INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACY AND CHEMISTRY

Available online at www.ijrpc.com

Research Article

EVALUATION OF ANTIINFLAMMATORY, ANALGESIC AND DNA BINDING STUDIES OF MANJANATHI FRUIT EXTRACT

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ABSTRACT

In continuation of our work on the isolation, preliminary phytochemical and antibacterial activity studies of the ethanol extract of manjanathi fruits (EEMF), Anti–inflammatory activity by Carrageenan induced paw edema model and Analgesic activity by tail immersion methods were carried out. DNA binding by Electrochemical methods and Hydrogen peroxide induced DNA Cleavage studies were carried out for the above extract. In the carrageenan-induced paw edema model using the oral doses of EEMF, the results proved that the dose dependent decrease in the size of the edema from 0.58 ± 0.0141 to 0.14 ± 0.0245 was observed. EEMF 200 mg/kg after two hours had an activity of 59.70% with a probability < 0.001 similar to diclofenac sodium (59.68%), the standard anti-inflammatory drug available. Analgesic activity of EEMF by tail immersion method, the results proved that there was an increase in the number of tail withdrawal ranging from 3.75 ± 0.25 to 10 ± 0.4082 for 100 mg/kg of EEMF and 4.5 ± 0.2887 to10.75 ± 0.4787 for 200 mg/kg. The administration of 200 mg/kg of EEMF brought about significantly equal activity with standard drug pentazocine at the fourth hour with a probability < 0.001. . The changes of the voltammeter currents in the presence of CT DNA can be attributed to diffusion of the Manjanthi fruit extract of ethanol bound to the large diffused DNA molecule and indicate that the extract possess DNA binding affinity.The result of agarose gel electrophoresis indicated that the EEMF exhibited cleavage capability of pUC19 DNA in the presence of H_2O_2.

Keywords: Manjanathi fruits, Anti–inflammatory, Analgesic and DNA binding.

INTRODUCTION

There are several plants families known for their medicinal properties found in India. Several elderly women in Tamil Nadu find plant parts useful in curing Mantham, Digestive disorders, especially in children, Gastropathy, Dyspepsia, Diarrhea, Ulcerative Stomatitis, Wounds, Gout, Inflammation, Hernia, Dysentery and Fever. We have selected Manjanathi fruit for our project work. Manjanathi called as indian Noni is a popular medicinal plant among the siddha practitioners of Tamilnadu. The genus Morinda (Rubiaceae) including the species Morinda citrifolia is made up of around 80 species. Different parts of the plant including stem, bark, root, leaf and fruits have been used in the system of traditional medicine to treat a broad range of diseases including hypertension[1,2], arteriosclerosis[3], colic and diarrhoea. Morinda citrifolia has been reported to possess antithrombotic[4], antioxidant[5], analgesic, anti-inflammatory[6] and xanthine oxidase inhibitory[7] activities. There are also preliminary studies reporting its blood pressure lowering and vasodilatory properties[8].

MATERIALS AND METHODS

Drugs

Diclofenac sodium (standard for anti-inflammatory), Pentazocine (standard for analgesic), CT DNA and pUC19 DNA were chosen for our work.

Animals used

Albino rats of Wistar strain (110-200 gm) maintained in SB College of Pharmacy animal house were used. All the experimental protocols were approved by the Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA), animal

ethics committee vide number SPCP/2009-2010/IAEC/CPCSEA/10.

METHODS

A. Determination of the anti-inflammatory activity by Carrageenan induced paw edema model and compare the activities of EEMF at different hours and to explore the extract activity comparing with the standard drug Diclofenac Sodium.

B. Analgesic activity of EEMF was determined by tail immersion method to identify its analgesic property. Standard drug pentazocine was selected for comparing the analgesic activities.

C. DNA Binding and Cleavage Studies

DNA binding studies was carried out by electrochemical methods. Disodium salt of calf thymus DNA was stored at 4°C. Solution of DNA in the buffer 50 mM NaCl, 5 mM Tris HCl (pH 7.2) in water gave a ratio 1.9 of UV absorbance at 260 and 280 nm, A260/A280, indicating that the DNA was sufficiently free from protein13. The concentration of DNA was measured using its extinction coefficient at 260 nm (6600 M-1) after 1: 100 dilutions. Stock solutions were stored at 4 °C and used not absorption data were analyzed for an evaluation of the intrinsic binding constant Kb using reported procedure[9-10]. Electrochemical studies were carried out using CHI Electrochemical analyzer, controlled by CHI620C software. CV measurements were performed using a glassy carbon working electrode and an Ag/AgCl reference electrode and supporting electrolyte was 50 mM NaCl, 5 mM Tris buffer (pH 7.2)and. All solutions were deoxygenated by purging with N2 for 30 min prior to measurements.

The cleavage of pUC19 DNA was determined by agarose gel electrophoresis. The gel-electrophoresis experiments were performed by incubation of the samples containing 30 µM, pUC19 DNA, 50 µM EEMFand 50 µM hydrogen peroxide (H2O2)in Tris-HCl/NaCl buffer (pH 7.2) at 37 oC for 2 h.

After incubation, the samples were electrophoresised for 2 h at 50 V on 1% agarose gel using Tris-acetic acid-EDTA buffer (pH 7.2). The gel was then stained using 1 µg cm-3 ethidium bromide (EB) and photographed under ultraviolet light at 360 nm. All the experiments were performed at room temperature unless otherwise stated.

A. Determination of Anti-Inflammatory Activity[11].

Carrageenan-induced hind paw edema is the standard experimental model of acute inflammation. The animals were divided into four groups as Control, Standard, group 3 (100mg/kg) and group 4 (200mg/kg) of the EEMF. Acute inflammation was produced by sub plantar injection of 0.1 ml of 1% suspension of carrageenan in normal Saline, in the right hind paw of the rats, one hour after oral administration of the drugs. The paw diameter was measured with the aid of a vernier caliper at 1, 2 and 3 hours after the injection of carrageenan. The difference between the readings at time zero minutes and the different time intervals were taken as the thickness of edema. With Diclofenac sodium (5mg/kg) as standard, group 3 and group 4 were treated orally with EEMF of 100 and 200 mg/kg and the paw diameter were measured at 1, 2 and 3 hours after the injection of the standard , were given in **Table-1.** Percentage inhibition of paw edema was calculated by comparing the control, for each dose at different hours as given below.

Percentage inhibition = 1- Vt / Vc *100

Where Vt = volume of paw edema in treated animals

Vc = volume of paw edema in control animals

Table 1:

S.NO	Animal weight	Drug &Dose	Paw volume measured in mm											% inhibition of paw oedema
			0 hour		1 hour			2 hour			3 hour			
			L	R	L	R	D	L	R	D	L	R	D	
1	H-160	Control	.32	.32	.32	.52	.20	.32	1.12	.80	.32	1.56	1.24	-
	B-130		.32	.32	.32	.50	.18	.32	1.10	.78	.32	1.54	1.22	
	T-150		.34	.34	.34	.54	.20	.34	1.14	.80	.34	1.58	1.24	
	C-170		.34	.34	.34	.56	.22	.34	1.16	.82	.34	1.60	1.26	
		mean					.20			.80			1.24	
2	H-160	Diclofenac sodium 10mg/kg	.34	.34	.34	.68	.34	.34	.76	.42	.34	.84	.50	59.677
	B-150		.32	.32	.32	.62	.30	.32	.78	.46	.32	.82	.50	
	T-120		.36	.36	.36	.56	.30	.36	.74	.38	.36	.86	.50	
	C-110		.38	.38	.38	.64	.26	.38	.72	.34	.38	.88	.50	
		mean					.30			.40			.50	
3	H-180	Extract 100mg/kg	.36	.36	.36	.54	.18	.36	.80	.44	.36	.96	.60	53.23
	B-170		.38	.38	.38	.56	.18	.38	.82	.44	.38	.92	.54	
	T-150		.34	.34	.34	.58	.24	.34	.86	.52	.34	.94	.60	
	C-170		.40	.40	.40	.52	.12	.40	.88	.48	.40	.98	.58	
		mean					.18			.47			.58	
4	H-170	Extract 200mg/kg	.38	.38	.38	.48	.10	.38	.78	.40	.38	.86	.48	59.68
	B-160		.34	.34	.34	.50	.16	.34	.74	.40	.34	.88	.54	
	T-150		.32	.32	.32	.52	.20	.32	.76	.44	.32	.84	.52	
	C-170		.36	.36	.36	.46	.10	.36	.72	.36	.36	.82	.46	
		mean					.14			.40			.50	

B. Determination of analgesic activity[12-13]

The tail immersion method was carried out as described by Janssen et al10. The animals last 3.5 cm of their tail were immersed in hot water thermo-statistically maintained at 55°C. The latency to withdraw the tail was recorded with a stopwatch, and a cut-off maximum latency of 15 sec was established in order to prevent tissue damage. Group 1 served as control which received only saline (1 ml/kg, p.o.) and Group 2 received standard drug Pentazocine(4mg/kg p.o.). Group 3 received 100mg/kg(p.o.) and Group 4 received 200 mg/kg (p.o.) of EEMF. The initial reading was taken immediately before administration of test samples and then at 1 hour, 2 hours, 3 hours and 4 hours after the drug administration, the time to latency withdrawal were noted and given in **Table- 2.**

Table 2:

S.No.	Body weight	treatment &Dose	Reaction time (sec)				
			0 hour	1hour	2 hours	3 hours	4 hours
1	H-160	Control 5ml/kg of saline p.o.	2	2	2	2	2
	B-130		2	2	1	2	2
	T-150		2	1	2	1	1
	C-170		2	2	1	2	2
		mean	2	1.75	1.5	1.75	1.75
2	H-160	Diclofenac sodium 10mg/kg	2	4	6	8	10
	B-150		2	5	7	9	11
	T-120		1	5	7	9	11
	C-110		1	5	8	10	12
		mean	1.5	4.75	7	9	11
3	H-180	Extract 100mg/kg	2	4	6	8	9
	B-170		1	4	6	8	10
	T-150		2	3	5	7	10
	C-170		1	4	7	9	11
		mean	1.5	3.75	6	8	10
4	H-170	Extract 200mg/kg	1	4	6	8	10
	B-160		2	5	7	9	11
	T-150		2	4	6	8	10
	C-170		1	5	7	9	12
		mean	1.5	4.5	6.5	8.5	10.75

p.o - per oral

C.DNA Binding and Cleavage Studies

DNA Binding and Cleavage Studies were carried out using Cyclic Voltameter(CV). CV measurements were performed using a glassy carbon working electrode, platinum wire auxiliary electrode and an Ag/AgCl reference

electrode. The supporting electrolyte was 50 mM NaCl/5mM Tris–HCl buffer (pH 7.2). All the solutions examined by electrochemical techniques were purged with nitrogen for 10 min prior to each set of experiments. All measurements were carried out at room temperature (25°C). The difference between forward and backward peak potentials can provide a rough evaluation of the degree of the reversibility of one electron transfer reaction. The decreased in the extents of the peak currents observed for EEMF upon the addition of CT DNA indicated that ethanol extract was found to interact with DNA through binding mode. The Cyclic votammogram was recorded for EEMF in buffer P^{H}7.2 at 25˚C in the presence and absence of CT-DNA were shown in **Fig.1.**

Agarose gel electrophoresis was used to carry out DNA cleavage studies using pUC19 DNA in the presence of H_2O_2. The cleavage capacity of Manjanthi fruit EEMF extracts to DNA is considerably interesting as it can contribute to understanding the toxicity mechanism of them and to develop novel artificial nuclease. DNA cleavage is controlled by relaxation of super coiled circular form of pUC19 DNA into nicked circular form and linear form. When circular plasmid DNA is conducted by electrophoresis the fastest migration would be observed for the supercoil form **(Form I),** if one strand was cleaved. The supercoils will relax to produce a slowed moving open circular from **(Form II),** if both strands were cleaved. DNA cleavage was analyzed by monitoring the conversion of supercoiled DNA (Form I) to nicked DNA (Form II) in the presence of oxidant H_2O_2 which was shown in **Fig.2.**

RESULT AND DISCUSSION

A. Anti-inflammatory activity

Anti–inflammatory activity of ethanol extract of Manjanathi fruit (EEMF) on carrageenan-induced paw edema in rats were given in **Table -3**., the following results were observed

Table 3:

Treatment	Dose	Mean changes in paw edema ± SEM, % edema inhibition		
		I Hour	2 Hours	3 Hours
Control		0.2 ± 0.0082	0.8 ± 0.0082	1.24 ± 0.0082
Standard Diclofenac Sodium	10 mg/kg	0.3 ± 0.0163** (**50%**)	0.4 ± 0.0258*** (**50%**)	0.5 ± 0*** (**59.68%**)
EEMF Extract	100 mg/kg	0.18 ± 0.0245^{NS} (**10%**)	0.47 ± 0.0191*** (**41.25%**)	0.58 ± 0.0141*** (**53.23%**)
EEMF Extract	200 mg/kg	0.14 ± 0.0245* (**30%**)	0.4 ± 0.0163*** (**50%**)	0.50 ± 0.0183*** (**59.7%**)
ANOVA				
F		15.44	107	735.5
df		3,15	3,15	3,15
P		< 0.01, < 0.5, < 0.05	< 0.001	< 0.001

*Probability values (calculated as compared to control using one way-ANOVA followed by Dunnet's "t" Test).
All values are means of individual data obtained from four rats (n = 4)
Data presented as Mean ± SEM; n=4; * P≤0.05, ** P≤0.01. *** P≤0.001
Figures in parenthesis represent percentage anti-inflammatory activity.

In the carrageenan-induced paw edema model using the oral doses of ethanol extract of Manjanathi fruit , the results proved that the dose dependent decrease in the size of the edema from 0.58 ± 0.0141 to 0.14 ± 0.0245 was observed. EEMF 200 mg/kg after two hours had activity of 59.70% with a probability < 0.001 similar to diclofenac sodium (59.68%), the standard anti-inflammatory drug available.

B.Analgesic activity

Analgesic activity of ethanol extract of Manjanathi fruit by tail immersion method in rats were given in **Table-4.**

Table 4:

Treatment	Dose	Tail flick latency in seconds (Mean ± SEM)			
		1 Hour	2 Hours	3 Hours	4 Hours
Control		1.75 ± 0.25*	1.5 ± 0.28*	1.75 ± 0.25	1.75 ± 0.25*
Standard pentazocine	4 mg/kg.i.p.	4.75 ± 0.25* (**63.15**)	7 ± 0.40* (**78.57**)	9 ±0.40* (**80.55**)	11 ± 0.40* (**84.09**)
EEMF Extract	100 mg/kg	3.75 ± 0.25* (**53.33**)	6 ± 0.40* (**75.00**)	8 ± 0.40* (**78.13**)	10 ± 0.40* (**82.50**)
EEMF Extract	200 mg/kg	4.5 ± 0.28* (**61.11**)	6.5 ± .28* (**76.92**)	8.5 ±0.28* (**79.41**)	10.75±0.47* (**83.72**)
OneWay Anova					
F		42.6	51.33	201.7	315
df		3,15	3,15	3,15	3,15
P		< 0.0001	< 0.0001	< 0.0001	< 0.0001

All values are expressed in mean ± standard error mean (n=4).
All data were found to be significant with P< 0.0001. Values in parenthesis ( ) indicates % activity.

The results proved that there was an increase in the number of tail withdrawal ranging from 3.75 ± 0.25 to 10 ± 0.4082 for 100 mg/kg of EEMF and 4.5 ± 0.2887 to10.75 ± 0.4787 for 200 mg/kg. The administration of 200 mg/kg of EEMF brought about significantly equal analgesic activity with standard drug Pentazocine at the fourth hour with a probability < 0.0001.

C. DNA Binding and Cleavage Studies

DNA Binding Studies

The Cyclic votammogram of [ethanol extract of Manjanthi Fruit] in buffer pH=7.2 at 25˚C in the presence and absence of CT-DNA as shown in **Fig.1.**

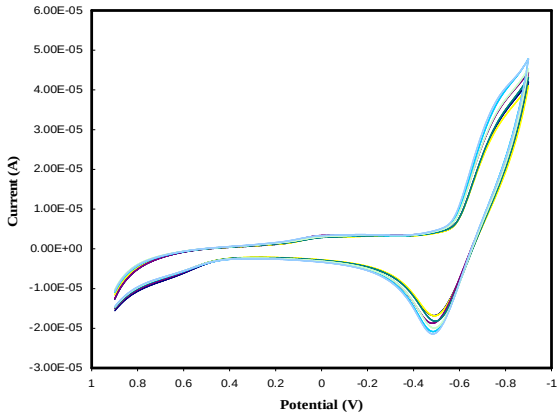

Fig. 1:

In the absence of CT DNA, the anodic peak appeared at -0.479V for Ethanol extract and then after the addition of DNA anodic peak decreases. The changes of the voltammeter currents in the presence of CT DNA can be attributed to diffusion of the Manjanthi fruit extract of ethanol bound to the large diffused DNA molecule. The changes of the peak currents observed for the extracts upon addition of CT DNA may indicate that extracts possess DNA binding affinity.

Hydrogen peroxide induced DNA Cleavage Study

The study on the cleavage capacity of Manjanthi fruit extracts to DNA was considerably interesting as it can contribute to understanding of the toxicity mechanism of them and to develop novel artificial nuclease. DNA cleavage was controlled by relaxation of super coiled circular form of pUC19 DNA into nicked circular form and linear form. When circular plasmid DNA was conducted by electrophoresis the fastest migration would be observed for the supercoild form (Form I),if one strand was cleaved.The supercoils will relax to produce a slowed moving open circular from (Form II),if both strands were cleaved. DNA cleavage was analyzed by monitoring the conversion of supercoiled DNA (Form I) to nicked DNA (Form II) in the presence of oxidant H_2O_2. **Fig.2.**clearly showed that the relative binding efficacy of the Manjanthi fruit extract to DNA. Therefore, we conclude that Manjanthi fruit extract of Ethanol with H_2O_2 had been found to be efficient oxidant as shown in **Fig.2.**

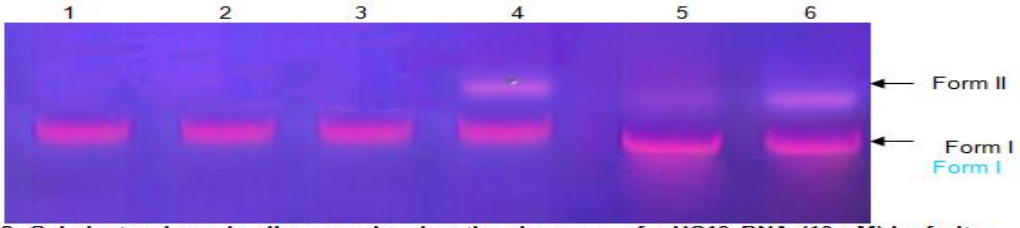

Fig. 2: Gel electrophoresis diagram showing the cleavage of pUC19 DNA (10 µM) by fruit extract in a buffer containing 50 mM Tris-HCl and 50 mM NaCl in the presence of ascorbic acid (AH_2, 10µM) at 37 ˚C. Lane 1, DNA control; lane 2, DNA+ H_2O_2, lane 3, DNA+ Ethanol extract; lane 4, DNA+ Ethanol extract+ H_2O_2; lane 5, DNA+ Ethanol extract+ H_2O_2; lane 6, DNA+Ethanol extract+ H_2O_2

CONCLUSION

Ethanol extract of Manjanthi Fruit (EEMF) 200 mg/kg after two hours had activity of 59.70% with a probability< 0.001 similar to diclofenac sodium (59.68%), the standard anti-inflammatory drug available. The analgesic activity of 200 mg/kg of EEMF brought about significantly equal analgesic activity (83.72) with standard drug Pentazocine(84.09) at the fourth hour with a probability < 0.0001.

The DNA-Binding study indicated that the Manjanthi fruit extract was found to possesses DNA binding affinity with CT-DNA. The result of agarose gel electrophoresis indicated that the ethanol extract Manjanthi fruit exhibited cleavage capability of pUC19 DNA in the presence of H_2O_2.

ACKNOWLEDGEMENT

The authors express their sincere thanks to the College Management, Principal of SFR College, Sivakasi for providing necessary research facilities and also to Prof. P.Solairaj and Prof. A.Thanga Thirupathi of SB College of Pharmacy Anaikuttam, for Pharmacological activity determinations. The authors also thank the University Grants Commission (UGC), New Delhi for financial assistance.

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