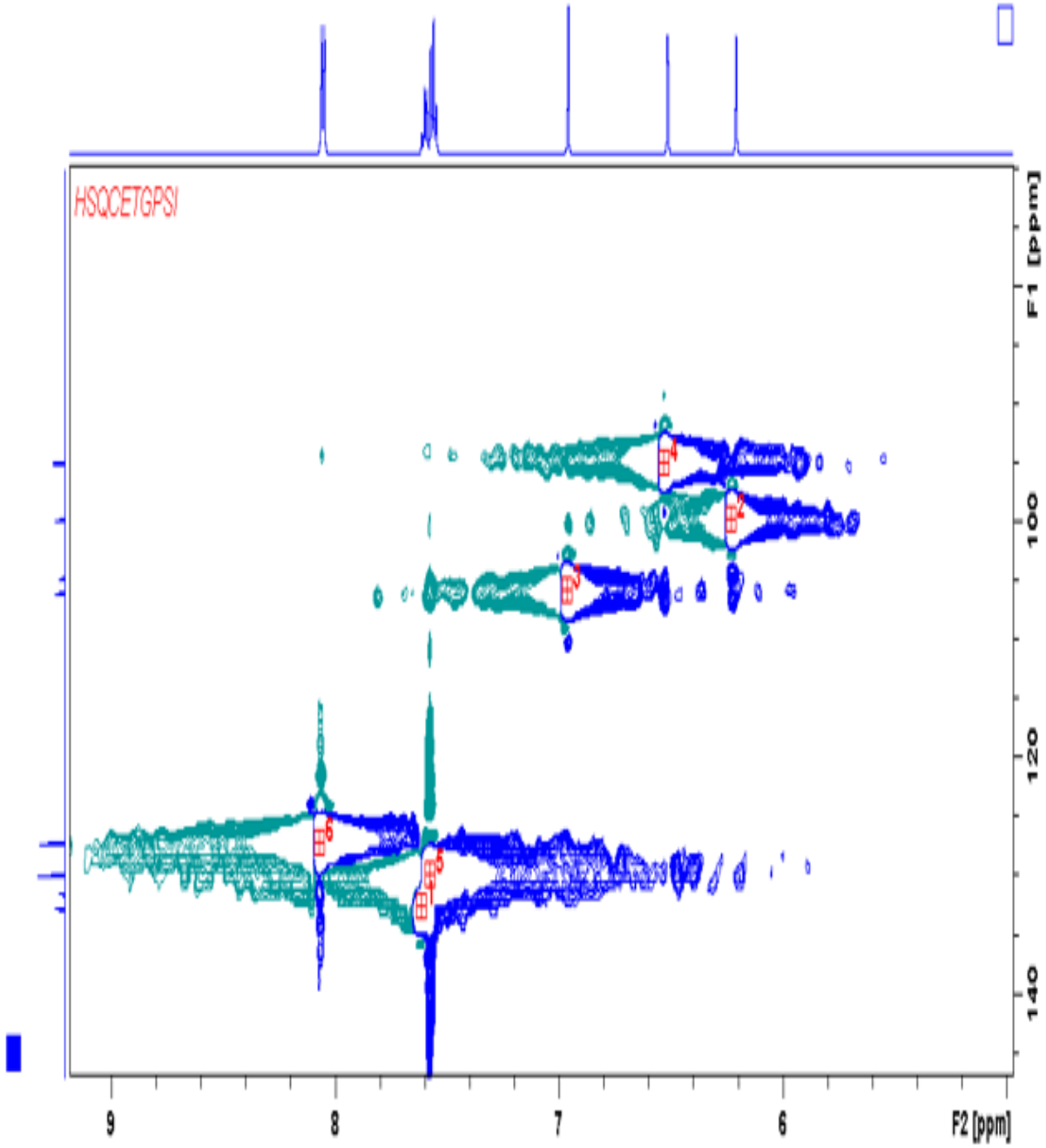


Figure.19 HSQC zoom spectra of isolated Chrysin.

HMBC

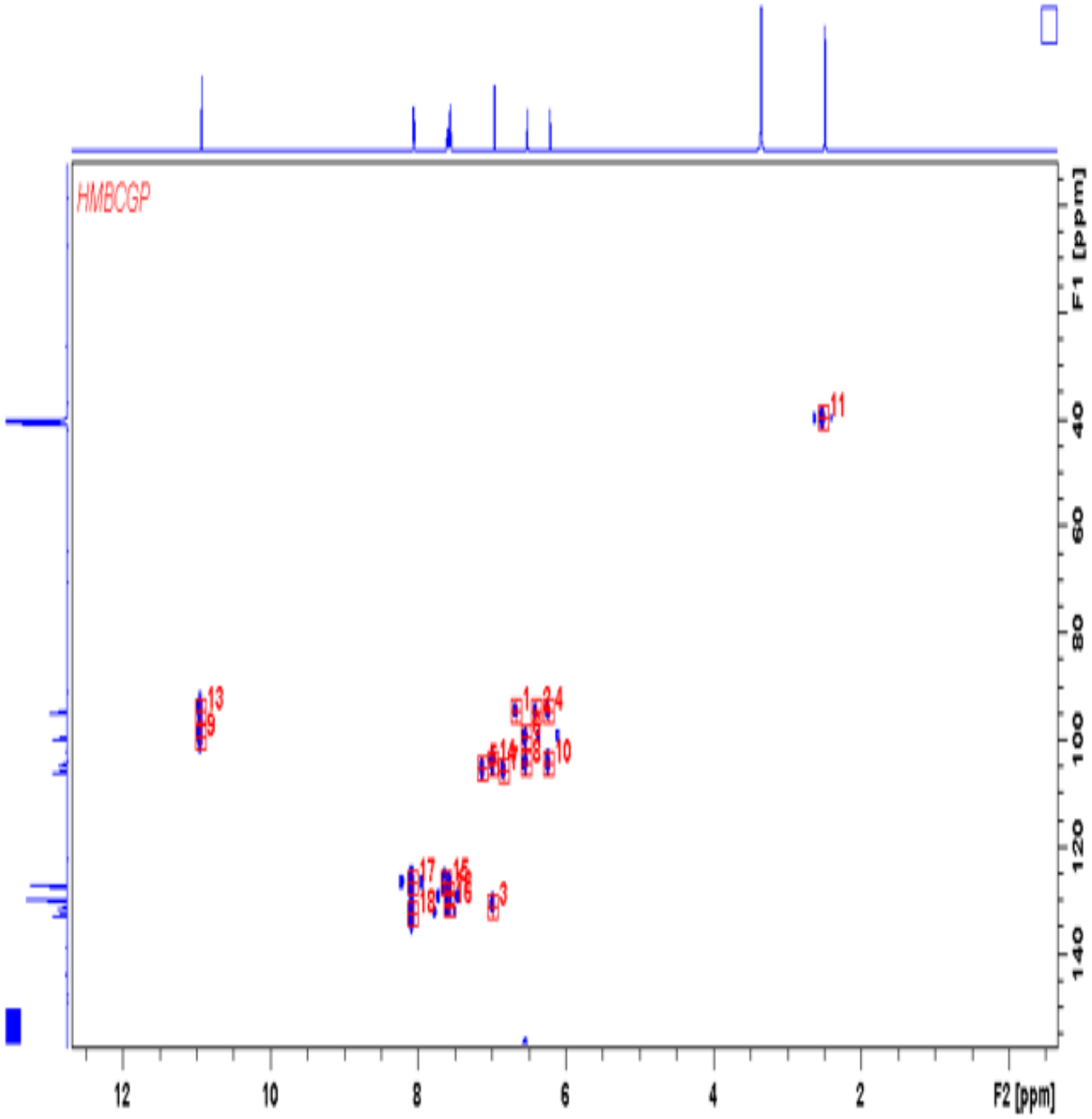


Figure. 20 HMBC spectra of isolated Chrysin.

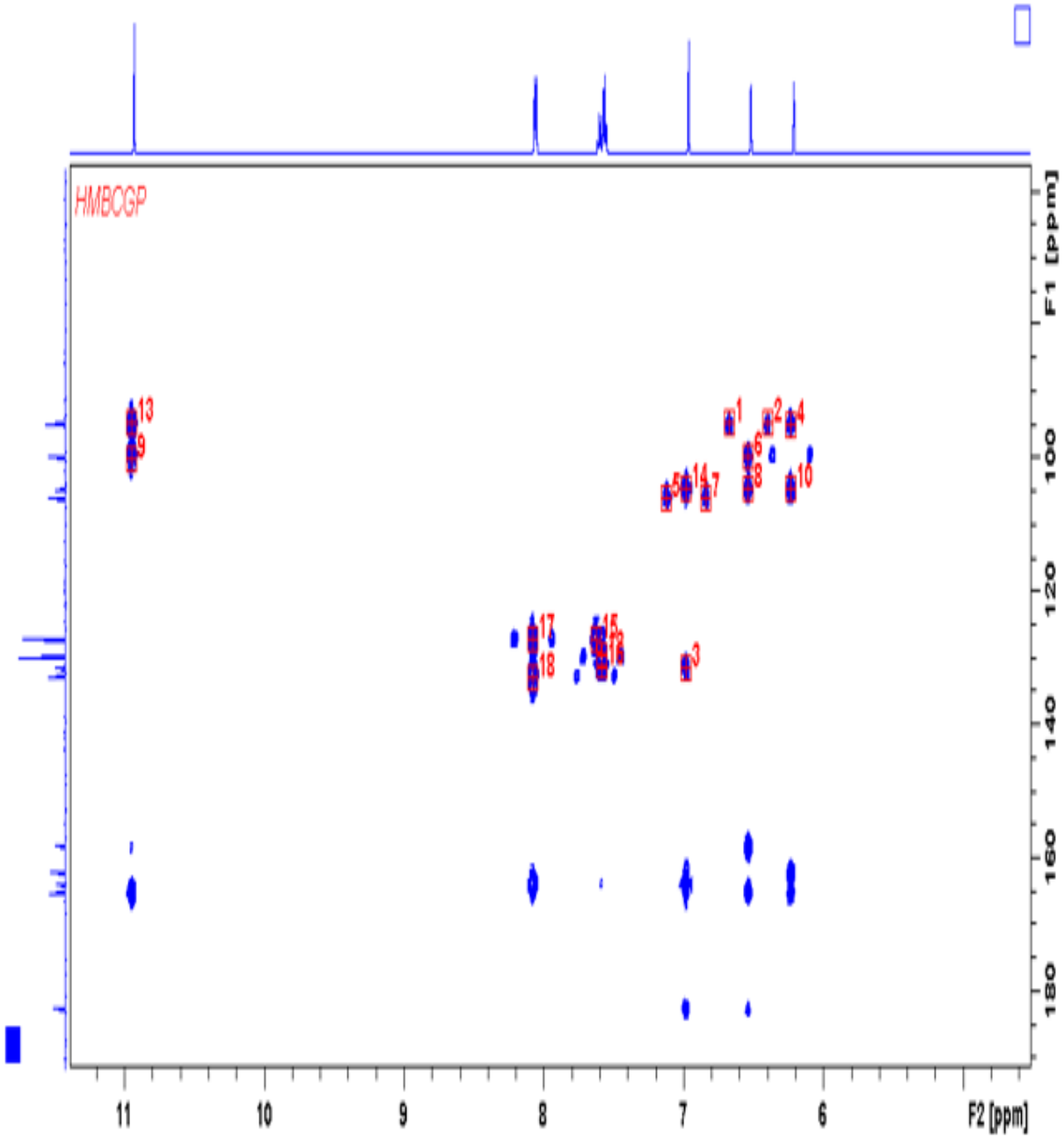


Figure. 21 A. HMBC zoom spectra of isolated Chrysin.

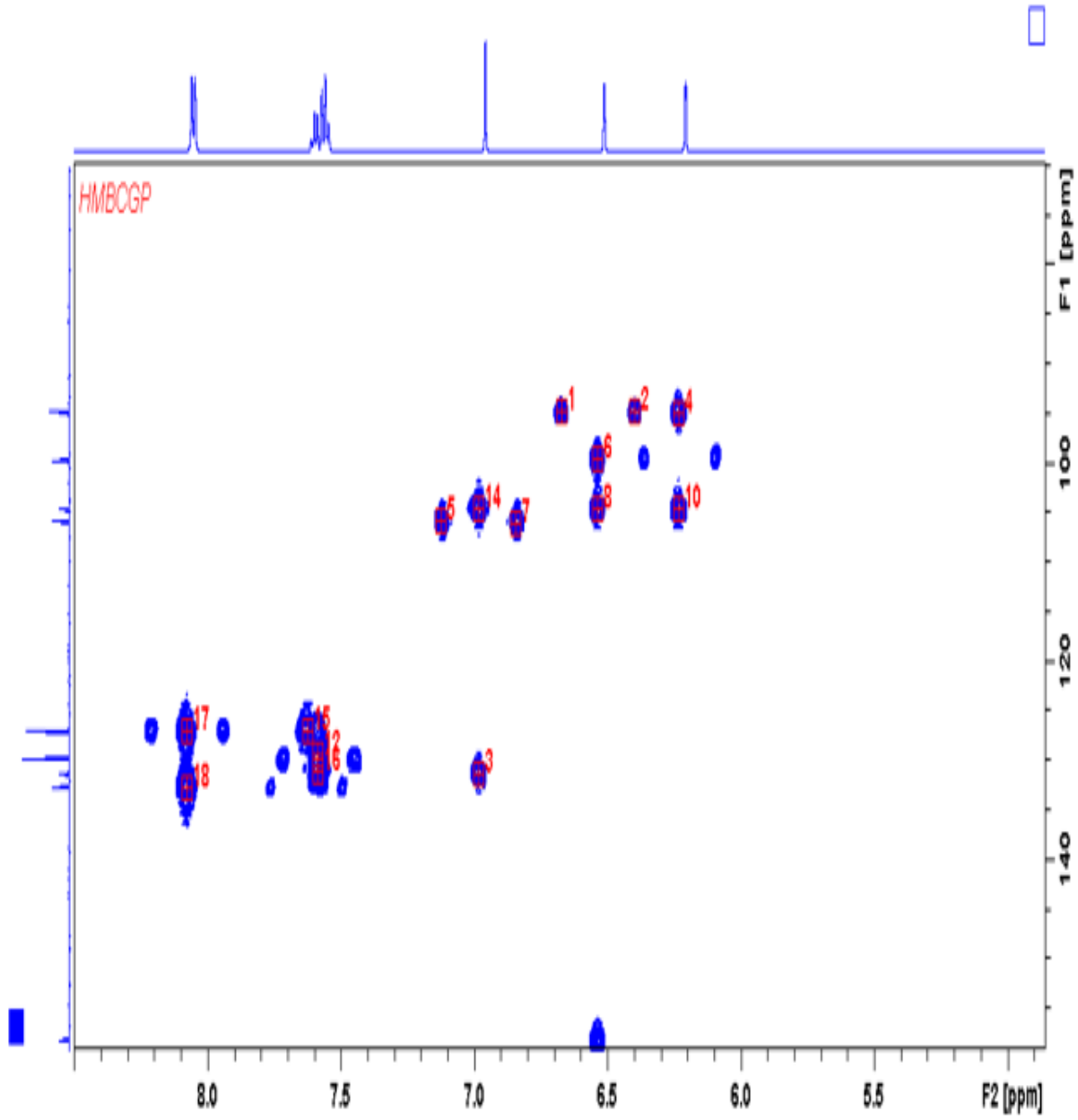


Figure 21 B. HMBC zoom spectra of isolated Chrysin.

HRMS (High Resolution Mass Spectroscopy) analysis of C1 (Chrysin)

Compound showed, M+1 Peak: 254.9299. Fig.22.

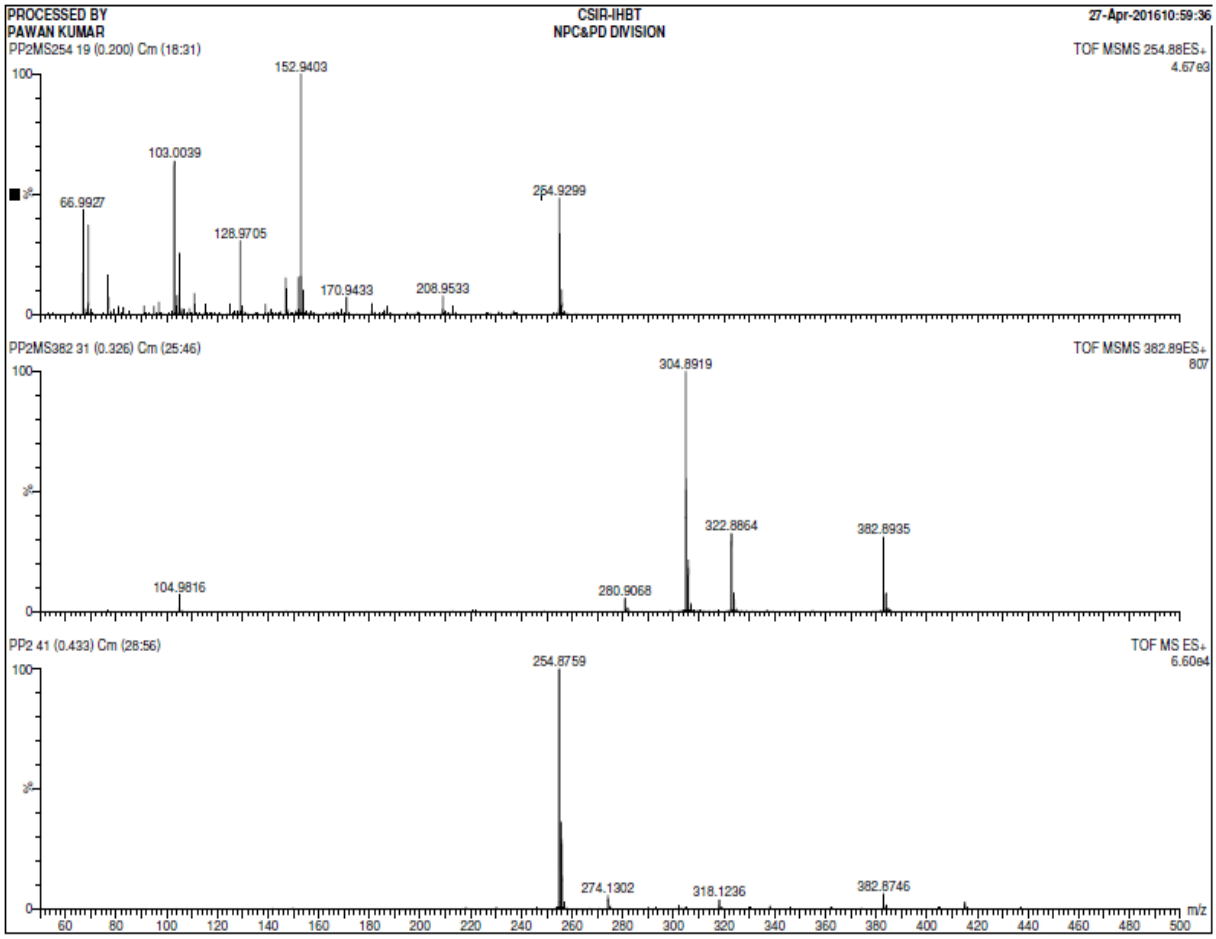
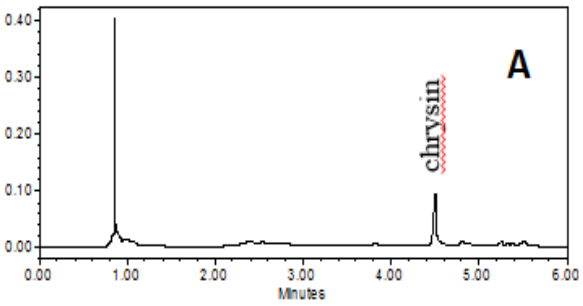


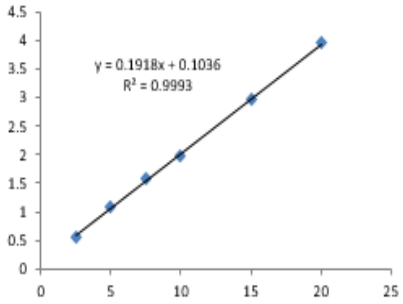
Figure. 22 HRMS spectra of isolated Chrysin

11. Quantification of chrysin in ethanolic extract of *Pyrus pashia* by UPLC

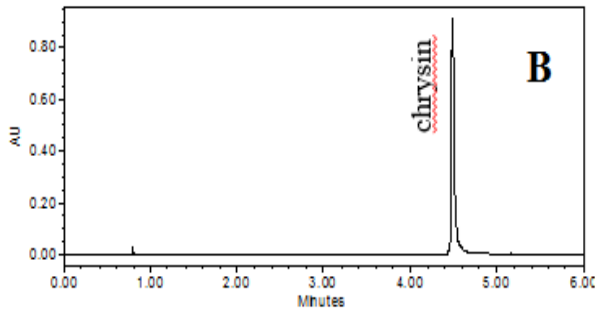
From the standard plot of chrysin and the linear regression equation, the content of chrysin in the crude ethanol extracts of *Pyrus pashia* was found to be 1.73% % w/w. Figure.23



Chrysin peak in sample



Calibration curve for standard chrysin



Standard

Figure. 23 UPLC chromatogram of ethanolic extract of *Pyrus pashia.* (A) Ethanolic extract of *Pyrus pashia*, (B) Standard Chrysin.

Above analytical analysis confirmed that isolated compound was chrysin.

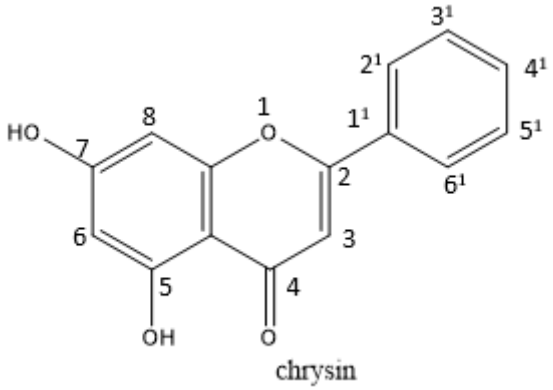


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Figure. 24 Structure of chrysin (5, 7- dihydroxy flavone)

1. Acute Toxicity Studies

The acute oral toxicity study revealed that the EPP in increasing doses of 5, 50, 300, and a maximum dose up to 2000mg/kg was found tolerable in the experimental animals. A low dose of 100 mg/kg (half of one-tenth of the maximum lethal dose), a median dose of 200 mg/kg (one-tenth of the maximum lethal dose), and a high dose of 400 mg/kg (twice that of one tenth dose) were selected for evaluation of pharmacological activities. Moreover, the rats did not show any gross behavioral, neurological or autonomic toxic effects after the first 3 h of EPP administration and any lethality after 24–72 h till 14 days.

2. **Evaluation of anticonvulsant activity of standardized EPP and chrysin**

Acute Model

2.1 Maximal electroshock (MES)-induced convulsion model

Fig. 25 illustrates the effect of EPP (100, 200, and 400 mg/kg) and (chrysin-2.5, chrysin-5 and chrysin-10 mg/kg) group on the duration of HLTE in MES induced seizures in rats. Statistical analysis revealed that there were significant differences in the duration of onset of HLTE in MES-induced seizures ($F (7, 40) = 10.4$, $P < 0.05$) among extract treated groups. Extract treatment group animals showed a significant decrease in the duration of onset of HLTE in MES induced seizures compared to NC. Moreover, there were no significant differences in the duration of onset of HLTE in MES-induced seizures between PHT-25, EPP-200, and EPP-400 group animals. However, chrysin failed to protect the animals against MES induced HLTE.

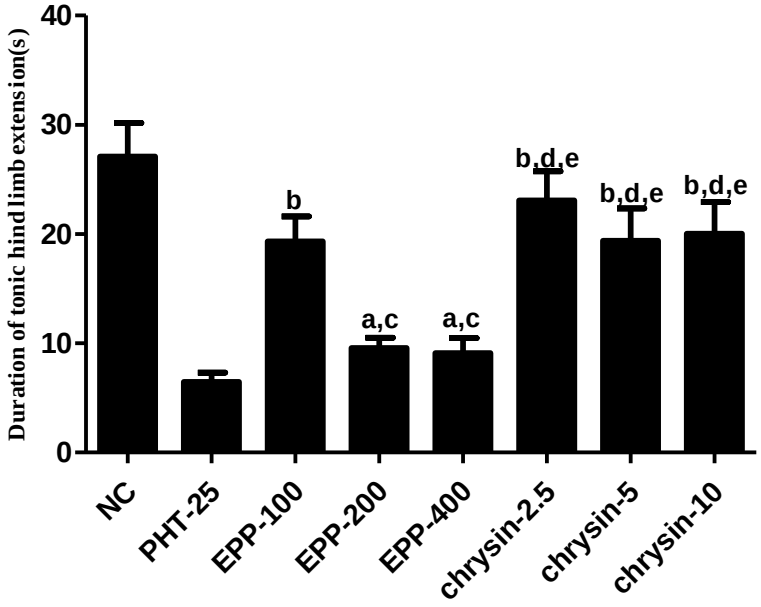

Figure. 25 Effect of EPP (100, 200, and 400 mg/kg) and chrysin (2.5, 5 and 10 mg/kg) on MES induced changes in the duration of convulsion in rats. All values are mean ± SEM (n = 6). $^{a}P < 0.05$, $^{b}P < 0.05$, $^{c}P < 0.05$, $^{d}P<0.05$,$^{e}P<0.05$,$^{f}P<0.05$,$^{g}P<0.05$ 0.05 compared to NC, DZ-5, and EPP-100, chrysin-2.5,chrysin-5, chrysin-10 respectively (one way ANOVA followed by Student–Newman–Keuls post hoc test.

2.2 Pentylenetetrazole induced convulsion (PTZ) model

Effect of EPP (100, 200, and 400 mg/kg) and (chrysin-2.5, chrysin-5 and chrysin-10 mg/kg) on the duration of onset of convulsion in PTZ-induced seizures in rats is depicted in Figure. 26. Statistical analysis revealed that there were significant differences in the duration of onset of convulsion in PTZ-induced seizures ($F (7, 40) = 43.7$, $P < 0.05$) among groups. All the drug treatment group animals showed a significant decrease in the duration of onset of convulsion in PTZ-induced seizures compared to NC. Moreover, there were no significant differences in the duration of onset of convulsion in PTZ-induced seizures between DZ-5, EPP-200, EPP-400, chrysin-5 and chrysin-10 group animals.

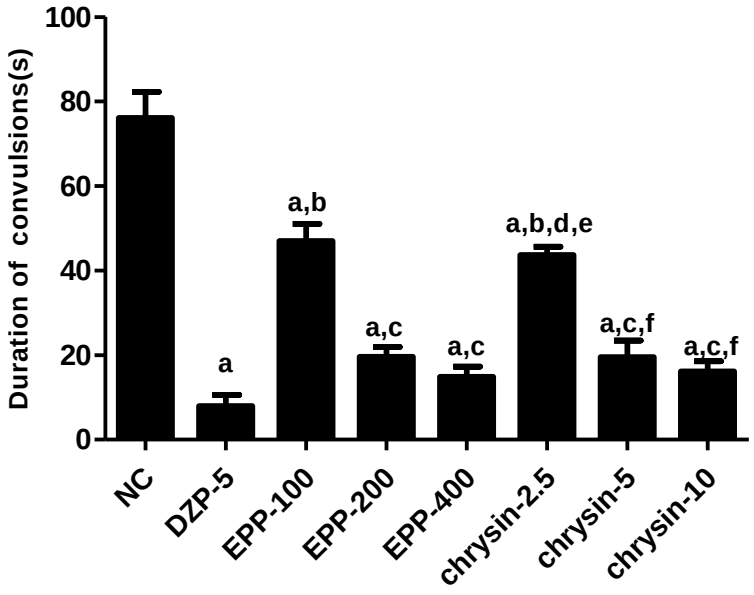

Figure. 26 Effect of EPP (100, 200, and 400 mg/kg) and chrysin (2.5,5 and 10 mg/kg) on PTZ induced changes in the duration of convulsion in rats. All values are mean ± SEM (n = 6). $^{a}P < 0.05$, $^{b}P < 0.05$, $^{c}P < 0.05$, $^{d}P < 0.05$, $^{e}P < 0.05$, $^{f}P < 0.05$, $^{g}P < 0.05$ 0.05 compared to NC, DZ-5, EPP-100, EPP-200, EPP-400, chrysin 2.5 chrysin-5, chrysin-10 respectively (one way ANOVA followed by Student–Newman–Keuls post hoc test).

2.3 Evaluation of anticonvulsant activity of EPP (100, 200, and 400mg/kg; p.o.) in PTZ challenged rats

Figure. 27 illustrates the effect of EPP (100, 200, and 400 mg/kg) on the duration of onset of convulsion in PTZ-induced seizures in rats. Statistical analysis revealed that there were significant differences in the duration of onset of convulsion in PTZ-induced seizures ($F(7, 40) = 43.7$, $P < 0.05$) among groups. All the drug treatment group animals showed a significant decrease in the duration of onset of convulsion in PTZ induced seizures compared to NC. Moreover, there were no significant differences in the duration of onset of convulsion in PTZ-induced seizures between DZ-5, EPP-200, and EPP-400 group animals. The effect of EPP (100, 200, and 400 mg/kg) on the percentage of power spectra to baseline of alpha, beta, delta, and theta waves in PTZ-induced seizures in rats is depicted in Figure. 27. Statistical analysis revealed that there were significant differences in the percentage of power spectra to baseline of alpha ($F(4, 25) = 47.0$, $P < 0.05$), beta ($F(4, 25) = 25.6$, $P < 0.05$), delta ($F(4, 25) = 80.8$,

P < 0.05), and theta (F (4, 25) = 14.5, P < 0.05) wave among groups. All the drug treatment group animals showed a significant decrease in the percentage of power spectra to baseline of alpha, beta, delta, and theta waves in PTZ-induced seizures compared to NC. Moreover, there were no significant differences in the percentage of power spectra to baseline of alpha, beta, delta, and theta waves in PTZ induced seizures between DZ-5, EPP-200, and EPP-400 group rats.

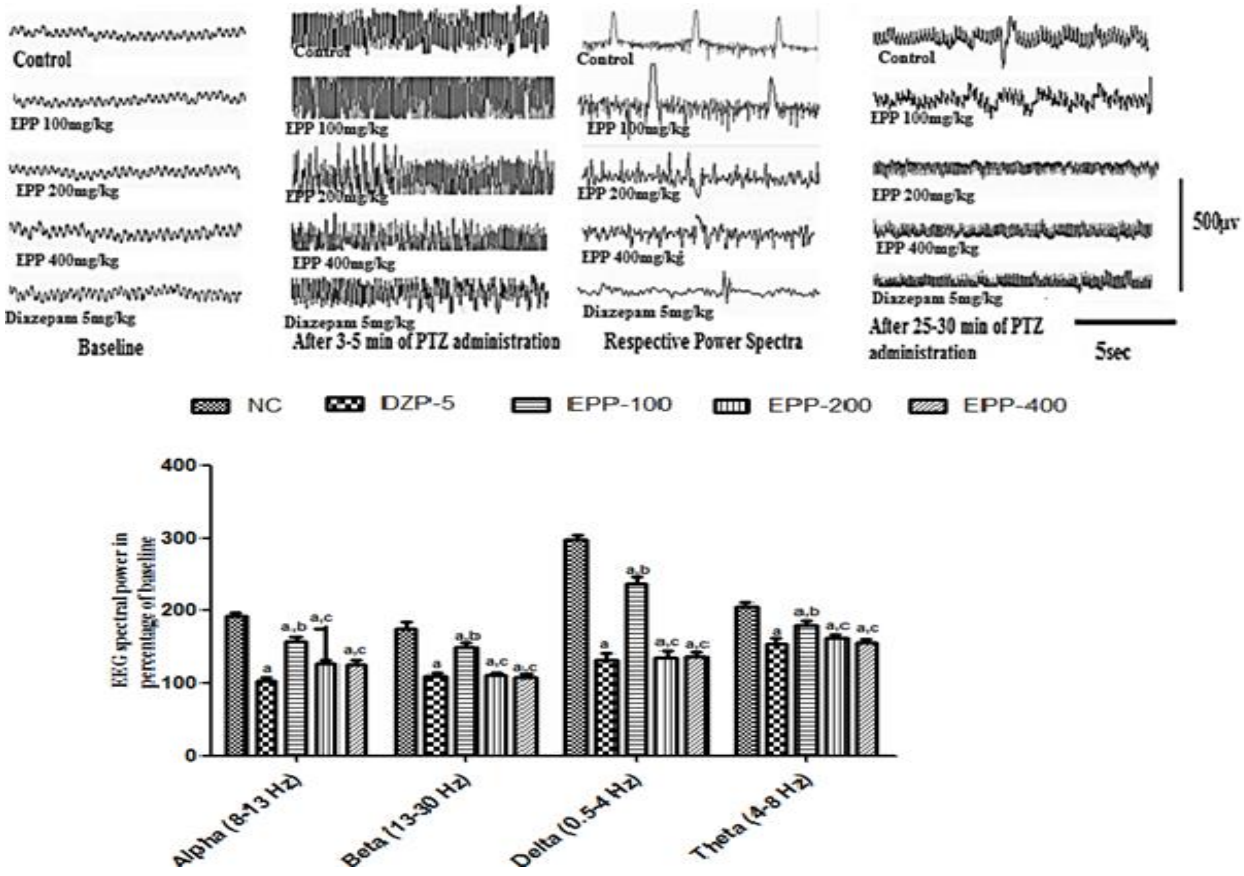

Figure. 27 Effect of EPP (100, 200, and 400 mg/kg) on PTZ-induced changes in the frequency of brain waves in EEG power spectra of rats. All values are mean ± SEM (n = 6). $^{a}P < 0.05$, $^{b}P < 0.05$, and $^{c}P < 0.05$ compared to NC, DZ-5, and chrysin-2.5, respectively (one-way ANOVA followed by Student–Newman–Keuls post hoc test).

2.4 Evaluation of anticonvulsant activity of chrysin (2.5, 5, and 10 mg/kg; p.o.) in PTZ challenged rats

Figure. 28 illustrates the effect of chrysin (2.5, 5, and 10 mg/kg) on the duration of onset of convulsion in PTZ-induced seizures in rats. Statistical analysis revealed that there were

significant differences in the duration of onset of convulsion in PTZ-induced seizures ($F(7, 40) = 43.7$, $P < 0.05$) among groups. All the drug treatment group animals showed a significant decrease in the duration of onset of convulsion in PTZ induced seizures compared to NC. Moreover, there were no significant differences in the duration of onset of convulsion in PTZ-induced seizures between DZ-5, chrysin-5, and chrysin-10 group animals. The effect of chrysin (2.5, 5, and 10 mg/kg) on the percentage of power spectra to baseline of alpha, beta, delta, and theta waves in PTZ-induced seizures in rats is depicted in Figure. 28. Statistical analysis revealed that there were significant differences in the percentage of power spectra to baseline of alpha ($F(4, 25) = 40.9$, $P < 0.05$), beta ($F(4, 25) = 52.1$, $P < 0.05$), delta ($F(4, 25) = 214.8$, $P < 0.05$), and theta ($F(4, 25) = 15.7$, $P < 0.05$) wave among groups. All the drug treatment group animals showed a significant decrease in the percentage of power spectra to baseline of alpha, beta, delta, and theta waves in PTZ-induced seizures compared to NC. Moreover, there were no significant differences in the percentage of power spectra to baseline of alpha, beta, delta, and theta waves in PTZ induced seizures between DZ-5, chrysin-5, and chrysin-10 group rats.

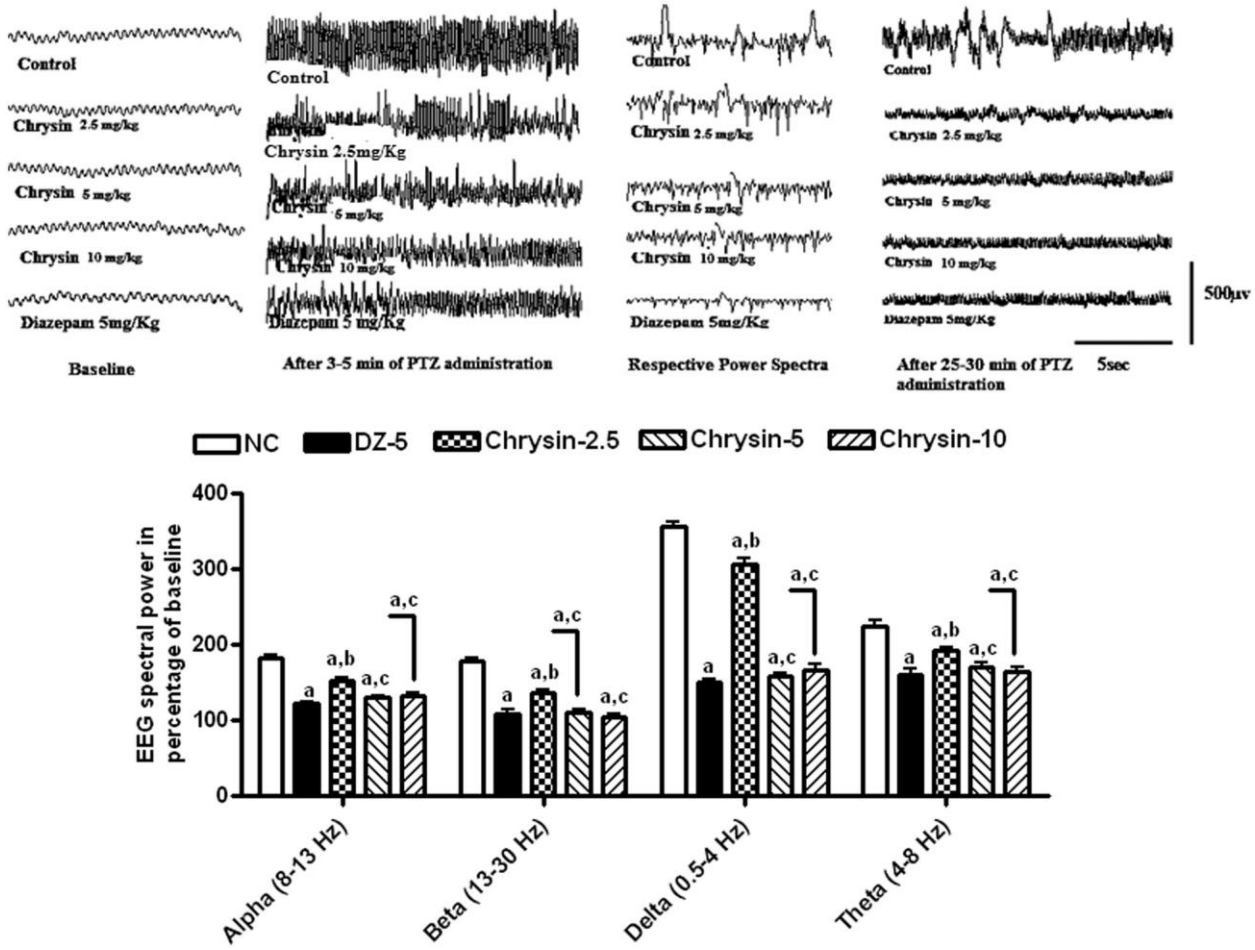

Figure. 28 Effect of chrysin (2.5, 5, and 10 mg/kg) on PTZ-induced changes in the frequency of brain waves in EEG power spectra of rats. All values are mean ± SEM (n = 6). $^{a}P < 0.05$, $^{b}P < 0.05$, and $^{c}P < 0.05$ compared to NC, DZ-5, and chrysin-2.5, respectively (one-way ANOVA followed by Student–Newman–Keuls post hoc test).

3. Effect of EPP (100, 200, and 400mg/kg; p.o.) and chrysin (2.5, 5, and 10 mg/kg) on PTZ induced oxidative stress in different brain regions.

Table 15. depicts the effect of chrysin (2.5, 5, and 10 mg/ kg) on PTZ-induced changes in the extent of LPO and protein oxidation, activity of SOD and catalase, and the level of protein sulfhydryl in the hippocampus, cerebellum, and cerebral cortex of rats. Statistical analysis revealed that there were significant differences in the extent of LPO and protein oxidation, activity of SOD and catalase, and level of protein sulfhydryl in hippocampus, cerebellum, and cerebral cortex. Post hoc test revealed that all the treatment group animals exhibited a significant decrease in the extent of LPO and protein oxidation, increase in the activity of SOD

and catalase, and level of protein sulfhydryl in all the brain regions of PTZ challenged rats. Moreover, there were no significant differences in the PTZ-induced changes in the extent of LPO and protein oxidation, increase in the activity of SOD and catalase, and level of protein sulfhydryl in all the brain regions of DZ-5, chrysin-5, and chrysin-10 group animals.

Brain region	Groups	Lipid peroxidation (nmol MDA/mg protein)	Carbonylated proteins (nmol DNPH/mg protein)	SOD (U/mg protein)	Catalase (µmol of H_2O_2 decomposed/ min/mg protein)	Protein sulfhydryl content (µmol DTNB/mg protein)
Hippocampus	**NC**	**1.7 ±0.13**	**3.1± 0.10**	**3.2± 0.21**	**4.4 ±0.68**	**0.1± 0.01**
	DZP-5	**0.4 ±0.07[a]**	**1.5± 0.07[a]**	**6.0± 0.17[a]**	**13.0 ±0.97[a]**	**0.4± 0.02[a]**
	EPP-100	**1.00±0.05[a,b]**	**2.0±0.73[a,b]**	**4.5±0.10[a,b]**	**9.4±0.39[a,b]**	**0.3±0.02[a]**
	EPP-200	**0.7±0.19[a]**	**1.8±0.17[a]**	**5.2±0.39[a]**	**11.5±0.23[a]**	**0.4±0.03[a]**
	EPP-400	**0.4±0.11[a]**	**1.6±0.11[a]**	**6.4±0.17[a]**	**12.7±0.73[a]**	**0.4±0.01[a]**
	Chrysin-2.5	**0.9 ±0.05 [a,b]**	**2.1± 0.19 [a,b]**	**4.9± 0.73[a,b]**	**8.4 ±0.83 [a,b]**	**0.2± 0.02 [a,b]**
	Chrysin-5	**0.5 ±0.05[a]**	**1.7± 0.23[a]**	**5.5± 0.11[a]**	**12.3 ±0.57 [a]**	**0.4± 0.02[a]**
	Chrysin-10	**0.3 ±0.06[a]**	**1.4± 0.10[b]**	**6.1± 0.10[a]**	**12.9 ±0.39 [a]**	**0.4± 0.03[a]**
Cerebellum	**NC**	0.9 ±0.04	3.3± 0.09	**4.1± 0.37**	**2.8 ±0.31**	0.2± 0.02
	DZP-5	**0.3 ±0.05a**	**1.9± 0.09a**	**8.4± 0.42a**	**7.0 ±0.52a**	**0.4± 0.01a**
	EPP-100	**0.7±0.08 [a,b]**	**2.5±0.03[a,b]**	**4.1±0.23**	**3.8±0.23**	**0.3± 0.02[a,b]**
	EPP-200	**0.4±0.03[a]**	**2.0±0.09[a]**	**8.5±0.48**	**6.9±0.31**	**0.4± 0.01[a]**
	EPP-400	**0.3± 0.05 [a]**	**1.9±0.11[a]**	**8.2±0.23**	**7.2±0.33**	**0.4± 0.02[a]**
	Chrysin-2.5	**0.6 ±0.08[a,b]**	**2.7± 0.15[a,b]**	**4.3± 0.27[a,b]**	**4.2 ±0.34[a,b]**	**0.3± 0.01[a,b]**
	Chrysin-5	**0.3 ±0.03[a]**	**2.1± 0.11[a]**	**7.9± 0.48[a]**	**6.5 ±0.33[a]**	**0.4± 0.03[a]**
	Chrysin-10	**0.3 ±0.03[a]**	**1.8± 0.09a**	**8.1± 0.68[a]**	**7.2 ±0.29[a]**	**0.4± 0.02[a]**
Cerebral cortex	**NC**	**1.6 ±0.07**	**1.7± 0.10**	**4.1± 0.37**	**5.1 ±0.54**	**0.1± 0.03**
	DZP-5	**0.8 ±0.07a**	**0.8± 0.08a**	**8.4± 0.42a**	**10.7 ±0.57a**	**0.3± 0.02a**
	EPP-100	**1.4 ±0.07a,b**	**1.2± 0.08a,b**	**6.9± 0.33a,b**	**7.9±0.54a,b**	**0.2± 0.03a,b**
	EPP-200	**1.0 ±0.08a**	**0.7± 0.05a**	**7.7± 0.48a,c**	**11.3 ±0.90a**	**0.3± 0.02a,c**
	EPP-400	**0.8 ±0.15a**	**0.6± 0.15a**	**8.3± 0.48a,c**	**10.8 ±0.90a**	**0.3± 0.02a,c**
	Chrysin-2.5	**1.3 ±0.08a,b**	**1.3± 0.08a,b**	**6.3± 0.37a,b**	**7.3 ±0.65a,b**	**0.2± 0.01a,b**
	Chrysin-5	**0.9 ±0.13a,c**	**0.9± 0.05a**	**7.7± 0.48a**	**10.3 ±0.83a**	**0.3± 0.02a**
	Chrysin-10	**0.6 ±0.15a,c**	**0.6± 0.12a,c**	**8.1± 0.68a,c**	**11.8 ±0.90a**	**0.3± 0.01a,c**

Table.15 Effect of EPP and chrysin on PTZ-induced oxidative stress in discrete rat brain region. All values are mean±SEM (n =6). [a]P< 0.05 compared to NC (one-way ANOVA

followed by Student–Newman–Keuls post hoc test). [b]P<0.05 compared to DZ-5 (one-way ANOVA followed by Student–Newman–Keuls post hoc test).

4. Evaluation of motor dysfunction test of EPP (100,200 and 400 mg/kg; p.o.) and chrysin (2.5, 5, and 10 mg/kg; p.o.) in rats

Effect of EPP (100, 200 and 400 mg/kg) and chrysin (2.5, 5, and 10 mg/kg) on change in the duration of movement in photoactometer (A), and duration of running in rotarod apparatus (B) is depicted in Figure. 29. Statistical analysis revealed that there were significant differences in the duration of movement in photoactometer ($F(7, 40) = 42.4$; $P < 0.05$) and duration of running in the rotarod apparatus ($F(7, 40) = 104.0$; $P < 0.05$) among groups. Post hoc test revealed that animals received DZ exhibited a decrease in duration of movement in the photoactometer and duration of running in the rotarod apparatus compared to vehicle-treated control rats. However, chrysin (2.5, 5, and 10 mg/kg) and EPP (100, 200 and 400mg/kg) treatment did not cause any significant change in the duration of movement in the photoactometer, and duration of running in the rotarod apparatus of the animals compared to control rats.

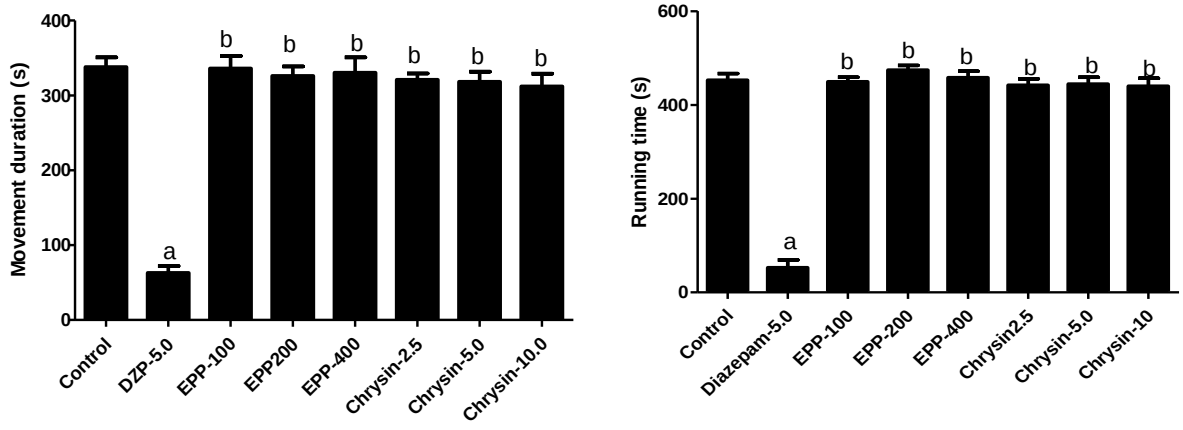

Figure. 29 **(A)** Effect of EPP (100, 200, and 400 mg/kg) and chrysin (2.5, 5 and 10 mg/kg) on actophotometer induced changes in the movement of duration in rats. All values are mean ± SEM (n = 6). [a]P < 0.05, [b]P < 0.05, [c]P <0.05, [d]P<0.05,[e]P<0.05,[f]P<0.05,[g]P<0.05 0.05 compared to NC, DZ-5, EPP-100, EPP-200, EPP-400, chrysin 2.5 chrysin-5, chrysin-10 respectively (one way ANOVA followed by Student–Newman–Keuls post hoc test). **(B)** Effect of EPP (100, 200, and 400 mg/kg) and chrysin (2.5, 5 and 10 mg/kg) on rotarod induced changes in running time in rats. All values are mean ± SEM (n = 6). [a]P < 0.05, [b]P < 0.05, [c]P <0.05,

[d]P<0.05,[e]P<0.05,[f]P<0.05,[g]P<0.05 0.05 compared to NC, DZ-5, EPP-100, EPP-200, EPP-400, chrysin 2.5 chrysin-5, chrysin-10 respectively (one way ANOVA followed by Student–Newman–Keuls post hoc test).

5. Mechanistic studies

5.1 Pentylenetetrazole-Induced Seizures

Chrysin and EPP significantly delayed the duration of clonic convulsions (F3, 20=263.5, P<0.05; Figure.30) which was significant at 5 mg/kg (P<0.05). ANOVA revealed that EPP-200 also significantly reduced the duration of clonic convulsions (F3, 20=263.5, P<0.05). Diazepam, the reference anticonvulsant used, delayed the duration of clonic convulsions (F3, 20=263.5, P<0.05; figure.30) with statistical significance at 1.0 mg kg/kg (P<0.05).

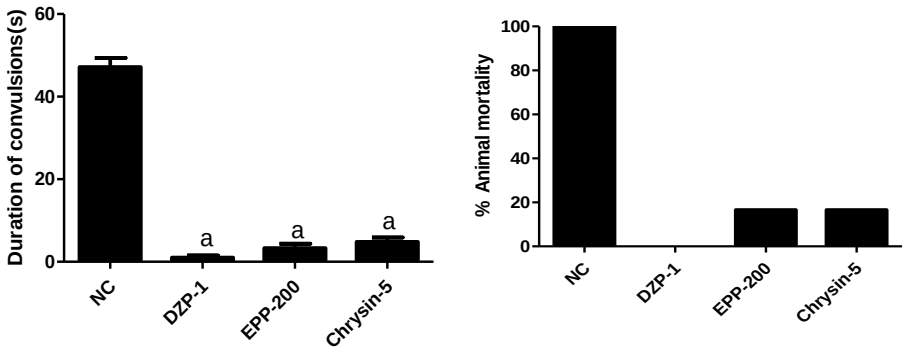

Figure. 30 Effect of EPP (200mg/kg p.o.) and chrysin (5 mg/kg p.o.) on PTZ induced changes in the duration of convulsions and animal mortality rate in (%). All values are mean ± SEM (n = 6). [a]P < 0.05, compared to NC.

5.2 Picrotoxin-Induced Seizures

Chrysin-5 exhibited anticonvulsant effect against picrotoxin-induced seizures significantly. EPP-200 also significantly delayed the duration of clonic convulsions (F3, 20=106.2, P<0.05 with statistical significance observed at 300 mg kg-1 (P<0.05). No mortality was observed for the entire duration of the experiment. Diazepam produced effects similar to that of the chrysin-5 and EPP-200. It significantly delayed the duration of convulsions (F3, 20=106.2, P<0.05; Figure 31).

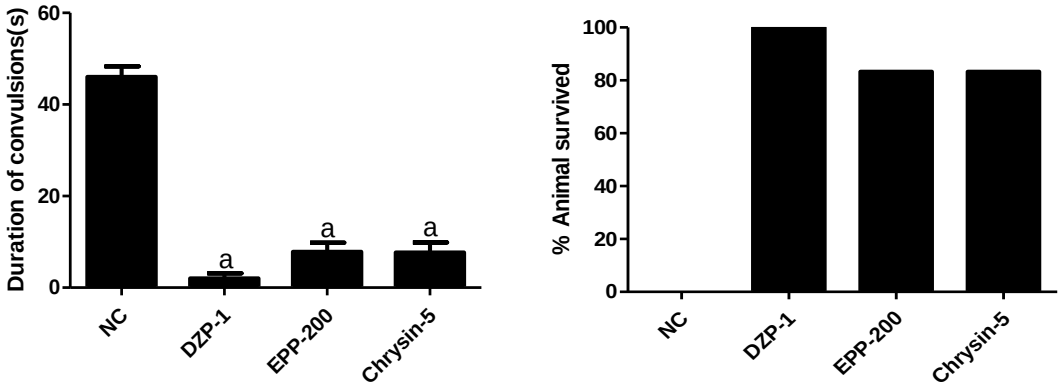

Figure. 31 Effect of EPP (200mg/kg p.o.) and chrysin (5 mg/kg p.o.) on picrotoxin induced changes in the duration of convulsions and animal mortality rate in (%). All values are mean ± SEM (n = 6). $^{a}P < 0.05$, compared to NC.

5.3 Isoniazid-Induced Seizures

Isoniazid (300 mg/kg, s.c.) elicited clonic convulsions followed by tonic hind limb extension and mortality in mice. Treatment with EPP (200 mg kg-1, p.o.) significantly increased the latency to seizures ($F_{3, 20}=73.3$, $P<0.0001$; Figure 32) as compared to negative control mice. Furthermore, EPP-200, and Chrysin-5 improved survival of the animals after induction of convulsions (Figure 32). As compared to vehicle control mice, diazepam (1.0 mg/kg, i.p.) treated mice showed significant protection against INH-induced mortality. Furthermore, in Figure.32 it significantly increased latency of convulsions ($F_{3, 20}=73.3$, $P<0.05$).

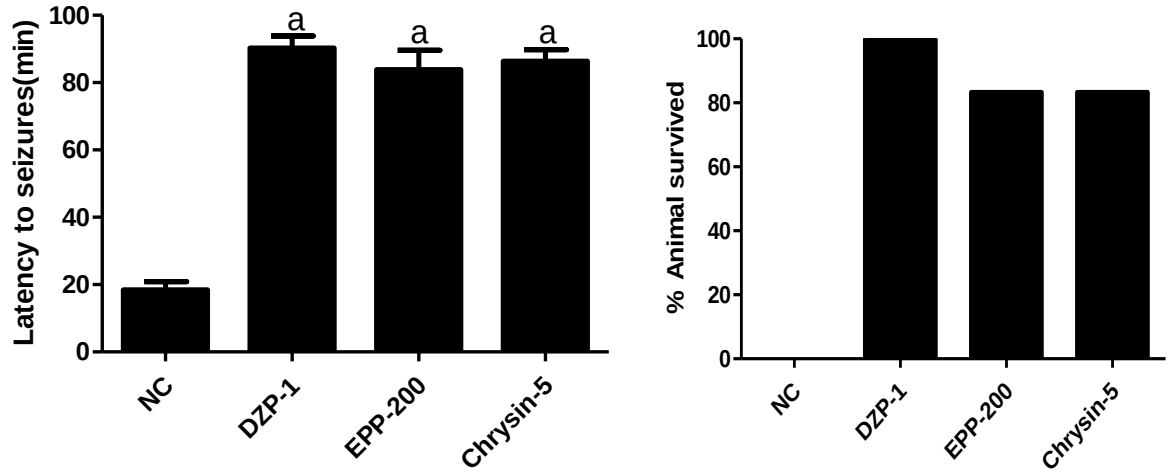

Figure. 32 Effect of EPP (200mg/kg p.o.) and chrysin (5 mg/kg p.o.) on Isoniazid induced changes in the latency to seizures, and animal mortality rate in (%). All values are mean ± SEM (n = 6). [a]P < 0.05, compared to NC.

5.4 Strychnine-Induced Seizures

Figure. 33 indicates the effects of EPP-200 mg kg^{-1}, p.o., chrysin-5 mg/kg, p.o. and diazepam (1 mg kg-1, i.p.) on duration of clonic convulsions induced by strychnine in mice. ANOVA revealed that the extract exhibited a dose-dependent anticonvulsant effect against strychnine-induced clonic seizures by significantly decreasing duration of convulsions (F 3, 20=109.7, P<0.05).

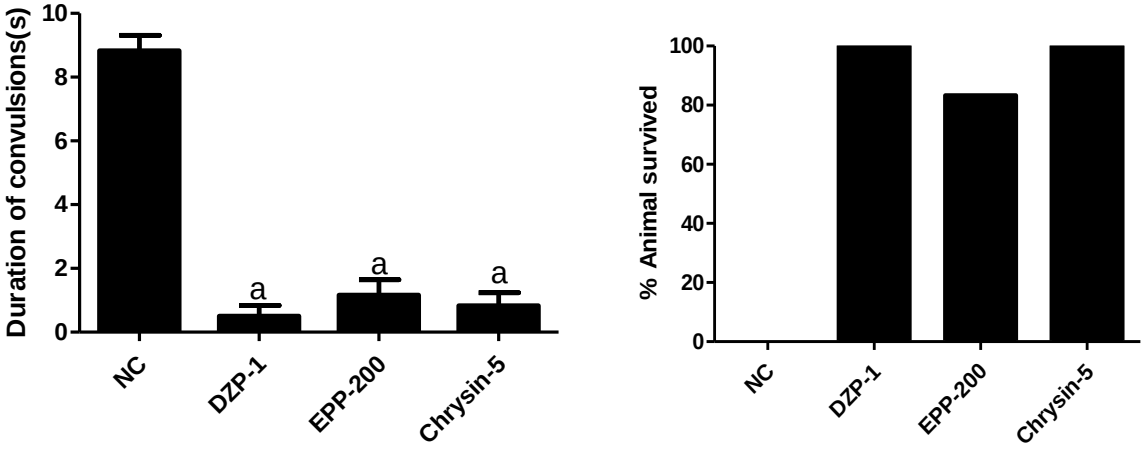

Figure. 33 Effect of EPP (200mg/kg p.o.) and chrysin (5 mg/kg p.o.) on strychnine induced changes in the duration of convulsions and animal mortality rate in (%). All values are mean ± SEM (n = 6). [a]P < 0.05, compared to NC.

5.5 4-Aminopyridine-Induced Seizures

A single administration of 4-AP (12 mg/kg, i.p.) caused seizures, and 100 % mortality in negative control mice. In contrast, pretreatment of animals with carbamazepine (CBZ) (30 mg/kg, p.o.) as shown in Figure. 34 caused a significant delay in the latency of clonic (F3, 20=58.98, P<0.05). EPP-200 and Chrysin-5 did not protect animals against seizures.

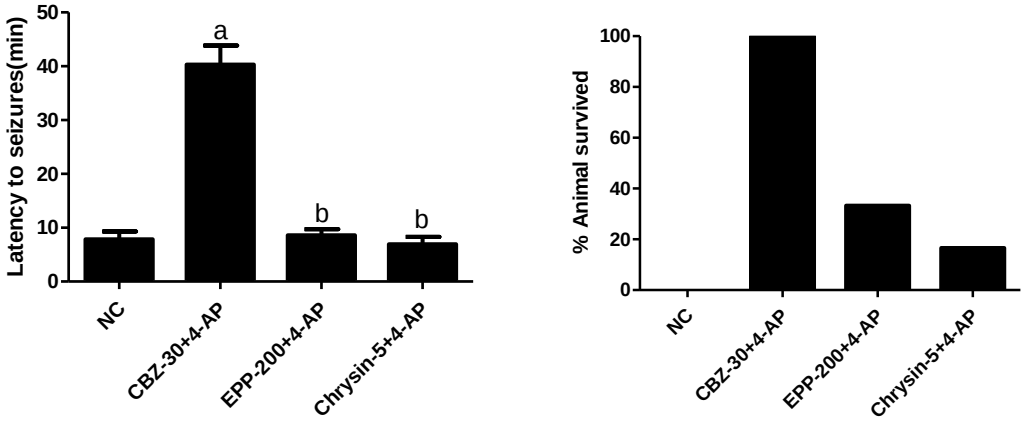

Figure. 34 Effect of EPP (200mg/kg p.o.) and chrysin (5 mg/kg p.o.) on 4- Amino Pyridine induced changes in the latency to seizures and animal mortality rate in (%). All values are mean ± SEM (n = 6). $^{a}P < 0.05$, $^{b}P < 0.05$compared to NC and DZ-5.

4.6 Effect on Maximal Electroshock Seizures

Electrical stimulation produced tonic hind limb extensions (HLEs) in all saline-control mice. The extract did not protect against tonic hind limb extensions. In contrast to EPP-200 and Chrysin-5 mg/kg. Phenytoin at the dose of 25 mg/kg completely protected mice against tonic hind limb extensions. In addition, no deaths were recorded in Phenytoin treated animal. (Figure.35).

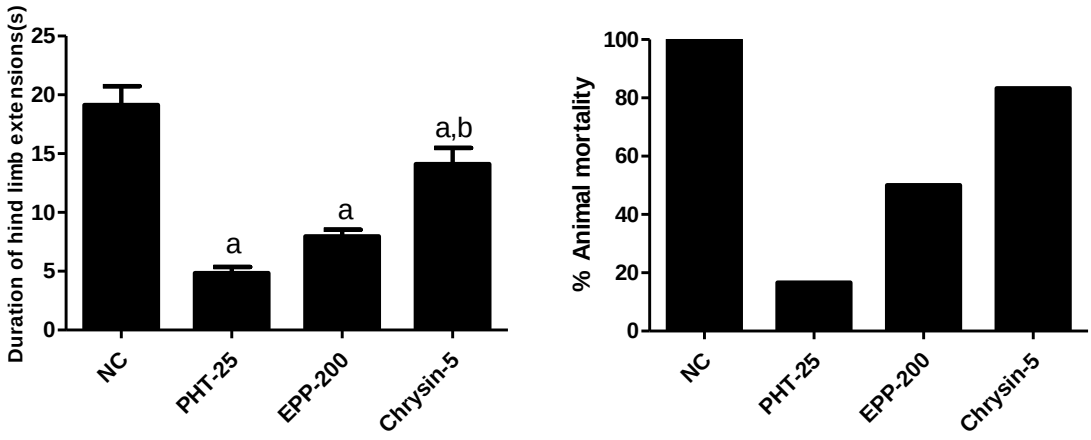

Figure. 35 Effect of EPP (200mg/kg p.o.) and chrysin (5 mg/kg p.o.) on MES induced changes in the duration of hind limb extensions and animal mortality rate in (%). All values are mean ± SEM (n = 6). $^{a}P < 0.05$, $^{b}P < 0.05$compared to NC and DZ-5.

5.7 N-Methyl-D-aspartate-induced convulsions

N-Methyl-D-aspartic acid-induced lethality test is a most commonly used test to explore the possible role of glutaminergic pathway in the anticonvulsant effect of the test drug. In the present study, none of the tested doses of EPP and chrysin (200 and 5 mg/kg) showed significant protection against NMDA-induced behavioral changes. However, the reference standard D-2-amino-7-phosphonoheptanoate (D-AP7; 33 nmol/kg, i.p.) showed significant (90%) protection against NMDA-induced mortality in mice. Figure. 36

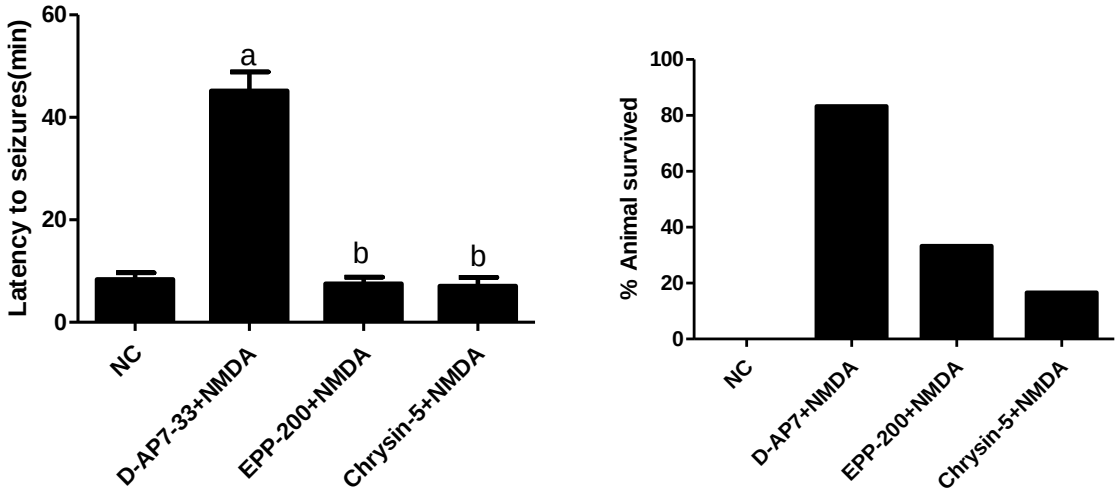

Figure. 36 Effect of EPP (200mg/kg p.o.) and chrysin (5 mg/kg p.o.) on NMDA induced changes in the duration of hind limb extensions and animal mortality rate in (%). All values are mean ± SEM (n = 6). $^{a}P < 0.05$, $^{b}P < 0.05$compared to NC and DZ-5.

5.8 Effect on $GABA_A$

EPP (200 mg/kg p.o) and chrysin (5 mg/kg p.o.) exerted anticonvulsant activity by significantly decreasing duration of clonic convulsions. However, pre-treatment with flumazenil significantly reversed the anticonvulsant effect of EPP and chrysin by increasing duration ($F_{3, 20}=64.15$), $P<0.05$).Similar results were obtained for diazepam (Figure.37).

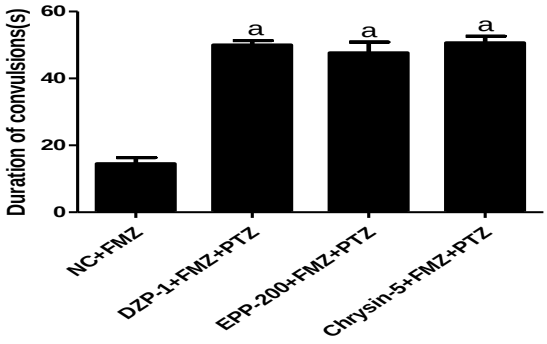

Figure.37 Effect of EPP (200mg/kg p.o.) and chrysin (5 mg/kg p.o.) on $GABA_A$ using benzodiazepine antagonist flumazenil. All values are mean ± SEM (n = 6). $^aP < 0.05$, compared to NC.

6. Chronic studies

6.1 Experimental Protocol

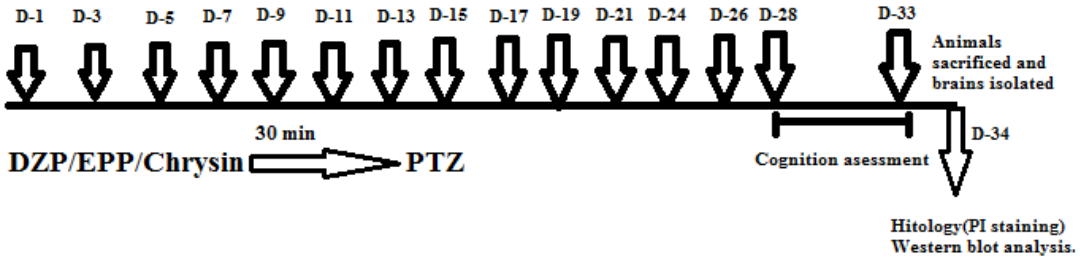

Figure. 38 Experimental design for chronic studies of PTZ induced kindling model.

6.2 PTZ induced kindling model

In our study, repeated administration of sub convulsive doses of PTZ (35mg/kg; *i.p.*) on every alternative day (14 injections) resulted in a steady increase in seizure score, subsequently resulting in generalized clonic-tonic seizures. Pre-treatment with EPP-200mg/kg; *p.o.* and chrysin-5mg/kg; *p.o.* prior to each PTZ injections was found to suppress the progression of

kindling significantly (p<0.05) as demonstrated by the decrease in seizure scores in comparison with the PTZ control group (Figure. 39).

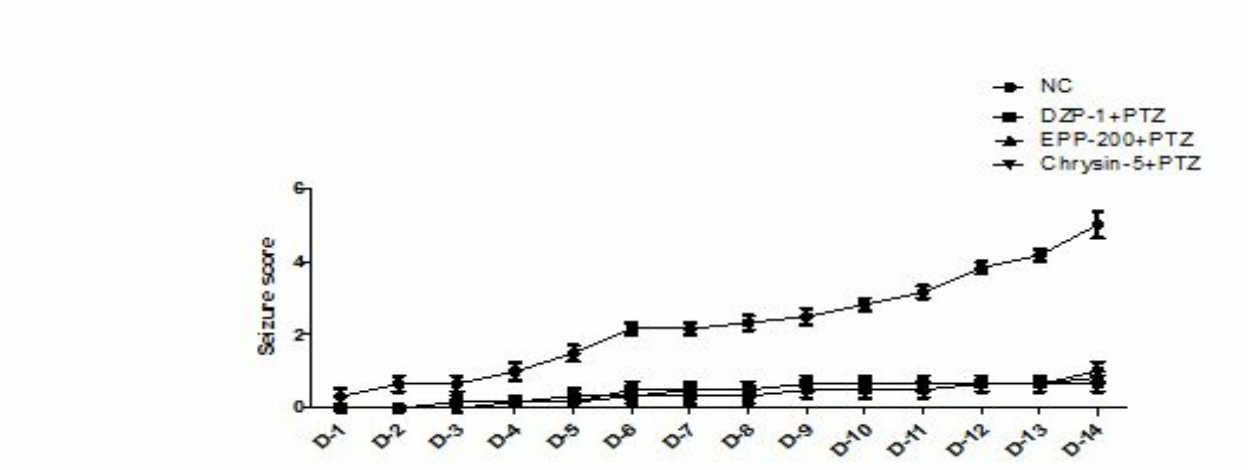

Figure. 39: Effect of DZP-1mg/kg, EPP-200mg/kg, and chrysin-5 mg/kg pre-treatment on behavior in PTZ kindled mice. Data expressed as mean±SEM with n=6.

7. Cognitive function test

7.1 Morris water maze

To determine whether EPP and chrysin alleviates PTZ induced cognitive impairment, we tested spatial learning and memory in MWM following drug treatment. PTZ kindling showed progressive decline in the escape latency with each training session. However, chronic pretreatment of DZP also exhibited the decline in escape latency. This behavior was alleviated upon EPP-200 and chrysin-5 mg/kg p.o. pre-treatment. In probe trial, the PTZ kindled mice and mice treated with DZP-1mg/kg i.p. spent less time in target quadrant compared to EPP and chrysin [F (3, 20) = 19.27, P<0.05] (Figure. 40).

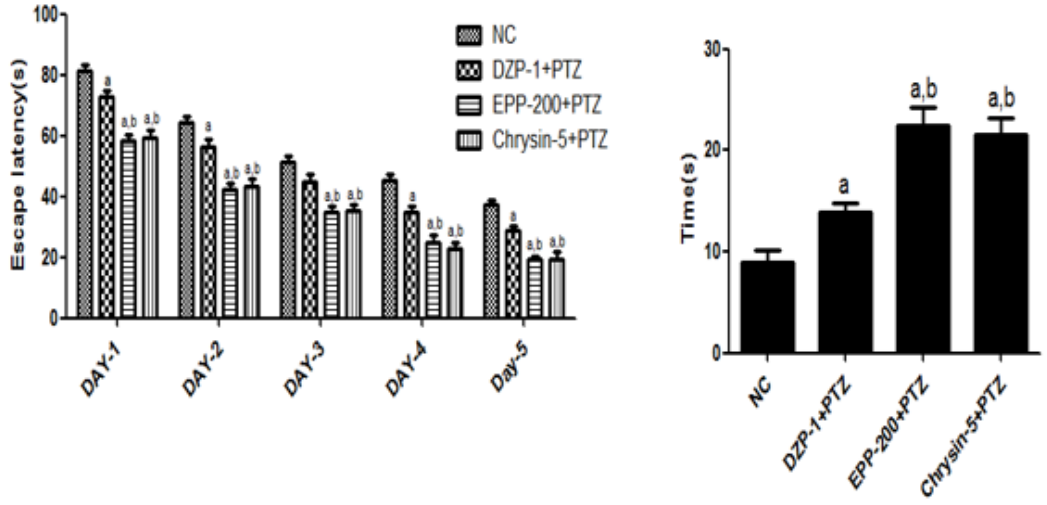

Figure.40 A: Escape latency of PTZ kindled and DZP-1, EPP-200+PTZ, chrysin-5+PTZ treated mice. Data expressed as mean ± SEM with n=6. $^{a}P < 0.05$, $^{b}P < 0.05$, and $^{c}P < 0.05$ compared to NC, DZ-1+PTZ, and EPP-200mg/kg, respectively (one-way ANOVA followed by Student–Newman–Keuls post hoc test). B: Learning and memory of mice following PTZ injections as determined by probe trials with MWM tests. All values are mean ± SEM (n = 6). $^{a}P < 0.05$, $^{b}P < 0.05$, and $^{c}P < 0.05$ compared to NC, DZ-1, and EPP-200mg/kg, (one-way ANOVA followed by Student–Newman–Keuls post hoc test)

8. Detection of cell death in brain tissue using propidium iodide staining

PTZ induced seizures resulted in apoptotic neurodegeneration. In order to assess the same histological analysis using PI was carried out. Fluorescence microscopic analysis revealed that there was robust staining throughout the hippocampal region in the brain of PTZ challenged mice [$F(3, 20) = 53.60$, $P<0.05$] in comparison to the treated groups. IDV/unit area of the hippocampus from the DZP-1mg/kg, EPP-200mg/kg, and chrysin-5 mg/kg was calculated using Alpha Ease FC software. (Figure. 41).

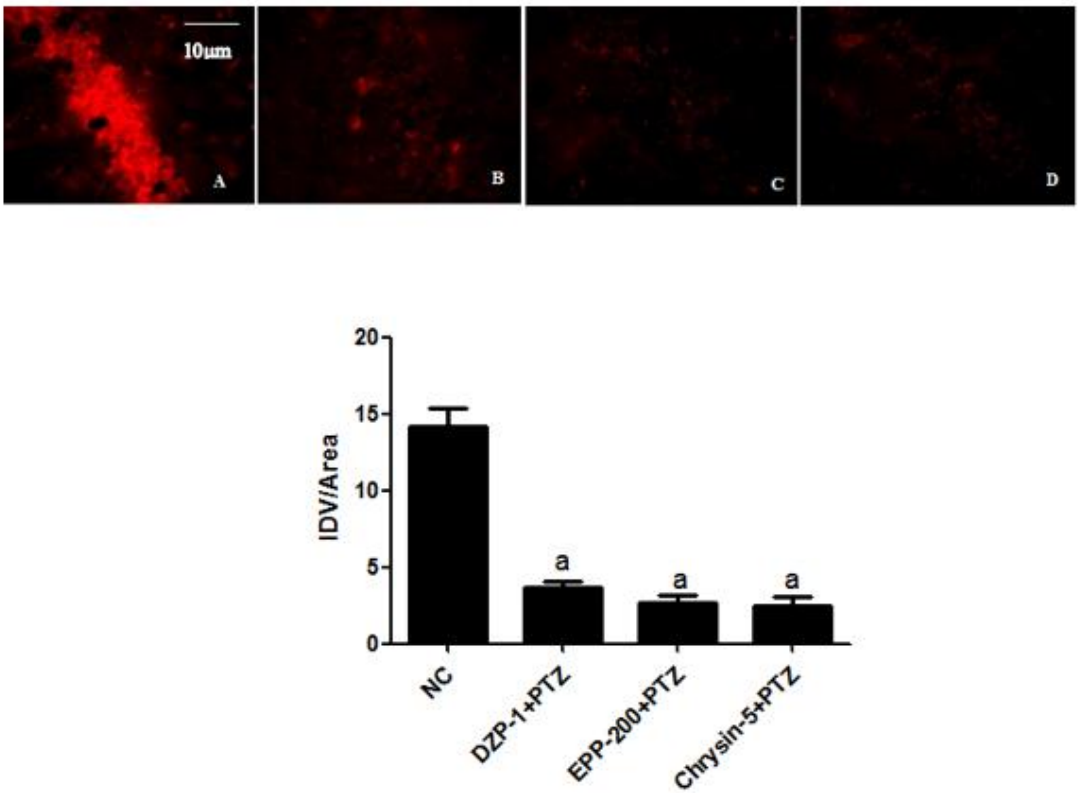

Figure. 41 PI staining showing cell death in hippocampus of NC mice. PI staining showing cell death in. A: NC, B: DZP-1+PTZ, C-EPP-200+PTZ, D-chrysin-5+PTZ, showing photomicrographs of ×10 magnification. Histogram represents IDV/area after deduction of negative control and background values of corresponding hippocampus from three independent experiments (mean ± SEM). $^{a}p < 0.05$ denotes significant differences as compared to NC one-way ANOVA followed by Student–Newman–Keuls post hoc test. IDV- Integrative density value.

9. Expression analysis of apoptotic biomarkers and BDNF levels by Western blotting

The translocation of mitochondrial Cyt *c* to the cytosol induces the formation of the apoptosomes, leading to apoptotic death. Cytoplasmic Cyt *c* concentration was markedly higher in the PTZ group [F (3, 20) = 15.34, P<0.05] in comparison to treated groups. While pre-treatment with DZP/EPP and chrysin reversed those levels compared to the PTZ group. There was no significant difference between the EPP, chrysin and Diazepam groups. Caspase-3 is the principle effector of the mitochondrial-dependent apoptotic pathway, and is activated during seizure-induced neuronal death. Western blot analysis revealed a significant group difference in both activated caspase-9 and caspase-3 expression in the hippocampus. Activated

caspase-9 and caspase-3 levels were significantly higher in the PTZ group [F (3, 20) = 19.08, P<0.05], [F (3, 20) = 67.35, P<0.05]. While pre-treatment with DZP/EPP/chrysin significantly decreased these levels compared to the PTZ group (Figure. 42). Taken together these results suggests that EPP-200 and chrysin-5 mg/kg; *p.o.* treatment attenuates the apoptosis of neuronal cells in pathogenesis of PTZ induced seizures by regulating the expression of the apoptosis related proteins.

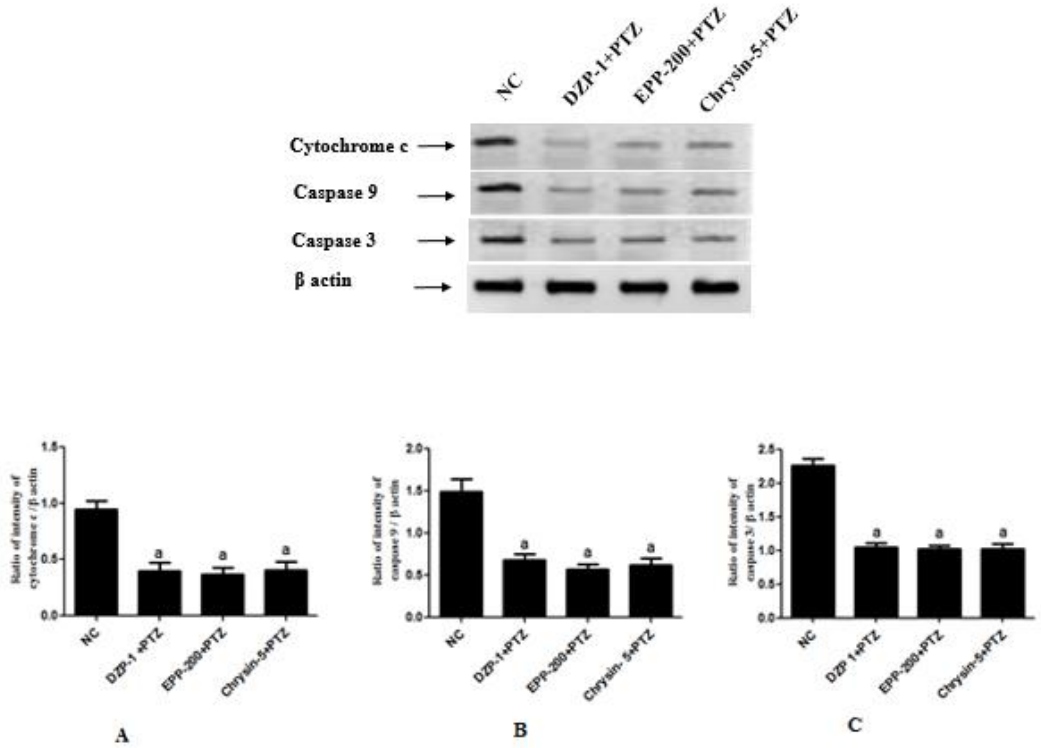

Figure. 42 Effects of DZP-1, EPP-200, chrysin-5 pretreatment on the expression of cytochrome c, caspase 9 and caspase 3 in the hippocampus (A) Realtive density of cytochrome c / β-actin. (B) Relative density of caspase 9/ β-actin. (C) Relative density of caspase 3/β-actin. Data expressed as mean ± SEM with n=6. $^{a}P < 0.05$, $^{b}P < 0.05$, and $^{c}P < 0.05$ compared to NC, DZ-1+PTZ, and EPP-200mg/kg, respectively (one-way ANOVA followed by Student–Newman–Keuls post hoc test).

9.1 Effects of EPP/chrysin on BDNF-related plasticity

The level of BDNF in the EPP/chrysin group animal was higher than that in the PTZ treated animals [F (3, 20) = 53.51, P<0.05].When the levels of pCREB and total CREB in the different

groups were compared by Western blot analyses, the results showed that the pCREB and total CREB levels in the (veh/ptz) and (DZP/PTZ) group were significantly lower than those in the (EPP-200/PTZ) group and (chrysin-5/PTZ) group rats [F (3, 20) = 28.00, P<0.05], [F (3, 20) = 13.08, P<0.05] (Figure 43).

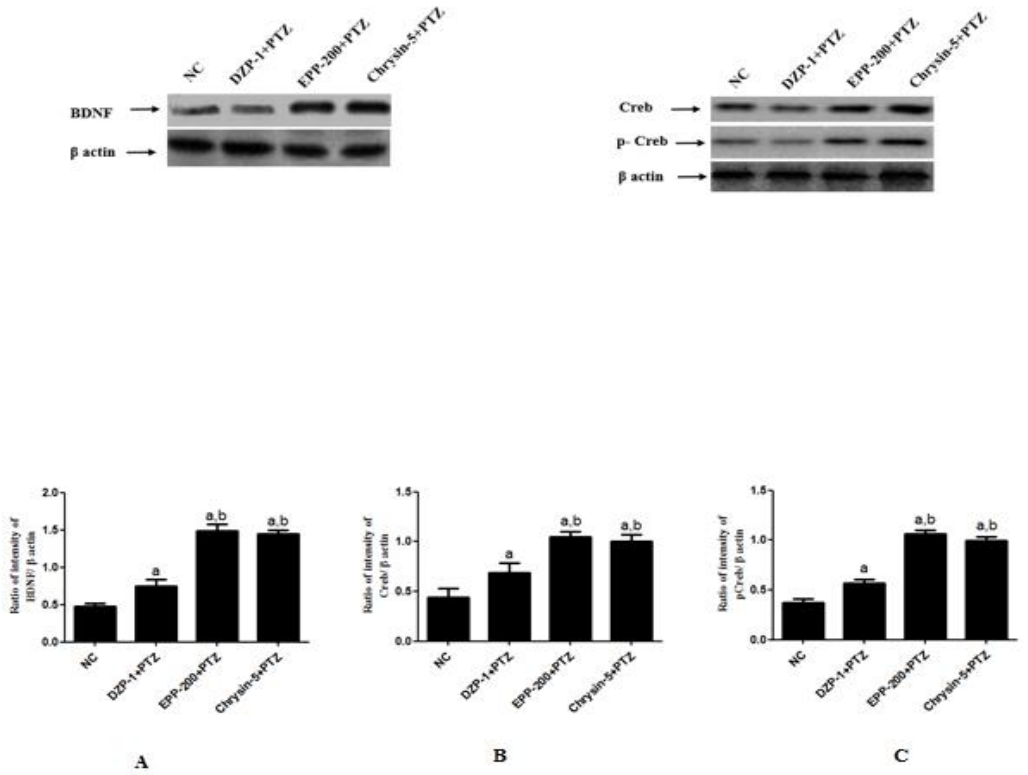

Figure. 43 Effects of DZP-1, EPP-200, chrysin-5 pretreatment on the expression of BDNF, Creb, p Creb in the hippocampus (A) Realtive density of BDNF/ β-actin. (B) Relative density of creb/ β-actin. (C) Relative density of p creb/β-actin. Data expressed as mean ± SEM with n=6. $^{a}P < 0.05$, $^{b}P < 0.05$, and $^{c}P < 0.05$ compared to NC, DZ-1+PTZ, and EPP-200mg/kg, respectively (one-way ANOVA followed by Student–Newman–Keuls post hoc test).

10. Expression analysis of gephyrin levels in hippocampus region of kindled animals

Expression level of gephyrin in the EPP/chrysin group animal was higher than that in the PTZ treated animals. [F (3, 20) = 27.3, P<0.05].When the levels of gephyrin in the different groups were compared by Western blot analyses, the results showed that gephyrin levels in the

(veh/ptz) and (DZP/PTZ) group were significantly lower than those in the (EPP-200/PTZ) group and (chrysin-5/PTZ) group rats . Figure.44

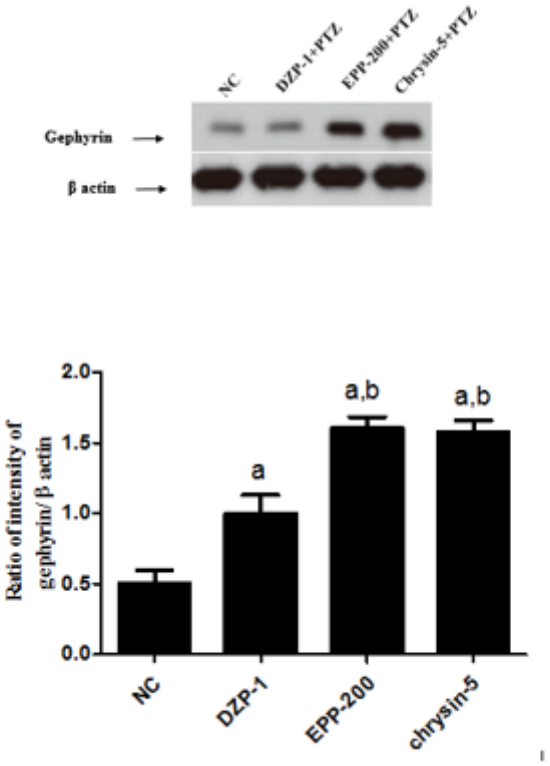

Figure.44 Effects of DZP-1, EPP-200, chrysin-5 pretreatment on the expression of gephyrin in the hippocampus (A) Realtive density of BDNF/ β-actin. (B) Relative density of creb/ β-actin. (Data expressed as mean ± SEM with n=6. $^{a}P < 0.05$, $^{b}P < 0.05$, and $^{c}P < 0.05$ compared to NC, DZ-1+PTZ, and EPP-200mg/kg, respectively (one-way ANOVA followed by Student–Newman–Keuls post hoc test).

11. Proposed Mechanism

Above findings suggest that EPP-200mg/kg and chrysin 5mg/kg possess anticonvulsant activity due to following mechanisms:

- Research outcomes confirmed the involvement of GABAergic mechanism behind the anticonvulsant activity of same. Moreover, chrysin acts as a ligand at the Benzodiazepine site of GABA receptors.
- EPP and Chrysin attenuated ROS induced oxidative damage in brain of experimental animals. Furthermore, both extract and compound downregulated the levels of apoptotic biomarkers and upregulated the levels of BDNF protein improving the cognition of epileptic animals.

- EPP and Chrysin upregulated the levels of gephyrin protein in the hippocampus of experimental animals, which is responsible for the integrity of GABA and glycine receptors (i.e. inhibitory synapses).

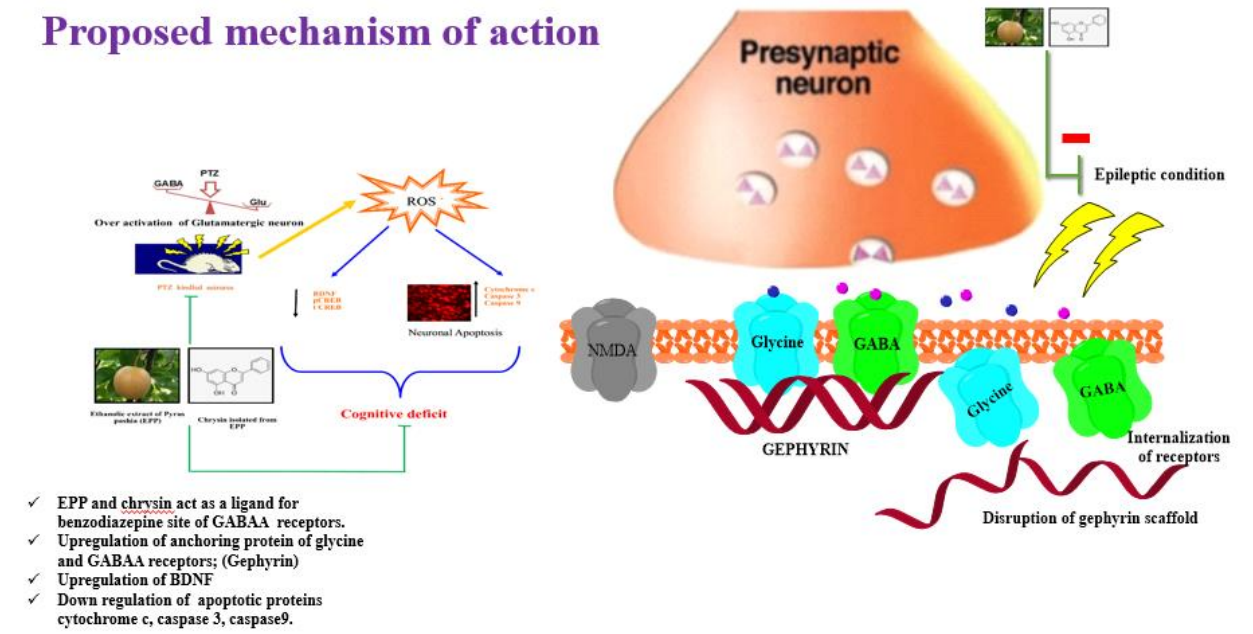

Figure. 45 Proposed hypothesis for mechanism of action of EPP and Chrysin.