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VIEWS

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Evaluation Of The Antipyretic Activity Of *Trapa Bispinosa* Root

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ABSTRACT

The methanolic extract of *Trapa bispinosa* root (METBR) was investigated for its antipyretic activity. Antipyretic potential of methanolic extract was evaluated by brewer's yeast-induced pyrexia test. The pyrexia in rats was reduced significantly ($P < 0.05$) compared to that of control. The results of the present study suggest that METBR (200mg/kg) possesses potent antipyretic activity.

Keywords: *Trapa bispinosa*, Antipyretic activity, METBR.

Introduction

Due to poor hygiene practices and malnutrition, children in developing countries frequently suffer from various forms of infections which present as fevers [1].These fevers are often accompanied by aches and pains which lead to morbidity and mortality. Herbal medicines are often used as remedies in these conditions since as the result of poverty orthodox medicine may be unaffordable [2].It is a well known fact that herbal medicine may be course of substance with better therapeutic potentials than some currently used orthodox medicines [3].The antipyretics-analgesics are chemically diverse, but most are organic acids [4].

Trapa bispinosa Roxb. (Trapaceae), commonly known as Indian Water Chestnut is a hydrophyte aquatic herb used in many Ayurvedic preparations in the Indian system of medicine. Stem flexuose, assending in the water, the submerged part furnished with numerous opposite pairs of green root-like spreading pectinate organs arising immediately below the position of the stipules of fallen leaves and reaching some times 3-6 cm. long. The fruit is sweetish; aphrodisiac, appetizer, useful in chronic fevers. The fruit is combination with other drug is recommended for snake-bite and scorpion-sting [5].This study was undertaken to evaluate the antipyretic properties of methanolic extract of *T.bispinosa* in rats at different doses.

Materials and Methods

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Plant materials

The roots of *T. bispinosa* were collected in Nov-Dec, from Bhaisatara village, near Raipur (Chattishgarh) and were authenticated by M.S. Mandal, Additional director, Botanical Survey of India (Kolkata). A voucher specimen (PCB/131/09) was deposited at Botanical Survey of India.

Preparation of the extracts

The powdered plant materials were extracted with methanol by Soxhlet apparatus at room temperature for 24 hrs. The solvents are then removed under reduced pressure which obtained a dark brown colored viscous residue.

Phytochemical screening

The methanolic extract of *T. bispinosa* was screened for various chemical constituents (saponins, glycosides, carbohydrates, phenolic compounds, flavonoids, phytosterols, fixed oils and fats) using established methods [6, 7].

Experimental animals

Wistar Albino Rats (100-150 gm) of either sex were used in this study. They were randomly distributed in to groups and housed in cages (6 par cage) and maintained under standard condition at 26 ± 2^0C and relative humidity 44-56% and 10h light: 14h dark cycles each day for one week before and during the experiments.

Acute toxicity study

The acute oral toxicity was performed according to OPPTS following up and down procedure. Wistar Albino Rats (100-150 gm) were maintained under controlled animal

house condition with access to food and water *ad libitum*. The limit test carried out first at 5000mg/kg b.w. all animal were observed for toxic symptoms and mortality for 72 h.

Antipyretic activity

The procedure of vogel's was used for the antipyretic study [8]. The body temperature of each rat was recorded by measuring rectal temperature at predetermined intervals. Albino Wistar rats weighing 150-200g of either sex were used for this study, they were fasted overnight with water *ad libitum* before the experiments. Pyrexia was induced by subcutaneously injecting 20% (w/v) brewer's yeast suspension (10 ml/kg) into the animal's dorsal region. The rectal temperature of each rat was again recorded after 18 h of yeast administration. Rats that did not show a minimum increase of 0.5 °C in temperature 18 h after yeast injection were discarded. Thirty selected rats were grouped into five and immediately treated as follows:

Group I received normal saline, group II received 100 mg/kg Paracetamol, while groups III, IV and V received methanol extracts 50, 100 and 200 mg/kg respectively intraperitonially. Rectal temperature of all the rats was then recorded by inserting 4 to 5 cm clinical thermometer into the rectum of each rat at thirty minutes and tabulated in Table-1.

Statistical analysis

Results were expressed as mean ± S.E.M. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Dunnett's test. $P < 0.05$ was considered statistically significant.

Results and Discussion

Effect of METBR extract on normal body temperature in rats is presented in the table 1.The antipyretic activity studied by using Brewers yeast solution shows significant reduction in elevated body temperature. This effect was maximal at the dose of 200 mg/kg of METBR in dose dependent manner. The antipyretic effect started as early as 1 hour and the effect was maintained for 4 hours after its administration. Both the standard drug paracetamol 200mg/kg and tested METBR extract significantly reduced the yeast elevated rectal temperature compared to that of control group.

Conclusion

In conclusion, the present study demonstrates that methanolic extract of *T. bispinosa* root has marked Antipyretic activity. The antipyretic activities of *T. bispinosa* root extract supports its use in the traditional medicine to reduce fever but further studies are needed to elucidate the exact mechanism by which *T. bispinosa* root extract exerts the antipyretic effect.

Table 1

Antipyretic activity of *T. bispinosa* Roxb on Brewer's yeast induced pyrexia in rats

Treatment	Dose	Rectal Temperature (°C)				
		0.0h	0.5h	1.0h	1.5h	2.0h
Control	-	38.35±0.06	38.52±0.07	38.5±0.07	38.47±0.08	38.25±0.06
Paracetamol	200mg/kg	38.68±0.16*	37.85±0.27*	37.08±0.08*	36.88±0.12*	36.68±0.08*
Test drug-I	50mg/kg	39.28±0.19	39.12±0.15	38.9±0.12	38.63±0.07	38.35±0.10
Test drug-II	100mg/kg	39.0±0.12	38.97±0.10	38.48±0.12	38.25±0.12	38.07±0.11
Testdrug-III	200mg/kg	38.83±0.10	38.1±0.21	38.03±0.20*	37.95±0.14*	37.43±0.16*

Significantly different from the control at P<0.05

Test drug- I, II&III- METBR (*Methanolic extract of T. bispinosa root*)

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RP-HPLC Method for the Estimation of Docetaxel in Bulk and Pharmaceutical Dosage Forms

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ABSTRACT

A simple, rapid, sensitive and precise High Performance Liquid Chromatographic (HPLC) method has been developed for the estimation of docetaxel in bulk and pharmaceutical dosage forms. In this method RP-C_{18} column (150mmx4.6mm I.D., 5ì m particle size) with mobile phase consisting of acetonitrile and 0.01M phosphate buffer pH 3.0 in the ratio of 60:40 v/v in isocratic mode was used. The detection wavelength is 230nm and the flow rate is 0.8ml/min. In the range of 10-140ì g/ml, the linearity of docetaxel shows a correlation coefficient of 0.9989. The proposed method was validated by determining linearity, sensitivity, accuracy, precision and system suitability parameters. The proposed method is simple, accurate, precise and reproducible hence can be applied for routine quality control analysis of docetaxel in bulk and pharmaceutical dosage forms.

Keywords: Docetaxel, HPLC, Validation.

Introduction

Docetaxel is a novel anticancer agent of the taxoid family. Docetaxel is a semi synthetic derivative of paclitaxel. Chemically [1] docetaxel is (αR,βS)-β-[[1,1-dimethylethoxy)carbonyl]amino]-α-hydroxybenzene-propanoic acid (2aR,4S,4aS,6R,9S, 11S,12S,12aR,12bS)-12b- (acetoxy)-12- (benzoyloxy)- 2a,3,4,4a, 5,6,9,10,11,12, 12a,12b-dodecahydro-4,6,11-trihydroxy-4a,8,13,13-tetramethyl-5-oxo-7,11-methano-1*H*-cyclo deca[3,4]benz[1,2-b]oxet-9-yl ester. Docetaxel promotes [2,3] tubulin assembly into the microtubules, stabilizes microtubules and inhibits microtubule depolymerization to free tubulin. This leads to disruption of the equilibrium within the microtubule system and ultimately cell death. Literature survey reveals that various HPLC [4-8] and LC-MS [9-14] methods have been reported for the estimation of docetaxel in bulk and pharmaceutical dosage forms. The proposed method is simple, rapid, sensitive, accurate and precise HPLC method for regular quality control analysis of docetaxel in bulk as well as in pharmaceutical dosage forms.

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Experimental

Instrumentation: The separation was carried out on isocratic HPLC system (Shimadzu) with Shimadzu Binary HPLC pump, Shimadzu LC- 10AT UV-Visible Detector, LC-solution software and RP-C_{18} column (150mmx4.6mm I.D; particle size 5µm).

HPLC conditions: The mobile phase consisting of acetonitrile (HPLC grade) and 0.01M phosphate (KH_2PO_4) buffer (pH adjusted to 3.0 with orthophosphoric acid) of AR grade in the ratio of 60:40v/v was pumped into the column at a flow rate of 0.8ml/min with ambient temperature. The detection was monitored at 230nm and the run time was 8min. The volume of injection loop was 20µl prior to injection of the drug solution the column was equilibrated for atleast 30 min. with the mobile phase flowing through the system.

Procedure: Stock solution of docetaxel was prepared by dissolving 25mg of docetaxel in 25ml standard volumetric flask containing 25ml of acetonitrile to get concentration of 1mg/ml. 2.5ml of the above solution was transferred to 25ml volumetric flask and the volume was made up to the mark with mobile phase. Subsequent dilutions of this solution were made with mobile phase to get concentration of 10-140µg/ml. The solutions were

injected into the 20µl loop and the chromatogram was recorded in Fig. 1. The calibration curve was constructed by plotting concentration Vs peak area ratio. The linearity experiment was carried out in triplicate to ascertain accuracy and precision of the method.

Assay: Ten injections of docetaxel were collected and transfer the contents accurately. A quantity equivalent to 20mg of docetaxel was transferred accurately into 25ml volumetric flask. About 15ml of acetonitrile was added and kept in ultrasonic bath for 15min. This solution is filtered through a membrane filter and the volume was made up to the mark to get 1mg/ml concentration. From the above solution 2.5ml was transferred to 25ml volumetric flask and the volume was made upto 25ml with mobile phase. From this solution, further dilutions were made to obtain concentration range of 10-140µg/ml. 20µl of the sample solution was injected under the chromatographic conditions and the chromatogram was recorded. The amount of docetaxel present in injection formulation was determined by comparing the peak area from the standard. The results are furnished in Table-1.

Table-1

Validation Summary

System Suitability	Results
Linearity range (µg/ml)	10-140
Correlation Coefficient	0.9989
Theoretical Plates (N)	22605
HETP	0.0066
LOD (µg/ml)	0.004
LOQ (µg/ml)	0.016
Symmetry factor	0.862
Tailing factor	1.20
Percentage recovery (Accuracy)	99.60

Validation of proposed method: Selectivity of the method was assessed on the basis of elution of docetaxel using the above mentioned chromatographic conditions. Precision was ascertained by the determination of intra-day and inter-day variabilities. The results are shown in Table-2.

Table-2

Intra- and Inter- day precision for Docetaxel by the proposed HPLC method

Concentration of Docetaxel (µg/ml)	Observed concentration of Docetaxel (µg/ml)			
	Intra-day		Inter-day	
	Mean (n=6)	% CV	Mean (n=6)	% CV
20	19.97	0.16	19.50	0.06
80	80.09	0.13	79.51	0.42
120	119.97	0.40	118.92	0.15

Table-3

Assay and recovery studies

Formulation	Label claim (mg)	Amount found (mg)	% Recovery	% C.V.
Brand-1	20	20.02	100.10	0.57
Brand-2	20	19.95	99.76	0.50

*Mean of Three Determinations

To study the accuracy, reproducibility, precision of the proposed method, recovery studies were carried out in triplicate by adding a known quantity of the sample and the percentage recovery was calculated. The results are furnished in Table-3.

Results and Discussion

By applying the proposed method, the retention time of desloratadine was found to be 3.667min (Fig.1). Linearity range was observed in concentration range of 10-140µg/ml. The regression equation of docetaxel concentration over its peak area ratio was found to be Y=-5.8135+12.583X (r^2=0.9989) where Y is the peak area ratio and X is the concentration of docetaxel (ìg/ml). The symmetry factor was found to be 0.862, which indicated symmetric nature of peak. The number of theoretical plates was found to be 22605, which indicates efficient performance of the column. The limit of detection and

limit of quantification was found to be 0.004ìg/ml and 0.016ìg/ml, indicates the sensitivity of the method. When docetaxel solution containing 20µg/ml, 80µg/ml and 120µg/ml were analysed by the proposed HPLC method for finding out intra- and inter-day variations, a low coefficient of variation was observed. The high percentage of recovery indicates that the proposed method is highly accurate.

Conclusion

The proposed HPLC method was found to be highly accurate, sensitive and precise. Therefore this method can be applied for the routine quality control analysis of docetaxel in its pharmaceutical dosage forms.

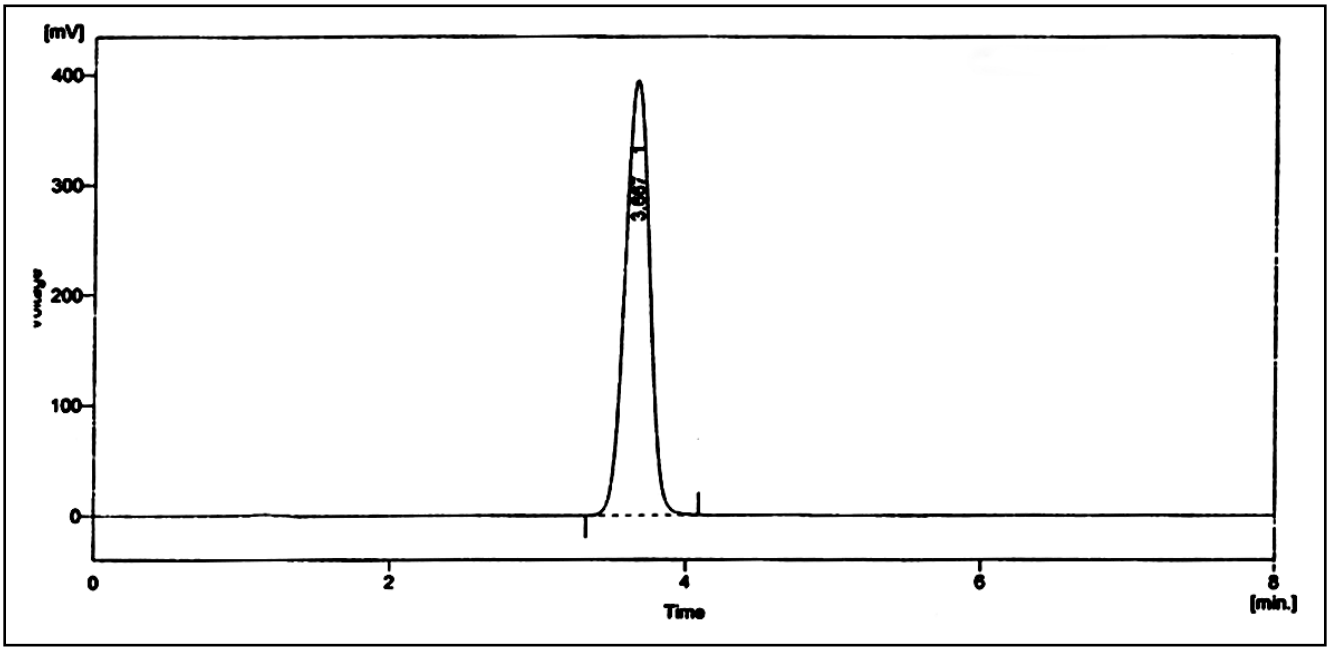

Fig. 1: Typical Chromatogram of Docetaxel

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Silica Nanoparticles and Multiwall Carbon Nanotubes as Solid Phase Sorbents Supports in Pre-concentration of Traces of Residues of Endosulfan and its Isomers in Water and Bovine Milk

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ABSTRACT

The silica nanoparticles prepared by stober's mechanism and multi-walled carbon nanotubes (MWCNT) generated by chemical vapor deposition method were used as solid sorbents supports in pre-concentration of residues of endosulfan in water and bovine milk. The synthesized nanoparticles were characterized by Scanning Electron Microscope (SEM) and X-Ray Diffraction (XRD) techniques. Size of the silica nanoparticles was 50 to 250 nm, MWCNT 20 to 50 nm diameter and 1 to 5 micrometer length. The pre-concentration capacity of the sorbents was studied by filling the empty SPE cartridges with 200 mg of nanoparticles. The residues of endosulfan (as a total of alpha, beta and sulfate moiety) were analyzed by a GC-ECD and confirmed by GC-NCI-MS (Gas Chromatography Negative Ion Chemical Ionization Mass Spectrometry). The lowest limit of detection (LLOD) established by GC-MS was 0.01 ng/mL and the limit of quantification 0.03 ng/mL. The recovery study conducted with the spiked water and bovine milk samples showed greater than 95% recovery. The nanoparticles based SPE cartridges were optimized for flow rate 3 to 4 mL/min, temperature <50°C, sample volume 1000 mL for silica nanoparticles; 2000 mL for MWCNT cartridges and pH 6 to 7. The change in ionic strength did not influence the pre-concentration capacity of the cartridges. The ground water samples collected from the irrigation wells where there is history of using the insecticide as part of crop protection practices showed the presence of residues of alpha endosulfan 0.6 ng/mL. The bovine milk samples collected didn't showed any residues.

Key words: MWCNT, silica nanoparticles, endosulfan, solid phase extraction.

Introduction

The widespread use of nanoparticles raised the eyebrows of researchers to utilize their versatile applications in the field of agriculture [1] environmental and health science. The residues of xenobiotics such as pesticides which are present in trace level have greater influence on environment and human health [2]. Analysis of water samples collected at Chennai showed the presence of organochlorine pesticides and endosulfan sulfate [3]. The water samples from river basins and agricultural wells from 1991 to 1998 showed the endosulfan residues from 0.18 to 48 µg/L [4]. Ground water samples collected from India [5], Malaysia [6], Taiwan [7] and Turkey [8] were also showed the presence of endosulfan residues.

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Several of the studies successfully used different solid phase extraction sorbents for the pre-concentration of residues. The SPE materials used in the enrichment of contaminants from environment includes Alumina, Iron [9] Carbon [10] and Silica [11]. The recent developments in nanotechnology brought out the use of MWCNT based SPE cartridges for the pre-concentration of residues of poly-halogenated pollutants [12], atrazine, methidathion, propoxur [13] and other highly leachable pesticide residues in environmental water [14, 15]. MWCNT based SPE adsorbent used for the enrichment of residues of pesticides reported the recovery 47 to 94% in milli Q water and mineral water [16]. The oxidized MWCNT has the higher adsorption capacity of residues in water than the non-oxidized [17]. The surface modified SPE cartridges were also used for the extraction of chlorinated pesticides, poly-chlorinated biphenyls and nitro compounds from water

[18-20]. The use of nanocomposites of SnO_2 nanoparticles and MWCNTs for detection of aldrin and DDT was also reported in the literature [21]. The research on the applications of nanoparticles based SPE techniques are still in nascent stage. In view of the paucity of information, present study was conducted to investigate the capacity of silica nanoparticles and MWCNT in pre-concentration of endosulfan a potential xenobiotic, and factors affecting the pre-concentration capacity in water and bovine milk samples which are the primary source for the entry of toxins in to human system.

Experimental

Synthesis and Characterization of Nanoparticles

Silica nanoparticles synthesized by stober's mechanism [22], MWCNT by chemical vapor deposition (CVD) technique [23] are used in this study. The SEM image of Silica nanoparticles (50 to 250 nm) and MWCNT (~ 50 nm diameter and 1 to 5 μm lengths) are presented in **Fig. 1** and **Fig. 2**. The size and shape of the nanoparticles was confirmed by SEM and XRD analysis.

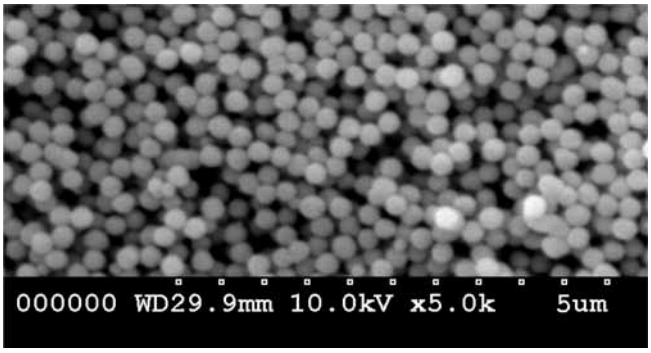

Fig. 1: Scanning Electron Microscope image of Silica nanoparticles

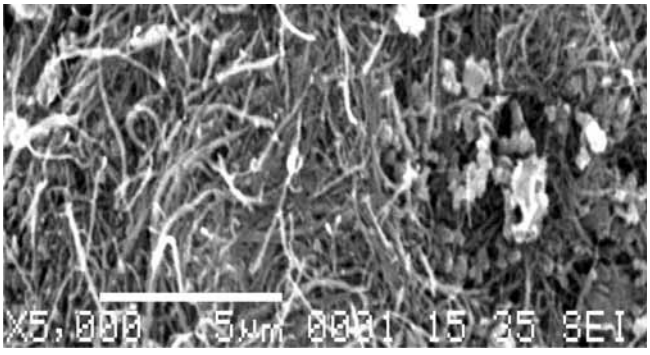

Fig. 2: Scanning Electron Microscope image of Multiwall carbon nanotubes

Preparation of SPE cartridges using nanoparticles

The SPE cartridges were prepared by filling different empty cartridges with 200 mg of MWCNT and silica nanoparticles separately. The material was packed in between two polystyrene impregnated PTFE frits and conditioned with methanol and milli Q water.

Pre-concentration:

The water sample was directly passed through the cartridge using a vacuum pump and Milk sample was initially acidified to pH 4.0 using phosphoric acid. Filtered and the collected aqueous phase was neutralized and passed through the cartridge. After passing the aqueous phase, the cartridges were dried under vacuum and residues were eluted using 5 mL of HPLC grade acetone for analysis.

Instrumentation

The residues of alpha endosulfan, beta endosulfan and endosulfan sulfate were quantified (Total endosulfan) by a validated GC-ECD method and confirmed by GC-NCI-MS method. The validation parameters include specificity, linearity, assay accuracy and repeatability.

GC-ECD conditions for the determination of residues of endosulfan

Shimadzu GC-17AAF gas chromatograph with electron capture detector and AOC 20i auto injector supported by Class GC software was used for the quantification of residues. DB-1 (100% dimethyl polysiloxane) fused silica capillary column (30 m length, 0.53 mm i.d. and 1.0 μm film), carrier gas nitrogen at flow rate 7.5 mL/min was used for the separation of residues. The set temperatures for injector, detector and column were 300, 310 and 280°C respectively. The sample and standard injection volume used for the analysis was 0.5 μL.

Confirmation of residues of endosulfan

The GC-NCI-MS conditions [24] established earlier was used in this study. A Shimadzu GC-MS QP5050A and AOC 20i auto injector, supported by GCMS solution software was operated in negative ion chemical ionization mode (NCI) with DB-1 (100% dimethyl polysiloxane) fused silica capillary column (30 m length, 0.25 mm inner diameter and 0.25 μm film). Helium was used as carrier gas at 1 mL/min flow rate and the reagent gas Isobutane 2.5 mL/min for soft ionization. The programmed temperature conditions were column oven 150°C for two minutes, ramp at the rate of 10°C/minute to 240°C, held for 5 minutes. The injector and interface was kept at 240°C and 250°C, split ratio 1:3 and injection volume 1.0 μL. The method has the lowest LOD 0.01 ng/mL and the limit of quantification 0.03 ng/ml.

Validation

Calibration solutions were prepared using alpha endosulfan (CAS No - 959-98-8), beta endosulfan (CAS No -33213-65-9) and endosulfan sulfate (CAS No -1031-07-8) of 99% pure analytical standards in acetone. The GC-ECD data showed the linearity over the concentration range 5 to 500 ng/mL with R^2 value 0.999. Based on signal to noise ratio 3:1 the LOD was established. The peaks of alpha endosulfan, beta endosulfan and endosulfan sulfate were eluted at 3.3, 4.3 and 5.1 minutes respectively. No

overlapping of peaks was observed as evidenced by specificity.

Assay accuracy and repeatability of the method was evaluated by spiking two different concentrations 0.03 and 0.1 ng/mL of endosulfan and its analogues in 500 mL of milli Q water and bovine milk. LOQ 0.03 ng/mL was established based on the recovery study. Five replicate determinations were made at each concentration levels.

Factors influencing the pre-concentration efficiency

The influence of flow rate on the pre-concentration was checked at different flow rates 1 to 10 mL/min varying the sample volumes 500 to 3000 mL. The effect of pH in pre-concentration of residues was investigated at pH levels 5 to 8, the effect of ionic strength with 0.1% and 1% sodium chloride solution; the temperature ranges tested are 10 to 50°C.

Results and Discussion

Assay Accuracy and repeatability

The recovery of alpha endosulfan from silica and MWCNT cartridges was 91.5 to 103.9 and 91.1 to 101.5 respectively. The recovery of beta endosulfan was 93.8 to 108.3 from silica and 97.7 to 105.7% in MWCNT cartridges and the recovery percentage of endosulfan sulfate was 89.1 to 112.7 from silica cartridge and 94.7 to 103.5 in MWCNT cartridge. From the results it was observed that no significant variation was observed in the recovery at lower and higher concentration. Repeatability of the method showed acceptable RSD% according to "Horwitz equation"

$$RSD\% < 2^{(1-0.5\ \log C)} \times 0.67$$

Where C is the concentration of the analyte expressed as percentage. The maximum acceptable RSD% calculated based on the above equation for the analyte concentrations 0.1 ng/mL was 42.88% and for 0.01 ng/mL 60.64%.

Representative GC-ECD chromatogram and GC-NCI-MS total ion chromatogram were presented in **Fig. 3** and **Fig. 4** respectively.

Influence of pH and ionic strength in the pre-concentration of residues

The recovery of endosulfan residues from Silica cartridge were 70.9 to 82.1% at pH 5.0; 91.7 to 98.1% at pH 6.0; 97.1 to 104.1% at pH 7.0 and 79.3 to 86.1% at pH

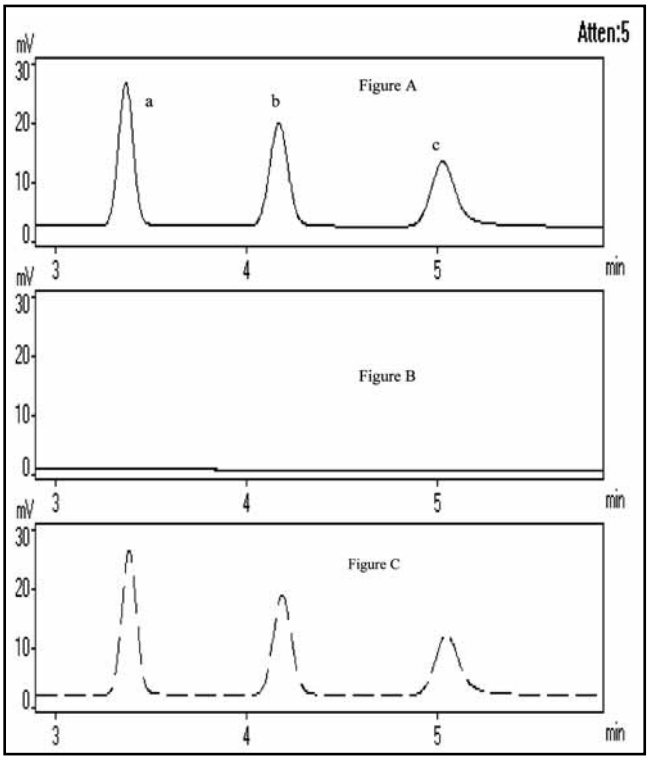

Figure A. Standard 10 ng/mL
Figure B. Control
Figure C. Recovery of residues - MWCNT cartridges (0.1 ng/mL in Water)
a. Alpha endosulfan, b. Beta endosulfan, c. Endosulfan sulfate

Figure 3. Representative GC-ECD chromatogram of endosulfan standard, control and recovery at 0.1 ng/mL

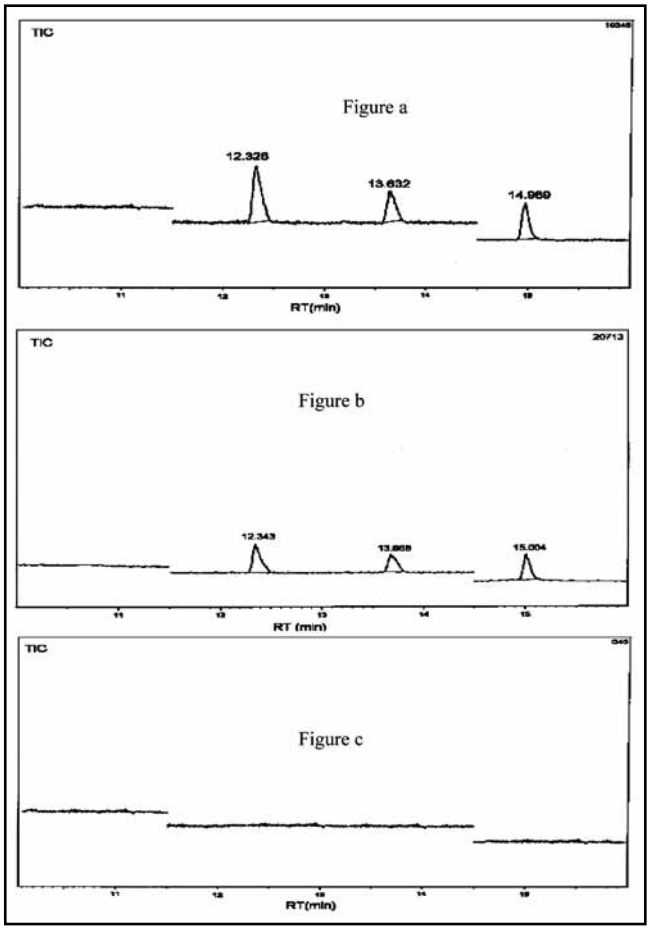

Figure a. Standard 10 ng/mL
Figure b. Recovery of residues - MWCNT cartridges (0.1 ng/mL in bovine milk)
Figure c. Control

Figure 4. GC-NCI-MS chromatogram for the confirmation of residues

8.0. Optimum pH was established as 6 to 7. No significant change in the pre-concentration capacity of residues due to variation in the ionic strength was observed. The recovery of endosulfan was 79.7 to 97.6% from silica cartridge and 81.6 to 108.1% from MWCNT cartridge.

Effect of temperature, sample volume and flow rate in pre-concentration of residues

The recovery of endosulfan residues from MWCNT cartridge at different temperatures were 96.7 to 102.4% at 10 ± 2°C; 91.2 to 106.1% at 20 ± 2°C; 92.7 to 102.4% at 30 ± 2°C, 82.1 to 90.7% at 40 ± 2°C and 78.3 to 85.3% at 50 ± 2°C. The optimum temperature for pre-concentration was less than 50°C. The optimum flow rate was established as 3-4 mL per minute. The optimum sample volume established for pre-concentration was ~2000 mL for MWCNT cartridge, ~1000 mL for silica and C18 cartridges.

Application to environmental samples

Water samples collected from six agricultural wells were tested. The pre-concentration was conducted at 20 ± 2°C. The residues of alpha endosulfan detected in the well water samples were in the range 0.5 to 0.6 ng/mL. The bovine samples collected from the market didn't show any residues. This was also confirmed by positive spiking aliquots of analytical standards in to the substrate matrix.

Conclusions

The silica nanoparticles and MWCNT were successfully demonstrated to be useful in pre-concentration of residues of endosulfan in water and in bovine milk samples. The residues of endosulfan were analyzed as a total of alpha endosulfan, beta endosulfan and endosulfan sulfate using GC-ECD. The residues of endosulfan was confirmed by GC-NCI-MS, the method has the LLOD 0.01 ng/mL and the LOQ 0.03 ng/mL. The method was also tested and found successful in identification and confirmation of residues of endosulfan and its analogues in water samples collected from different irrigation wells. From the analysis it was concluded that the nanoparticles based SPE cartridges has greater advantage in pre-concentration of residues of pesticides in water and bovine milk when compared with conventional cartridges. The MWCNT cartridges showed high recovery, retention efficacy, pH stability and able to tolerate large volume of sample when compared with silica nanoparticles based cartridge and C18 cartridge.

Acknowledgement

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Anti-hyperlipidemic Activity of *Bauhinia Purpurea* Extracts in Hypercholesterolemic Albino Rats

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ABSTRACT

The ethanolic extract of unriped pods and leaves of *Bauhinia purpurea* at a dose of 300mg/kg body weight were evaluated for anti-hyperlipidemic activity in cholesterol high fat diet (CHFD) induced hyperlipidemia. Changes in body weight and the analysis of serum lipids were carried out at the end of the study. Marked decrease in body weight, total cholesterol (TC), triglycerides (TG), low density lipoprotein (LDL), very low density lipoprotein (VLDL) where as a significant increase in the level of high density lipoprotein were observed after treatment with *Bauhinia purpurea* extracts. Ethanolic extract of unriped pods showed a marked effect over body weight reduction where as ethanolic extract of leaves had a significant effect on the lipoprotein profile. The lowered atherogenic index, TC: HDL-c and LDL: HDL-c ratios in the extract groups suggest the anti-hyperlipidemic and cardio protective potential. The present work indicated that *Bauhinia purpurea* extracts in a dose of 300mg/kg/day significantly suppressed the CHFD induced hyperlipidemia in rats, suggesting the need for a further in depth evaluation.

Key words: *Bauhinia purpurea*, Anti-Hyperlipidemic, leaf and unriped pod extracts

Introduction

Cardiovascular diseases remain by far the number one cause of death for both men and women of all ethnic backgrounds. Although many causative factors of these diseases are recognized (smoking, high blood pressure, genetic background, diabetes), total cholesterol levels are the most prevalent indicators for susceptibility to atherosclerotic heart disease [1], [2]. Atherosclerosis is a disorder of the arterial wall characterized by accumulation of cholesterol esters in cells derived from the monocyte macrophage line, smooth muscle cell proliferation and fibrosis and results in narrowing the blood vessel [3]. An association of dietary cholesterol with cardiac and cerebral vascular diseases is based on several lines of evidence [4].

Medicinal plants are an indispensable part of the traditional medicine practiced all over the world. The advantages are low costs, easy access and long safety experience [5]. Many different medicinal plants have been reported for their hypolipidemic activity against animal models [6]. *Bauhinia purpurea* is a flowering plant (Family: Fabaceae). Several species of this plant are known to possess pharmacological activities. Aqueous extract of leaves have antinociceptive, anti-inflammatory and antipyretic [7], hypoglycaemic [8], antimalarial, antimycobacterial, antifungal and cytotoxic activities [9]. Antioxidant and hepatoprotective activities of *Bauhinia* species have also been reported [10]. Methanolic extract obtained from *Bauhinia purpurea* led to the isolation and identification of 6-butyl-3-hydroxy flavone [11]. On the basis of this information, the present study was designed to investigate the effect of ethanolic extract of leaves and unriped pods of *Bauhinia purpurea* on body weight, serum lipid and lipoprotein profile in cholesterol high fat diet (CHFD) induced hyperlipidemia.

*Address for correspondence

Materials and Methods

Plant material

Bauhinia purpurea leaves and unriped pods were collected from Dhullapally, Rangareddy district, Hyderabad. The plant was authenticated by the department of Botany, Osmania University. A voucher specimen (MRCP-106) is deposited in the department of pharmacognosy for further reference. Leaves and unriped pods were air dried, powdered to 40 mesh and subjected to soxhlet extraction with ethanol. The extract was concentrated under reduced pressure. Leaf extract (23%w/w) was suspended in 1% Tween-80 and unriped pod extract (17%w/w) with 1% gum acacia for oral administration.

The Phytochemical analysis revealed the presence of flavonoids, carbohydrates, glycosides, tannins, volatile oils, anthocyanidins, lactones and terpenoids [12].

Animals

Albino Wistar rats weighing 200-250 g of either sex, 4 months of age were used for this study. The experimental animals were housed in polypropylene cages and maintained under standard conditions (12 h light and dark cycles, at 25±3° C and 35-60% humidity). Standard pelletized feed and tap water were provided *ad libitum*. The Institutional Animal Ethical Committee of Malla Reddy College of Pharmacy, Hyderabad, with Reg. No. 1217/a/08/CPCSEA, approved the study.

Acute Toxicity studies

Rats were divided into isolated groups of six in each lot. After an over night fast, the suspension of ethanolic extract of unriped pods and leaves was administered orally to the isolated groups in divided doses of 0.2-4 g/kg body weight and were under continuous observation for the first 2 hours for observing the toxic symptoms and later up to 24 hours to study the mortality rate. The number of dead/ survived animal after 24 hours was recorded and accordingly the LD_{50} was calculated. The effective dose of both the extracts was fixed at 300 mg/kg.

Anti-Hyperlipidemic activity

Animals were divided into 5 groups with 6 animals per group.

Group 1: Normal control.

Group 2: Hyperlipidemic control (Vehicle 1ml/100gm/day p.o)

Group 3: Hyperlipidemic treated with unriped pods extract (300mg/kg, p.o)

Group 4: Hyperlipidemic treated with leaf extract (300mg/kg, p.o)

Group 5: Hyperlipidemic treated with Atorvastatin (5mg/kg, p.o)

The treatment period for all these groups was for one month.

Preparation of high cholesterol diet [13]:

The high cholesterol diet was prepared by mixing-Thiouracil-0.4%, High fat-30 %(Amul butter), Cholesterol-1%, Cholic acid-0.5% and Proteins-11%.

Biochemical assays for lipids:

At the end of treatment period, all the animals were tested for biochemical lipid markers. Blood was collected by cardiac puncture method under ether anaesthesia. Serum total cholesterol (TC), triglycerides (TG) was estimated by method of CHOD-PAP and high-density lipoprotein-cholesterol (HDL-c) by the method of GPO-PAP using span diagnostic kits. Serum LDL-c [14], VLDL-c level and atherogenic index was determined by calculation [15].

Statistical Analysis

All the results were expressed as mean±SEM and subjected to One way Analysis of variance followed by dunnet's test for comparision between the groups and $P<0.05$ was considered significant.

Results and Discussion

Rats fed with CHFD, for one month displayed an increase in body weight as compared to normal rats. Treatment with ethanolic extract of unriped pods (300mg/kg/day) and leaves (300mg/kg/day) showed only slight increase in body weight to 2.0% and 7.4% respectively as compared to hyperlipidemic group (13.11%). Unriped pod extract shows significant reduction in body weight, which was comparable to atorvastatin treatment (3.06%). A reduction of body weight suggests the potential of *B.purpurea* against obesity (Fig. 1).

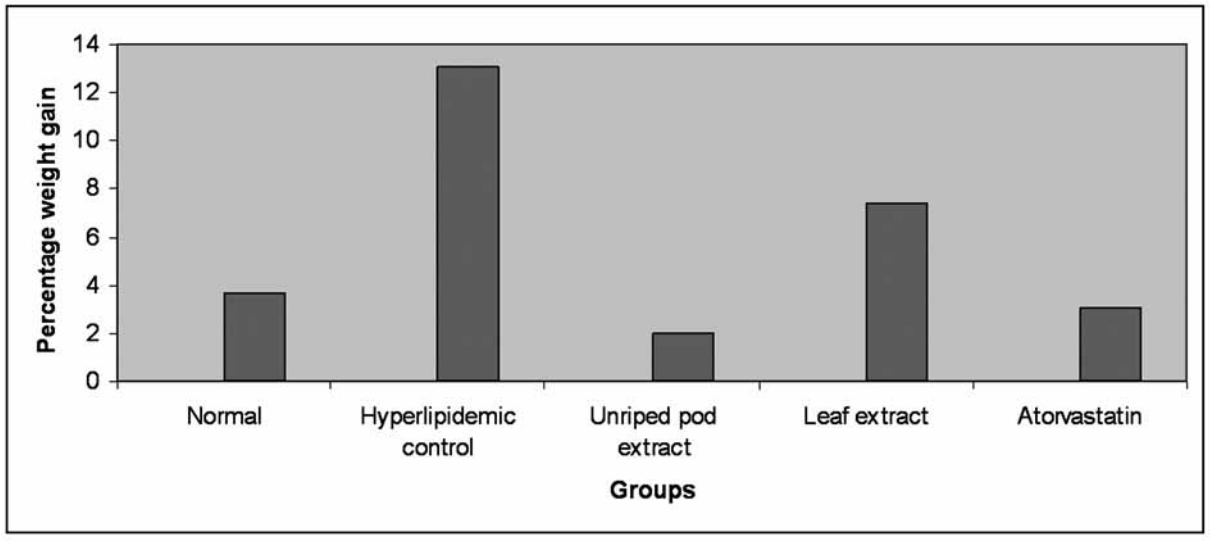

Fig. 1: Effect of ethanol extract on the body weight in high cholesterol diet

There was significant increase in the levels of serum TC (300.31±2.4), TG (286.47±3.0), LDL-c (200.62±2.5), VLDL-c (60.44±1.2), although the levels of HDL-c (30.41±2.9) were found to be diminished when compared to normal control animals. Treatment with unriped pods extract and leaf extract showed a marked reduction in TC level (29.26%, 39.60%), TG level (27.99%, 41.90%) and LDL-c levels (48.32%, 72.63%) respectively. But there was a significant rise in HDL-c levels in all the groups. Atorvastatin produced significant reduction in serum TC (53.90%), TG (55.17%), LDL-c levels (81.39%) and a rise in HDL-c levels .The results are shown in Table 1.

There was a marked reduction in TC: HDL-c ratio, LDL: HDL-c ratio and the atherogenic index after the treatment of rats with 300mg/kg dose of ethanolic extract of unriped pods and leaves of *bauhinia purpurea* (Table 2). TC: HDL-c ratio, LDL: HDL-c ratio is an effective predictor of coronary risk [16]. Atherogenic index is an important indicator of CHD risks at both high and low serum cholesterol level [17]. The cholesterol lowering effect of the extracts might be due to inhibition of dietary cholesterol absorption and/or esterification. Since two enzymes are involved in these two processes pancreatic cholesterol esterase [18] and intestinal acyl Co-A-Cholesterol acyl transferase enzyme (ACAT) [19]. Thus it could be suggested that the extracts inhibits one or both enzymes activity. In the present study, the activity of the extracts may be due to direct inhibition of cholesterol absorption or due to increased biliary excretion of sterol and /or bile acids and the block of cholesterol movement from the liver to the blood; as cholic acid was one of the ingredients of cholesterol high fat diet.

Conclusion

The activity may be due to the presence of polyphenolic compounds flavonoids, tannins and proanthocyanidines in the ethanolic extracts, which reduce oxidation of LDL-c [20]. This needs to be studied further by assay of oxidized LDL. The ethanolic extract of unriped pods has significant weight reduction property than leaf extract and which was comparable to that of Atorvastatin. Ethanolic extract of leaves had a marked effect on anti-hyperlipidemic activity.

Table-1

Effect of *Bauhinia purpurea* on serum lipid level in CHFD induced hyperlipidemia

Groups	Dose (mg/kg)	TC	TG	HDL	LDL	VLDL
Normal	—	120.81±1.1	107.13±2.9	51.45±2.7	42.14±5.0	23.91±3.9
Hyperlipidemic Tween-Control	80 1%	300.31±2.4	286.47±3.0	30.41±2.9	200.62±2.5	60.44±1.2
Unriped pod extract	300	212.43±2.9 (-29.26%)*	206.26±1.6 (-27.99%)	68.42±3.0	103.67±6.5 (-48.32%)*	41.25±2.9 (-31.75%)
Leaf extract	300	181.37±3.6 (-39.60%)*	166.43±1.8 (-41.90%)*	70.55±2.4*	54.90±3.8 (-72.63%)*	35.41±2.0 (-41.41%)*
Atorvastatin	5	138.44±2.8 (-53.90%)*	128.41±2.1 (-55.17%)*	75.42±1.8*	37.33±2.1 (-81.39%)*	25.60±4.0 (-57.64%)*

Values are expressed as mean±SEM. n=6, *P<0.05; significantly differently from control.

Table-2

Effect of *Bauhinia purpurea* on serum lipoprotein ratio

Groups	Dose (mg/kg)	Atherogenic Index	TC:HDL-c	LDL:HDL-c
Normal	—	1.2	2.3	0.8
Hyperlipidemic Tween-control	80 1%	8.5	9.8	6.5
Unriped pod extract	300	2.1	3.1	1.5
Leaf extract	300	1.3	2.5	0.7
Atorvastatin	5	0.83	1.8	0.5

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Antidiabetic and Hypolipidemic Potential of *Amaranthus Spinosus.* Linn. in Streptozotocin-Induced-diabetic Rats

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ABSTRACT

Antidiabetic, hypolipidemic effects were studied with methanolic extract of leaves of *Amaranthus spinosus* .Linn (Family: Amaranthaceae) in diabetic rats. The invivo hypoglycemic activity was evaluated against streptozotocin-induced type-2 diabetic rats. Oral administration of methanolic extract of *Amaranthus spinosus* leaves at 200 and 400 mg/kg body weight per day, 21 days to diabetic rats was found to possess dose dependent antidiabetic and hypolipidemic activity in type-2 diabetic rats. Histologically, focal necrosis was observed in diabetic rat pancreas, but was less obvious in treated groups.

Key words: Antidiabetic, hypolipidemic, *Amaranthus spinosus*, streptozotocin, type-2 diabetes, pancreas.

Introduction

Diabetes mellitus is one of the common metabolic disorders and 1.3% of the population suffers from this disorder through out the world [1]. Insulin and oral hypoglycemic agents are still the major players in the management of this disorder. Many herbal products, including several metals and minerals have been described for the cure of diabetes mellitus in ancient literature. Herbal preparations alone or in combination with oral hypoglycemic agents sometimes produce a good therapeutic response in some resistant cases where modern medicines alone fail [2]. Currently available treatment is far from satisfactory and is expensive. Recently, there has been a renewed interest in the plant remedies for the treatment of diabetes. *Amaranthus spinosus*.L is a glabrous herb (Amaranthaceae) found in tropical and sub tropical regions of India. The root of this plant is used as a diuretic and febrifuge [3]. The plant was reported to possess anti-malarial [4], anti-diarrheic [5], stimulation of proliferation of β-lymphocytes [6] and haematological properties [7]. The present study was undertaken in STZ-diabetic rats to evaluate the antidiabetic activity and to determine changes in body weight, lipid profile and histology of pancreas.

Materials and Methods

Plant material

Amaranthus spinosus.L (Amaranthaceae) leaves, collected in May-June, 2008 from GKVK, Agricultural university, Bangalore, were authenticated by Dr. Rajanna, the taxonomist of the university, voucher specimen was deposited in the herbarium of the Pharmacognosy department, PES College of pharmacy, Bangalore, Karnataka, India, with reference no. MAS-26.

Extraction

The leaves (60g) shade dried, powdered, soxhlet-extracted with methanol (400 ml). The extract was then concentrated using rotary evaporator under reduced pressure (yield- 4.8 % w/w) was stored in a refrigerator at 4ºC, until use for the biological testing and phytochemical screening.

Preliminary phytochemical screening

Preliminary phytochemical screening [8] revealed the presence of glycosides, proteins, triterpenoids, flavonoids and tannins.

Animals Through out the experiment, experimental rats were processed in accordance with the instructions given by our institutional animal ethical committee CPCSEA [9]. Healthy wistar rats between 2-3 months of age and weighing 180-200g were used for the study. Rats were kept in standard polypropylene cage and maintained under standard laboratory conditions of temperature (25±1ºC), relative humidity (50±15%), 12hrs light-dark cycles, standard diet and water ad-libitum.

Acute toxicity studies

The acute oral toxicity study was carried out according to the guidelines set by OECD. Starting dose was selected to be 2000 mg/kg body weight and finally a dose of 5000 mg/kg body weight was evaluated for toxicity.

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Effect of methanolic extract in normoglycemic rats (NG)

Animals in the control group received normal saline (orally).The test group of animals was treated with the methanolic extract of *Amaranthus spinosus* at a predetermined therapeutic doses of 200 and 400 mg/kg per orally. Blood samples were collected at 1st, 7th, 14th, 21st day, after the administration [11]

Oral Glucose tolerance test in normal rats (OGTT)

Rats were divided into five groups (n=6) and were administered normal saline, diabetic control (STZ,70 mg/kg), Glibenclamide (10 mg/kg) and dose of 200 mg/kg and 400 mg/kg per oral of methanolic extract. Glucose solution 2g/kg was administered 30 min after the administration of the extract. Blood samples were withdrawn from the retro-orbital at intervals of 60,120,180 min of glucose administration. Blood glucose levels was estimated using GOD-POD kit (Acuuurex, India) OECD 2001-guideline on (AOT) No.425 [11]

Induction of diabetes

The streptozotocin diabetic rat model was performed as per the method described by Kadnur and Goyal, 2005 [10]. Rats were injected intraperitoneally (i.p.) with 70 mg/kg body weight, STZ (sigma chemical Co. U.S.A) dissolved in 0.1M cold citrate buffer (P^H4.5). The animals exhibiting fasting glucose levels of 140-180 mg/dl were screened for the study.

Experimental design

Animals were divided into five groups of six rats each.

Group I: Normal rats, administered saline 10 ml/kg orally for 21 days

Group II: Diabetic control and received 3 % v/v tween 80 in water 10 ml/kg orally for 21 days

Group III: Diabetic rats, administered standard drug Glibenclamide, 10 mg/kg orally for 21 days

Group IV: Diabetic rats, administered MEAS 200 mg/kg orally for 21 days

Group V: Diabetic rats, administered MEAS 400 mg/kg orally for 21 days

Blood samples were drawn by retro-orbital puncture and fasting blood glucose levels were estimated on days 1, 7, 14 and 21 with the help of GOD-POD kit (Acuurex, India). Blood lipid profiles, body weights were determined [11].

Histopathology of the pancreas of STZ induced diabetic rats

On the last day of the study, pancreas samples were fixed in 10% formalin. Thin sections of the tissue, 5-7µm, were cut and these were stained with haematoxylin-eosin. The tissue sections were subjected to rehydration by exposing them to decreasing concentrations of alcohol, 100-30% and then stained with haematoxylin. The sections were dehydrated by using increasing concentrations of alcohol and then stained with eosin. They were then treated with diphenyxylene (DPX) and examined under the microscope [12]

Statistical analysis: The results are expressed as mean ± SEM. Statistical difference was tested by using one-way analysis of variance (ANOVA) followed by Dunnett's test. A difference in the mean P value < 0.05 was considered as significant.

Results

Acute toxicity study

The various observations showed the normal behaviour of the treated rats. No toxic effects were observed at a higher dose of 5g/kg body weight. Hence, there were no lethal effects in any of the groups.

Effect of MEAS on blood glucose in normoglycemic rats

At dose 200 mg/kg and 400 mg/kg of MEAS on fasting blood glucose level were assessed in normal rats at various time intervals is shown in the Table.1. The mean blood glucose level decrease from 93.32 mg/dl to 68.28 mg/dl at dose of 200 mg/kg body weight of MEAS and 91.68 mg/dl to 60.69 mg/dl at dose of 400 mg/kg body weight in rats treated with MEAS.

Table-1

Effect of MEAS on blood glucose in normoglycemic rats

Treatment (mg/kg body weight)	Blood Glucose Levels (mg/dl)			
	1st day	7th day	14th day	21st day
Normal control	89.29 ± 2.41	87.88 ± 1.19	88.29 ± 1.49	86.28 ± 1.53
Vehicle control (3%v/v Tween80 10ml/kg)	89.78 ± 2.92	93.76 ± 1.22	91.42 ±2.86	90.89 ± 2.98
Diabetic + Glibenclamide (10mg/kg)	89.80 ± 1.31	59.69 ± 1.98**	51.62± 1.28**	48.41 ± 2.92**
Diabetic + MEAS (200mg/kg) p.o.	93.32 ± 1.63	85.55 ± 1.82	72.91 ± 1.86	68.28 ± 1.72*
Diabetic + MEAS (400mg/kg) p.o.	91.68 ± 2.97	80.68 ± 1.96	66.68 ± 1.68	60.69 ± 1.62*

Values are given as mean ± SEM, n=6 significant values are compared with diabetic control *P<0.05, **P<0.01 vehicle control Vs all groups

Effect of MEAS on blood glucose in glucose fed hyperglycemic rats

At dose 200 mg/kg and 400 mg/kg of MEAS blood glucose level were assessed in glucose fed rat at various intervals as shown in Table.2. The blood glucose levels decreased from 198.83 mg/dl to 197.81 mg/kg body weight and 189.44 mg/dl to 182.68 mg/dl at 400 mg/kg body weight.

Anti-diabetic activity of MEAS in STZ induced diabetic rats

The results from the study clearly indicated that the methanolic extract exhibited significant hypoglycemic activity in STZ induced diabetic rats, while there was no significant effect observed on normoglycemic rats. However, at the end of 21 days of treatment, there was a 62.47%, 64.65% decrease (P<0.01) of serum glucose levels with the methanolic extract(200 mg/kg and 400 mg/kg) Table.3. The standard drug Glibenclamide also indicated a significant decrease (70.71 %) of serum glucose levels.

Changes in body weight

At the end of 21 day treatment, the body weight of normal rats, methanolic extract at 200 mg/kg and 400 mg/kg and standard drug treated group, increased significantly (P< 0.01) by 23.10 %, 6.29%, 9.20%, 2.34% where as the body weight of diabetic control group decreased by 33.04 % table.4.

Table-2

Effect of MEAS on blood glucose in glucose fed hyperglycemic rats

Treatment (mg/kg body weight)	Blood Glucose Levels (mg/dl)			
	Initial	60 min	120 min	180 min
Normal control	96.84 ± 1.66	135.26 ± 2.81	116.32 ± 1.32	98.22 ± 1.24
Diabetic control	292.28 ± 5.61	428.62 ± 9.78	369.28 ± 6.29	298.62 ± 3.98
Diabetic+Glibenclamide (10 mg/kg)	118.58 ± 3.21	148.93 ± 3.62**	124.61 ± 3.14**	108.28 ± 2.12**
Diabetic+MEAS (200 mg/kg) p.o.	198.83 ± 2.81	242.81 ± 2.18	216.21 ± 2.02*	197.81 ± 2.28**
Diabetic+MEAS (400 mg/kg) p.o.	189.44±4.56	229.28 ± 2.01	199.89 ± 5.29**	182.68 ± 4.21**

Values are given as mean ± SEM, n=6 significant values are compared with diabetic control *P<0.05, **P<0.01 Diabetic control Vs all groups

Table-3

Anti-diabetic activity of MEAS in STZ induced diabetic rats

Treatment (mg/kg body weight)	Blood Glucose Levels (mg/dl)			
	1st day	7th day	14th day	21st day
Normal control	92.6 ± 1.9	93.2 ± 2.2	92.8 ± 2.3	92.2 ± 1.6
Diabetic control	322.0 ± 6.4	343.3 ± 6.1	381 ± 8.2	404.3 ± 9.1
Diabetic+Glibenclamide (10mg/kg)	316.6 ± 3.9	228.3 ± 4.2**	178 ± 2.8**	118.4 ± 1.6**
Diabetic+methanolic extract (200mg/kg) p.o.	329.2 ± 6.2	245.4 ± 6.6**	192.2 ± 6.1**	151.7 ± 5.8**
Diabetic+methanolic extract (400mg/kg) p.o.	326.4 ± 5.9	237 ± 6.2**	186.3 ± 5.4**	142.9 ± 4.9**

Values are given as mean ± SEM, n=6 when compared with diabetic control *P<0.05, **P<0.01

Table.4:

Changes in body weight in the treatment of MEAS in STZ induced diabetic rats

Treatment (mg/kg body weight)	Changes in body weight (g)		
	Initial	7th day	21st day
Normal control	161.0 ± 0.4	189.0 ± 1.2	198.2 ± 3.1
Diabetic control	164.0 ± 0.2	128.1 ± 1.4	109.8 ± 2.1
Diabetic+Glibenclamide (10mg/kg)	162.0 ± 0.4	143.4 ± 1.3*	158.2 ± 1.9*
Diabetic+methanolic extract (200mg/kg) p.o.	162.0 ± 0.4	148.2 ± 1.4**	151.8 ± 1.5**
Diabetic+methanolic extract (400mg/kg) p.o.	164.0 ± 0.4	161.1 ± 1.9**	179.1 ± 2.4**

Values are given as mean ± SEM, n=6 when compared with diabetic control, *P<0.05 , ** P<0.01

Changes of histopathology of the pancreas

After 21 days treatment period, it was observed that in non diabetic animals showed round and elongated islets were evenly distributed throughout the cytoplasm (Fig.1A). In diabetic control animals, the cells were irregular, not well defined and necrosis of the cells is very clear (Fig.1B).

The standard group showed a mild protection from STZ induced changes in the pancreatic islets.(Fig.1C) Methanolic extract at 200 mg/kg showed no significant changes in the cells were seen when compared with the diabetic control (Fig.1D). Methanolic extract at 400 mg/kg showed slight regeneration of beta cells when compared with the diabetic control. (Fig.1E)

Fig.1: Photomicrographs of histological changes of rat pancreas of islets of langerhans

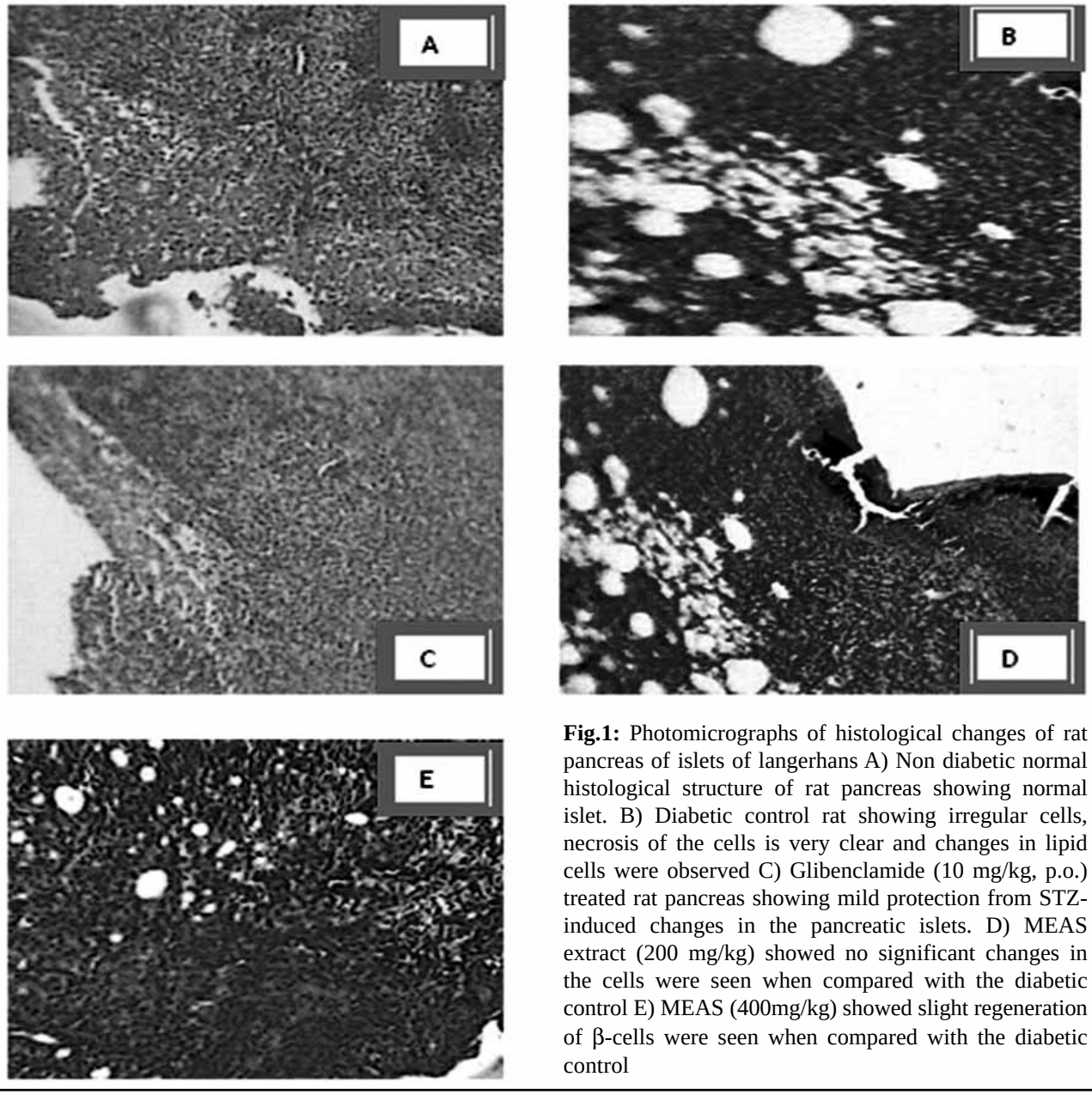

Fig.1: Photomicrographs of histological changes of rat pancreas of islets of langerhans A) Non diabetic normal histological structure of rat pancreas showing normal islet. B) Diabetic control rat showing irregular cells, necrosis of the cells is very clear and changes in lipid cells were observed C) Glibenclamide (10 mg/kg, p.o.) treated rat pancreas showing mild protection from STZ-induced changes in the pancreatic islets. D) MEAS extract (200 mg/kg) showed no significant changes in the cells were seen when compared with the diabetic control E) MEAS (400mg/kg) showed slight regeneration of β-cells were seen when compared with the diabetic control

Lipid profile

When compared to the diabetic control rats, significant ($P<0.01$) reductions of 20.3%, 28.08% CHL (Cholesterol), 37.8%, 43.61% LDL (Low density lipoprotein), 31.84%, 40.12% VLDL (Very low density lipoprotein) and 28.20%, 29.79% TGL(Triglyceride) were found after treatment of methanolic extract of *Amaranthus spinosus* leaves at doses of 200 mg/kg and 400 mg/kg. Also, there was a significant ($P<0.05$) increase of (21.50%, 27.41%) HDL (High density lipoprotein) cholesterol in treated diabetic rats. In case of untreated diabetic rats, there was a fall in HDL level Table.5.

Table-5

Anti-hyperlipidemic effect of MEAS in STZ induced diabetic rats

Treatment (mg/kg body weight)	Changes in mg/dl				
	Total cholesterol	Tri-glycerides	S.HDL	S.LDL	S.VLDL
Normal control	92.5 ± 6.8	88.6 ± 7.1	29.8 ± 2.9	39.4 ± 4.1	14.3 ± 1.9
Diabetic control	211.5 ± 9.9	169.8 ± 4.9	18.6 ± 0.8	64.2 ± 6.3	31.4 ± 1.2
Diabetic+Glibenclamide (10mg/kg)	143.5 ± 8.6**	107.6 ± 1.9*	24.7 ± 2.2*	32.8 ±3.8**	17.4 ± 2.0
Diabetic+Methanolic extract (200mg/kg) p.o.	168.4 ± 8.9*	121.9 ± 4.1	22.6 ± 1.9	39.9 ± 4.4*	21.4 ± 2.2
Diabetic+Methanolic extract (400mg/kg) p.o.	152.1 ± 7.4	119.2 ± 2.9	23.7 ± 1.9	36.2 ± 4.1**	18.8 ± 1.9**

S.HDL- Serum high density lipoproteins, S.LDL- Serum low density lipoproteins, S.VLDL- Serum very low density lipoproteins. Values are given as mean ± SEM, n=6 when compared with diabetic control, *P<0.05 , ** P<0.01

Discussion and Conclusion

The present manuscript discusses about the antidiabetic and hypolipidemic effects of the methanolic leaf extract of *Amaranthus spinosus* on normal and STZ-induced diabetic rats. Acute toxicity studies revealed the non-toxic nature of the methanolic leaf extract of *Amaranthus spinosus*. There was no lethality or any toxic reactions found with the selected dose until the end of the study period. The basal food intake of normal group rats were found to be 14.3 ± 0.2 g/rat/day, whereas the food intakes were significantly (18.4 ± 0.2) increased in the diabetic group of rats (compared with normal), but no change in food intake was observed (14.5 ± 0.2) in the standard and samples of treated rats. From the above result, we can confirm that the methanolic extract of leaves of *Amaranthus spinosus* at doses of 200 mg/kg and 400 mg/kg possesses significant antidiabetic activity on long-term (21 day) treatment in rats. The sample drug showed optimum activity at 400 mg/kg.

The results of the study have shown a significant (P<0.01) difference between the initial and final fasting plasma glucose levels of methanolic leaf extract of *Amaranthus spinosus* and Glibenclamide treated groups. STZ, slightly cytotoxic agent of pancreatic β-cells [14], induces diabetes by damaging the cells that causes reduction in insulin release. It is reported that treatment of diabetic animals with medicinal plant extracts resulted in activation of β-cells and granulation return to normal, showing an insulinogenic effect [15]. The possible mechanism through which MEAS extract exerts antidiabetic effect might have been due to the increased release of insulin from regenerated β-cells. In diabetes, hyperglycemia is accompanied with dyslipidemia [16] i.e., characterised by increase in TC, LDL, VLDL, TG and fall in HDL. This altered serum lipid profile was reversed towards normal after treatment with methanolic extract of *Amaranthus spinosus.* The hypothesis is further supported by the pancreatic histology which showed protection of pancreatic β-cells from toxic effects of STZ and focal necrosis was observed in the diabetic rat pancreas but was less obvious in treated groups.

On the basis of the current investigation, it was noted that the methanolic extract of *Amaranthus spinosus* acted in a similar fashion to the Glibenclamide (standard drug) in reducing the elevated blood glucose level and lipid profile of STZ induced diabetic rats and it can be suggested that these results provide pharmacological evidence as an antidiabetic and hypolipidemic agent. Further studies to isolate, identify and characterize the active principle(s) are in the progress.

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Evaluation Of Analgesic Activity Of *Jasminum Grandiflorum* Linn Leaf Extracts

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ABSTRACT

The extracts of leaf of *Jasminum grandiflorum* L. (Family Oleaceae) were investigated for analgesic activity in albino rat and albino mice using formalin test and hot plate method. The dried power of leaf, extracted with different solvents according to their polarity such as petroleum ether, ethanol and water. All the extracts given at a dose of 100 and 200 mg/kg *b.w* through orally. Indomethacin and pentazotocin used as a standard drug. Extracts shows dose dependent analgesic activity. Water extract of *Jasminum grandiflorum* leaf at a dose of 200 mg/kg produce highest activity is both the models.

Key words: *Jasminum grandiflorum*, analgesic, formalin test, hot plate, indomethacin, pentazotocin.

Introduction

Jasminum grandiflorum Linn (Oleaceae) commonly known as chameli or yasmin with fragrant flower. Plant is available tropical, plain and hilly places like India, France, Italy, China, Japan, India, Morocco and Egypt [1, 2]. Traditionally different parts of the plant possess a lot of activities like odontalgic, thermogenic, aphrodisiac, antiseptic, emollient, anthelmintic, deobstruant, suppurative, tonic, in fixing loose teeth, ulcerative stomatitis, leprosy, skin diseases, ottorrhoea, otalgia, wounds, corns and aromatherapy [3, 4]. Spasmolytic, antiinflammatory, antimicrobial, antioxidant, antiulcer, cytoprotective, chemo preventive, wound healing and antiacne activities of plant has been reported [5-11]. Different phytochemicals like sambacein I-III, 200-epifraxamoside, demthyl-200-epifraxamoside, jasminanhydride, indole oxgyenase, kaempterol-3-O-α-L-rhamnopyranosyl(1-3) α-L-rhamnopyranosyl (1-6) β-D-galactopyranosyl, 7-ketologanin, kaempferol-3-O- rutinoside, oleoside-11-methyl ester, 7-glucosyl-11-methyl ester, ligstroside and oleuropein are isolated from the leaves so far [12].

In modern era drugs which are in use presently available for the management of pain are either narcotics analgesics (eg: opioids), NSAIDs (eg. salicylates) and corticosteroids (eg. hydrocortisone). But theses synthetic drug are expensive and also possess serious side and toxic effects. Attention is being focused on the investigation of drugs from plant origin which are used traditionally. Plant based drugs are cheap and safer than the synthetic drugs and still more than 80% of the world population believe in herbal medicines [13, 14].

The lack of potent analgesic drugs with fewer side effects in use prompted the present study. In the present study, our aim was to evaluate the analgesic potential of the different extracts of the leaves of *Jasminum grandiflorum* L.

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Materials and Methods

Plant material

Jasminum grandiflorum Linn leaves were collected from Bengalore and authenticated by Regional Research Institute (Ay.), Bangalore (no.2008-09/318). A voucher specimen was deposited in the herbarium of Department of Pharmacognosy, The Oxford College of Pharmacy, Bangalore.

Extraction procedure

Leaves of the *Jasminum grandiflorum* (470 g) were dried in shed, coarsely powdered and subjected to successive solvent extraction by continuous hot extraction (soxhlet). The extraction was done with different solvents in their

increasing order of polarity such as petroleum ether (60-80°C), ethanol and water. Each time the marc was air dried and later extracted with other solvents. All the extracts were concentrated by distilling the solvent in a rotary flash evaporator. The yield was found to be 2.36, 4.67 and 9.26% w/w with reference to the air dried plant. The dried extracts were dissolved in dimethyl sulphoxide (DMSO) and subjected to analgesic activity.

Preliminary phytochemical screening

The coarse powder of *Jasminum grandiflorum* leaves (50g) was extracted by successive extraction method using different solvents i.e. petroleum ether, ethanol and water in their increasing order of polarity. The extracts were concentrated and used for various chemical tests to detect the presence of different phytoconstituents [15].

Animals

Adult Wister rats of either sex, weighing between 100-200 gm and albino mice of either sex (20-25 gm) were used for the study. All the animals were housed in animal house of the institution in polypropylene cages maintained under standard conditions (12 hourlight/12 hour dark cycle; 25±2°C, 35-60% humidity). The animals were fed with standard food pellets (Hindustan Lever Ltd, India) and water *ad libitum*. Prior permission from the Institutional Animal Ethical Committee was obtained as per the prescribed guidelines.

Analgesic Activity

Formalin test

Formalin test was conducted as described by Dharmasiri et al. [16]. Rats were divided into 6 groups each contain 6 animals. Group I (control) treated with 2 ml/kg normal saline, group II as serves as standard and treated with 10 mg/kg indomethacin. Group III, IV were treated with petroleum ether extract of *J. grandiflorum* leaf (PEEJG) (200 and 400 mg/kg), ethanol extract of *J. grandiflorum* leaf (EEJG) administered to group V, VI at a dose of 200 and 400 mg/kg and water extract of *J. grandiflorum* leaf (WEJG) (200 and 400 mg/kg) given to group VII and VIII respectively. Extracts and standard drug were given orally. Thirty minutes later, the rats were injected with 0.05 ml of 2.5% formalin into the right hand foot paw and were immediately placed in a transparent plastic cage separately, the licking time and frequency of the injected paw were recorded for 30 min. (Table-1. Fig.1)

Table-1

Analgesic effect of different extract of *Jasminum grandiflorum* leaves on formalin induced paw licking in rat

Treatment	Duration (sec)	Frequncy/ 30 min
Control	15.12±2.31	25.33±3.08
Indomethacin(10 mg/kg)	5.93±1.01**	14.25±2.59*
PEEJG (100 mg/kg)	10.54±2.61	21.71±3.11
PEEJG (200 mg/kg)	8.09±2.11*	18.79±2.79*
EEJG (100 mg/kg)	8.11±2.10*	16.06±1.80*
EEJG (200 mg/kg	6.64±1.37**	14.74±1.32*
WEJG (100 mg/kg)	7.73±1.14*	15.72±1.19*
WEJG (200 mg/kg)	6.22±1.31**	14.41±1.17*

All values are expressed as mean ± S.E.M.; (n=6) animals in each group $^{*}P < 0.05$, $^{**}P < 0.01$, when drug treated group were compared with control.

Hot Plate Method

The analgesic activity was assessed by Hot plate method. Albino mice (20-25 g) were selected, weighed and divided into six groups, each having six animals. Animals were fasted 18 hrs prior to commencement of experiment but water was provided *ad libitum.* Normal saline (2 ml/kg) given orally to the control group (group I), pentazocin (10 mg/kg, i.p) given to the standard group (group II). PEEJG (200 and 400 mg/kg), EEJG (200 and 400 mg/kg) and WEJG (200 and 400 mg/kg) given orally to the groups III-VIII respectively. The responses are in the form of jumping, paw withdrawal or paw licking when animals are placed on Eddy's hot plate maintained at a constant

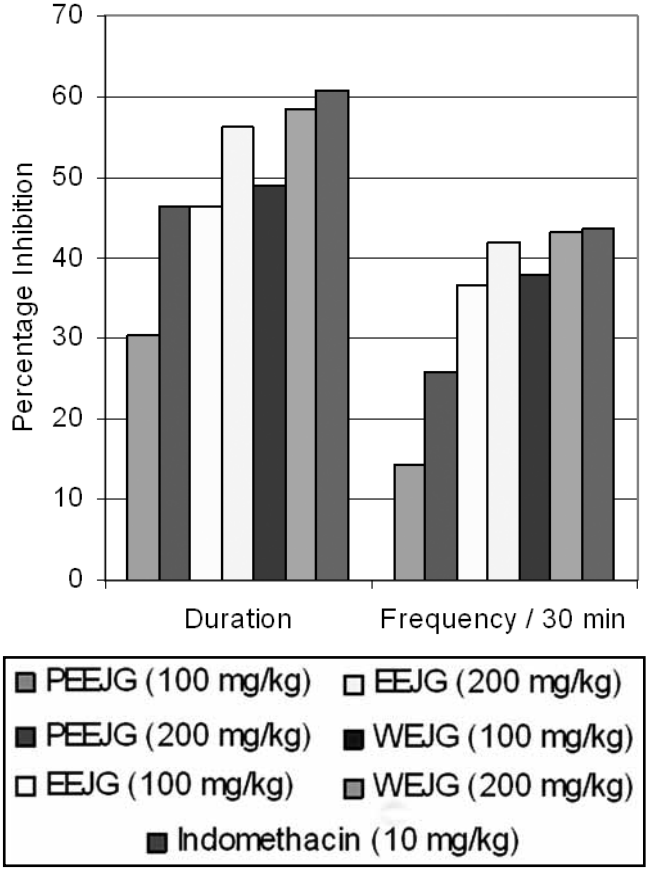

Fig.1: Effect of *Jasminum grandiflorum* leaf extracts against formalin induced paw licking

temperature of 55°C. Basal reaction time taken before the drug administered. To avoid injury to the paws, cut off time of 15 sec was taken as maximum analgesic response. Reaction time in seconds was recorded after 30 min, 60 min, 90 min and 120 min following the treatment [17]. (Table-2)

Statistical analysis

Values are expressed as mean ± standard error mean (S.E.M) and analyzed using statistical package for social science (SPSS) version 10.0 using ANOVA followed by Dunnett's test, $P< 0.05$ were considered statistically significant.

Results and Discussion

In this present study analgesic activity of different extracts of *Jasminum grandiflorum* leaves investigated using formalin test method and hot plate method. Results of analgesic activity by formalin method using rats are given in Table no 1 and Figure. 1. Water extract of *Jasminum grandiflorum* leaves producing highest activity at a dose of 200 mg/kg. Pre-administration of the extract significantly ($P<0.01$) reduces both duration and frequency of licking after formaline injected to paw when compare to control. Ethanolic extract (200 mg/kg) of plant leaves produces similar effect to that of WEJG. WEJG (200 mg/kg) and EEJG (200 mg/kg) produces 58.46% and 56.08% inhibition in duration in response and 43.11% and 41.80% inhibition in frequency/30 min against formalin induction respectively, whereas indomethacin produces 60.78% and 43.74% inhibition in duration and inhibition in frequency/30 min. Analgesic activity of both the extract is similar to that of standard drug indomethacin (10 m/kg). Both the extract at a dose of 100 mg/kg also produce significant ($P<0.05$) analgesic effect. But PEEJG at a dose of 100 mg/kg produce insignificant effect though at dose of 200 mg/kg it produces significant effect. PEEJG (200 mg/kg) produces 46.49% and 25.81% inhibition in duration and inhibition in frequency/30 min respectively, but it is less then the effect of other two fractions.

Formaline test is one of the most valid analgesic models which can be better correlated with clinical pain. In the early phase of formalin test pain occur due to the direct stimulation of the sensory nerve fibres by formalin while in the late phase pain was due to inflammatory mediators, like histamine, prostaglandins, serotonin and bradykinins [18, 19, 20]. This study showed that, the extracts produced a dose-dependent decrease in licking time and licking frequency by the rats when injected with formalin signifying the analgesic effect of the extracts.

Extracts also produces dose dependent analgesic activity against thermal model of nociception in mice. The results of the activity on mice by hot plate method are presented in Table no 2. WEJG (200 mg/kg) produces highest activity in after 60 min. EEJG (200 mg/kg) also produce similar activity. Extracts produces significant analgesic activity after 30, 60, 90 and 120 min. PEEJG produces lesser activity then WEJG and EEJG. All the extract produces dose dependent activity as they increases latency period in hot plate method.

Hot plate method usually employed to investigate those compounds which may act through opiod receptor [21]. Analgesic activity against hot plate method suggesting that extracts may also have central analgesic activity.

Preliminary phytochemical screening showed the presence of alkaloids, tannins, saponin, flavonoids in ethanol extract; aqueous extract contain alkaloids, tannins and flavonoid glycosides. Therefore, presence of different constituents may be responsible for analgesic activity of *Jasminum grandiflorum* leaves.

Table-2
Analgesic effect of different extract of *Jasminum grandiflorum* leaves on hot plate method

Treatment	Reaction time in second at time (min)				
	0	30	60	90	120
Control	4.11±0.5	4.20±0.6	4.44±0.5	4.68±0.6	4.56±0.6
Pentazocine (10 mg/kg)	3.98±0.3	4.91±0.9*	8.23±1.1**	8.03±1.2**	7.89±1.1**
PEEJG (100 mg/kg)	4.27±0.8	4.32±1.0	6.01±0.7	5.71±0.6*	5.66±0.7*
PEEJG (200 mg/kg)	4.22±0.3	4.54±0.7*	6.87±0.9*	6.79±0.8*	6.48±0.8*
EEJG (100 mg/kg)	4.08±0.8	4.46±0.8*	6.96±0.9*	6.71±0.7*	6.58±0.8*
EEJG (200 mg/kg	3.89±0.6	4.64±1.1*	7.90±1.1**	7.95±1.2**	7.56±1.0**
WEJG (100 mg/kg)	4.13±0.9	4.54±0.8*	7.12±1.0*	7.18±0.9*	6.72±0.9*
WEJG (200 mg/kg)	3.91±0.7	4.68±0.7*	8.18±1.3**	8.05±1.4**	8.01±1.1**

All values are expressed as mean ± S.E.M.; (n=6) animals in each group $^{*}P < 0.05$, $^{**}P < 0.01$, when drug treated group were compared with control.

In conclusion, the results of present study suggest that water and ethanolic extract of the *Jasminum grandiflorum* leaves possess analgesic activity though further work need to isolate the active compound and to investigate proper mechanism of action.

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Hypoglycemic And Anti Diabetic Effect Of *Alternanthera Sessilis* In Normal And Streptozotocin Induced Diabetic Rat

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ABSTRACT

Various herbs have been found beneficial in the management of NIDDM and are gaining considerable recognition in the management of NIDDM worldwide. The present study was planned to search, standardize, and evaluate the efficacy of indigenous herbal ingredient *Alternanthera sessilis* which is used in some areas for the treatment of Diabetes mellitus and whose hypoglycemic effects have not yet been scientifically studied. The ethanolic extract of whole plant of *Alternanther sessilis* was subjected for hypoglycemic and anti diabetic activity on both normal and streptozotocin induced rats. The result shows 34 % reduction in blood sugar levels in normal rats and the water extract showed 28.9 % reduction in blood sugar levels in normal rats. The alcoholic extract of Alternanther *sessilis* reduced blood sugar levels in streptozotocin induced diabetic rats, from 323.35 to 111.05 (8hr) and the water extract of *Alternanther sessilis* reduced blood sugar levels in streptozotocin induced diabetic rats, from 365.92 to 123.96(8hr). The results indicate that this plant has interesting possibilities as source of oral hypoglycemic agent.

Keywords: *Alternanthera sessilis*, Hypoglycemic activity, Anti diabetic activity, Amaranthecea.

Introduction

The prevalence of Diabetes Mellitus is increasing globally day by day. The past two decades have seen an explosive global increase in the number of people diagnosed as non-insulin dependent diabetics. In India it is estimated that 19.4 million individuals are affected by non-insulin dependent diabetes mellitus, which is likely to go up to 57.2 million by the year 2025. It was observed that in urban India, prevalence of diabetes has risen to 12.1 percent and there was an equally large pool of individuals with Impaired Glucose Tolerance (IGT), many of them will eventually develop NIDDM in the coming future. [1]

Alternathera sessilis is a species belongs to the plant family Amarantaceae very widespread throughout the tropics and subtropics: and occurs throughout the hotter parts of India, ascending to an altitude of 1200 m in the Himalayas, cultivated as a pot herb. It is a prostrate herb much branched and rooting at the lower nodes. Leaves simple, opposite, fleshy, lanceolate, oblanceolate or linear oblong, sub acute. Flowers are small, white, shining, in small axillary sessile clusters. Fruits compressed, obcordate utricles with suborbicular seeds. The leaves are widely used as vegetable. Majorly the whole plant is used for medicinal purpose. The Wealth of India says young shoots are nutritious and contain protein 5% and iron 16.7 mg\100 gms. The young shoots are nutrious and contain carotenoids, triterpene [2],saponins[3],flavonoids, steroids, stigmasterol, â-sitosterol [4].It is accredited with galactogouge properties and antibacterial properties [5] useful in night blindness due to its high carotene content. Siddha literature mentioned *Alternanthera sessilis* as Kaya Kalpa drug (i.e. drug which prevents and cures chronic diseases and rejuvenates the body) and as compatible diet. The antioxidant carotene is found in large amounts in *Alternanthera sessilis*.[6,7] It is used for the treatment of biliousness, dyspepsia, sluggish liver in Sri Lanka [8]. The plant is also used traditionally as cooling, digestive, intellect promoting, in burning sensation, liver disorders, skin diseases, as antipyretic [9] and in children for overall development. Ayurveda and Siddha medical systems consider *Alternanthera sessilis* as Rasayana drug.

**Address for correspondence*

Methodology

The whole plant of *Alternanthera sessilis* was collected from local areas of Mahaboobnagar district of Andhra Pradesh, India and the same was authenticated by Pharmacognosy Department of J & J Dechane Laboratories Pvt. Ltd., Hyderabad, India. The plants were shade dried and ground to get coarse powder of 40-mesh size and extracted with ethanol in soxhlet extractor. The extract was concentrated under reduced pressure and it was dissolved in a minimum amount of ethanol and adsorbed on already extracted plant material. The material air-dried successively extracted in a soxhlet extractor with ethanol and water. After each extraction the solvents were evaporated and concentrated under reduced pressure. The different extracts so obtained were subjected to qualitative and quantitative chemical analysis and subjected to pharmacological studies.

Hypoglycemic activity of various extracts on rats

Dose of extracts was fixed 200-mg/kg concentrations considering the results of the toxicity and behavioral study as suitable for hypoglycemic activity. The male Albino wistar rats weighing between 100-150 g were divided into three groups each group having 6 animals. These were housed under standard environmental conditions. Prior to experiment carried out, the animals were fasted for 18 h and water *ad libitum.* First group of animals received average 0.2 ml volume of Tween-20 and other groups of animals received 200 mg/kg body weight of extract and 90 µg/kg body weight Glibenclamide mixed with Tween-20 by single oral administration. And 0 h blood samples were collected from tail vein before drug administration to estimate fasting blood glucose. Blood samples were collected at 0.5, 1, 2, 4, 8, 12, 18 and 24^{th} h after administration of extract, Glibenclamide, solvent and glucose is estimated.

Antidiabetic Activity

Experimental induction of Diabetes

The animals were kept fasting overnight and Diabetes was induced by a single intraperitonial injection of freshly prepared solution of streptozotocin (STZ) (70 mg/kg body weight) in 0.1 M citrate buffer (pH 4.5).[10] In order to avoid the streptozotocin induced hypoglycemic mortality, 5% glucose solution was given for 24 h to streptozotocin treated animals. The control rats received the control vehicle in amounts equivalent to the drug treated group. A rest period 2 days was allowed for the blood glucose levels to stabilize. During this period the animals used to have free access to both food and water. The animals having blood glucose values above 250 mg/dl on the 3^{rd} day after STZ injection were considered as Diabetic. The various extracts were given on the 4^{th} day after STZ injection and it was considered as first day of treatment.

The blood glucose lowering activity of alcoholic and aqueous extracts were determined in STZ – induced Diabetic rats, after oral administration at the dose of 200 mg/kg body weight in comparison to glibenclamide and control. The blood samples were collected from the tail vein of rats before and also at 0.5, 1, 2, 4, 8, 12, 18 and 24^{th} h after drug administration and the samples were analyzed for blood glucose by using glucose-oxidase/peroxidedase method.

Experimental Design

The rats were divided into three groups comprising of six animals each group as follows:

Group I: Diabetic controls + Tween-20.

Group II: Diabetic rats given 200-mg/kg body weight of extract orally.

Group III: Diabetic rats given 90µg/kg body weight of Glibenclamide orally.

Results

Results of various phytochemical parameters of the plant summarized in table-1 and the quantitative data in table-2.The results indicate the presence of active constituents in the solvents extracted from medicinal plants material. The water-soluble ash value, acid insoluble ash value, the total ash value were reported in table-2. Ash values measures the residue of the extraneous matter (e.g. Sand and soil) adhering to the plant surface. The acid insoluble ash value measures the amount of silica present especially as sand and siliceous earth. Their results were given in NMT limit as quality control parameters of crude drugs. The hypoglycemic activity results of the alcoholic extract of *A. sessilis* shown in Fig. 1. and the results of the water extract of *A. sessilis* in Fig 2.

Table-1

Studies on Phytochemical constituents

Phytoconstituent	*Alternanthera sessilis* (Water extract)	*Alternanthera sessilis* (Ethanol extract)
Alkaloids	_	-
Flavonoids	+	+
Saponins	+	+
Steroids	+	+
Tannins	-	-
Coumarins	-	-
Phenols	-	-
Glycosides	+	+
Proteins	+	+
Triterpenoids	+	+
Amino acids	+	+
Carbohdrates	+	+

Table-2

Studies on quantitative parameters of selected parameters

Parameters	*Alternanthera sessilis* (Water Extract)	*Alternanthera sessilis* (Alcohol Extract)
Total ash (NMT)	5%	5%
Water soluble ash (NMT)	0.7%	0.8%
Acid insoluble ash (NMT)	0.4%	0.6%
Water soluble extractive (NLT)	35%	20%
Ethaol soluble extractive (NLT)	18%	26 %

The study on STZ induced diabetic rats of alcoholic extract of *Alternanthera sessilis* is shown in Fig. 3 Similarly, of the water extract of *A. sessilis* in Fig. 4.

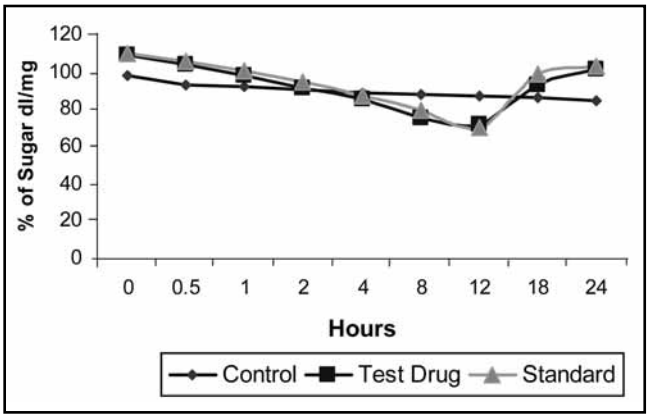

Fig.1: Hypoglycemic Effect of Alcohol Extract of Alternanther sessilis

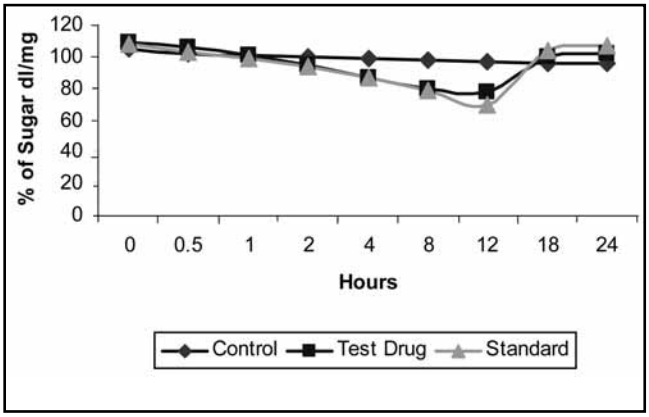

Fig.2: Hypoglycemic Effect of Water Extract of Alternanther sessilis

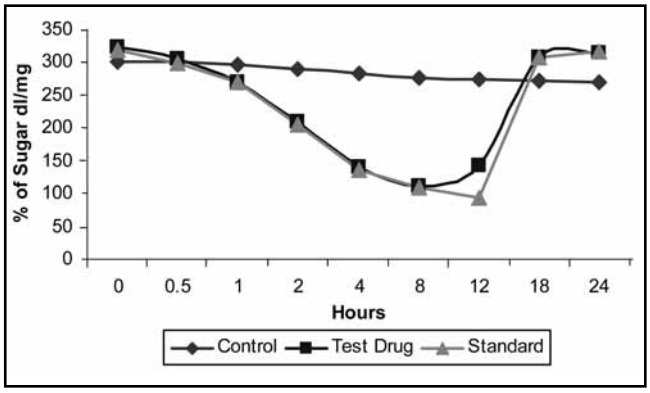

Fig.3: Anti Diabetic Effect of Alcohol Extract of Alternanther sessilis

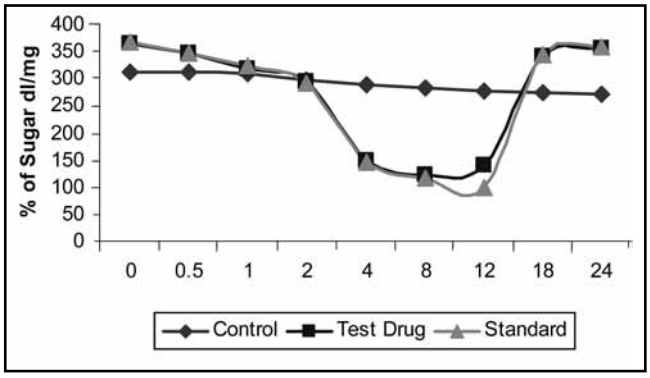

Fig.4: Anti Diabetic Effect of Water Extract of Alternanther sessilis

Discussion

The data in table-1 reveal the results of the preliminary identification tests for the phytoconstituents of ethanolic and aqueous extracts of the plants. Alcoholic and water extracts of *Alternanthera sessilis* on silica gel G plate in different mobile phases showed different bands at various Rf values indicating the presence of different chemical constituents like glycosides, triterpenes, saponins, protein and amino acids ,which may serve as responsible constituents for the management of diabetes. In the present study the alcoholic and aqueous extracts of the plant were used for screening of Antidiabetic activity in rats. The role of flavonoids, polyphenols, triterpenoids, saponins and glycosides as Antidiabetic agents was well established. [11-13]

The pure alcoholic and aqueous extracts of *Alternanthera sessils* were used for screening Antidiabetic and hypoglycemic activities in rats. The hypoglycemic activity results of the extracts revealed that, the alcoholic extract of *A. sessilis* reduced sugar levels from 109.32 to 71.67 (12 h) and the significant effect of the extract started from 4 h and the activity was increased further and sustained till 12 h . The extract shown significant hypoglycemic activity in comparison to control and standard.

The water extract of *A. sessilis* reduced the sugar levels from 108.70 to 77.30 (12 h), and the significant effect of the extract started from 4 h and the activity was increased and sustained till 12 h. the extract shown significant hypoglycemic activity in comparison to control and standard.

The statistical analysis revealed that all the values were significant at 5% significance level ($P<0.05$). The results further indicate the efficacy of alcoholic and aqueous extracts of the plant. The study on STZ induced diabetic rats showed that the alcoholic extract of *Alternanthera sessilis* reduced the blood glucose levels from 323.35 to 111.05 (8 h), significant effect of the extract started from 4 h and effect was increased and sustained till 8 h. Similarly, the water extract of *A. sessilis* reduced the blood glucose levels from 365.92 to 123.96 (8 h), and this significant effect of the extract was started from 4 h and the effect was increased and sustained till 8h. The extracts shown significant hypoglycemic activity in comparison to control and standard.

Conclusion

The study clearly demonstrated that the alcoholic and aqueous extracts of *Alternanthera sessilis* showed significant reduction in blood glucose levels of STZ-induced diabetic rats and the activity of both the extracts was quite significant and encouraging. The Antidiabetic activity of *A. sessilis* can be attributed to the presence triterpenoids, phytosterols and glycosides. The results also further revealed that the aqueous extract is slightly less effective when compared with alcoholic extract.

Acknowledgments

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Evaluation of Wound Healing Potential of Ethanolic Extracts of Various Parts of *Phyllanthus Simplex* Retz

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ABSTRACT

Wound healing is a complex dynamic process that results in the restoration of anatomic continuity and function. The present study has proved that the wound healing potential of ethanolic extract obtained from roots of *phyllanthus simplex* Retz showed a significant effect than the same extract obtained from other parts of the plant. That extract showed a facilitatory epithelisation and wound contraction than other parts extracts. The animals administered with the root extract showed a high collagen content than other extracts and the control groups. Hexosamine content and ascorbic acid levels showed an increased level of the extract. Ascorbic acid is reported to have scavenging activity and inhibition of lipid peroxidation, which helps to show a better healing effect for the test groups.

Key words: Wound healing, Collagen, Hydroxy proline, Hexose amine, Epithelization.

Introduction

Phyllanthus simplex Retz. is commonly called as "Kaya-an", "Bhuiamali" and "Kayut-bulang". (Fam: Euphorbiaceae). It is a glabrous twining perennial herb, which has a taproot and its branches are compressed. Its flowers are solitary and its leaves are distichous. The siddha and ayurvedhic form of medications text showed that the Hindus used equal parts of the fresh leaves, flowers, fruit and cumin seeds with sugar, made into an electuary for the treatment of gonorrhea by taking a teaspoonful for twice a day. The fresh leaves, bruised and mixed with buttermilk, make a wash to cure itches in children. The root is used in Chota Nagpur as an external application for abscesses [1-3].

Many Indian indigenous plants have a very important role in the process of wound healing. Those plants are more potent healers because they promote the skin repair mechanisms in natural way. Wounds cause the activation of a cytokine cascade that result in the formation of oxygen free radicals to lipid per oxidation. Any drug that shows inhibition of lipid peroxidation is believed to increase the viability of cells by improving circulation, preventing cell damage, promoting DNA synthesis, increase in tensile strength of collagen, increases lysyl oxidase formation, contraction of the wound and showing null effect of necrosis have excellent wound healing activity [4-7].

The present study is undertaken to investigate the wound healing activity of ethanolic extracts obtained from various parts of *phyllanthus simplex* Retz.

Materials and Methods

Collection of Plant material

Fresh whole plant materials of *phyllanthus simplex* Retz had been collected from Chota-Nagpur of Jharkhand and was identified and authenticated by Dr.S.M.Khasim, Asst. Professor, Department of Botany, Acharya Nagarjuna University in Guntur.One of the plant specimen had been planted in KVSR Siddhartha College of Pharmaceutical Sciences Vijayawada and a voucher (No: PS/PCRL/No: 0041/ BN) had been deposited after planted in the herbal garden.

Preparation of Ethanolic extract

The various parts of the plant material such as leaves including flowering tops, stem part closed with barks and roots had been separated and shade-dried. About 1kg of the milled powder of powdered parts was extracted successively with 95% ethanol (60-70°C) by Soxhlet apparatus and was concentrated under reduced pressure to semisolid consistency. Their yield and amount used for evaluation are given in table-1. A preliminary phytoscreening was also done to identify the presence of various phytoconstituents by using standard tests.

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Preparation of Ointment Base

About 35% of Bees wax was melted in a china dish using a water bath at a temperature of 60-80°C. Hot paraffin oil (5%) was also boiled in a water bath at the same temperature. Then transferred into the china dish with constant stirring using a glass rod. Finally 60% of petroleum gel was added and boiled for 15 minutes with constant stirring until the ointment base was cooled and semisolid in nature.

Experimental work

The experiment protocol was duly approved by Institutional Ethical Committee. Adult wistar rats of either sex (200-250gms) were procured from King's Institute of Technology and Vaccination (Regd.No.993/a/06/CPCSEA). They were maintained for 12hrs light / dark cycle at an ambient temperature and fed with chow pellets (Hindustan lever) and water ad libitum. The animals were grouped into 6 batches with 4 animals per group.

Group I Wound control (WC)

Group II Wound + ointment prepared from Flowering tops and leaves extract (WFTLE)

Group III Wound + ointment prepared from Stem bark extract (WSBE)

Group IV Wound + ointment prepared from Root extract (WRE)

Group V Wound + ointment prepared from Standard drug –Soframycin (WSDS)

Wound Creation

A steel rod of diameter 2.5cm was heated to 80-85°C and burn wounds were created on the dorsal side of the rat. After 24h, dead tissues were excised using surgical blade and the wound was sterilized with surgical spirit. Control rats were dressed with paraffin and gauze. The tested groups were treated with formulation. 1gm of each formulation was used to treat the wounds. A similar quantity of soframycin was used to treat the Group-V animals. All the rats were kept under an observation period of 20 days. The rate of wound contractions was also monitored by measuring the wound areas for every 5days until the healing of the wound. The contraction was studied by tracing the raw wound area on a tracing sheet and measured. Its diameter was recorded.

Collection of Granulation tissue

Granulation tissues were collected from both control and drug treated rats and washed with cold saline (0.9%w/v NaCl) to remove blood tissues and stored for analyzing various parameters such as collagen and hexosamine after lyophilized.

Blood sample

The blood samples were collected by decapitation in the cervical region using a sterile syringe rinsed with EDTA. Then the plasma was separated from blood by centrifugation for 15 minutes at 1500rpm. The plasma was subjected for estimation of malondialdehyde and ascorbic acid.

Evaluation of Biochemical Parameters

1. Hydroxy Proline [8]

Samples of varying concentrations were taken for analysis. Hydroxy proline was oxidized by adding 1ml of chloramine T to each tube. The contents were mixed thoroughly by shaking and allowed to stand for 20minutes at room temperature. Then 1ml of 70% perchloric acid was added to each tube for destruction of chloramines T. The contents were mixed and allowed to stand for 5minutes. Finally 1ml of PDAB (Para dimethyl amino benzaldehyde) solution was added and the mixture was shaken well. The colour developed was read by UV spectrophotometer (JASCO-Japan) at an absorbance of 530nm. The collagen content was then calculated by multiplying the hydroxy proline by the factor 7.46 and was expressed as mg/100mg of dry weight of the sample.

2. Hexose amine [9]

Samples of various extracts were taken for analysis. The solutions were treated with 1ml of freshly prepared 2% acetylacetone in 0.5M Na_2CO_3 in capped tubes and kept in boiling water bath for 15 minutes. After cooling in tap water, 5ml of 95% ethanol and 1ml of Ehrlich's reagent were added and mixed thoroughly. The purple red colour formed was read by UV spectrophotometer (JASCO-Japan) at 530nm after 30 minutes.

3. Malondialdehyde[10]

To 0.1ml of the supernatant liquid 0.9ml of 10% TCA (Trichloroacetic acid) and 2.0ml of 67% thiobarbituric acid reagent were added and kept in boiling water bath for 20minutes. The tube was cooled after centrifugation and the mixture was measured at 532nm by UV spectrophotometer.

4. Ascorbic acid [11]

To 0.5ml of plasma, 0.5ml of ice cold 10% TCA was added and mixed thoroughly and centrifuged for 20 minutes. About 0.5ml of the supernatant was mixed with 0.1ml of DTC reagent, mixed well and incubated at 37°C for 3hours. Then 0.75ml of ice-cold 65% conc. H_2SO_4 was allowed to stand at a room temperature for 30minutes. The yellow colour was read at 520nm by using UV spectrophotometer. Ascorbic acid was used as a standard.

Statistical Analysis

All the results were expressed as mean ± SEM. $P<0.05$ was considered statistical significant.

Results and Discussions

The wounds treated with plant extracts showed a higher rate of wound contraction and they are significant to soframycin. Also the extracts obtained from roots are showing an increase in the wound contraction. The rate of contraction

of the wounds increased with treatment showed an appreciable decrease in wound size as showed in table-2. The tensile strength of the collagen fibre was found to increase in tensile strength and hence showed an increrase in biochemical parameters showed by the ointment prepared from Root extract treated animals (Group IV) as showed in table-3.

Hexose amine content in group IV animals indicate that the fibroblasts actively biosynthesized, ground substances on which the collegen can be laid on[12]. The cytokine cascade activated after a burn injury with stimulation of phagocytic cells that results in the formation of oxygen free radicals and lipid peroxidation. The control group showed an elevation in the lipid peroxidation levels which indicates the decreased free radical scavenging capacity of the wounded tissues. Lipid peroxidation is oxidative deterioration of PUFA. It leads to cell injury leading to generation of peroxides and lipid peroxides. Ascorbic acid was used as a standard drug for scavenging activcity. In the present study it was found that the ascorbic acid levels were higher in the test group when compared to the control group and hence a decline in the lipid peroxidation.

Finally the present study showed that the wound healing potential of the root extract showed the significant results with the standard drug soframycin. Though many synthetic medications are available for the treatment of wounds these natural sources would serve better in the treatment of wounds at a faster rate.

Table 1

Amount of extract obtained and quantity of extract used for the observation

S.No	Parts Used	Amount yield w/w	Quantity used
1.	Flowering tops & leaves extract	0.425	40%extract
2.	Stem bark extract	0.724	40%extract
3.	Root extract	1.235	40%extract

Table 2

Various groups of animals showing the wound contraction during treatment period

S.No	Days	Group I	Group II	Group III	Group IV	Group V
1	0	4.97±0.004	4.97±0.004	4.97±0.004	4.97±0.004	4.97±0.004
2	5	4.02±0.032	4.17±0.042	3.86±0.014*	3.78±0.019*	3.98±0.012
3	10	3.71±0.023	3.28±0.0042	2.97±0.088*	2. 43±0.79*	2.36±0.0092
4	15	2.98±0.076	2.75±0.032	2.14±0.043*	1.75±0.043*	1.84±0.015
5	20	1.35±0.014	2.14±0.069	1.74±0.014*	0.96±0.065*	1.23±0.069

Values are mean ± S.E.M. (n = 4)
*P<0.05 statistically significant when compared with control group.

Table 3

Biochemical parameters observed from various control, extract treated (3 various extracts) and standard groups

Biochemical Parameters	Group I	Group II	Group III	Group IV	Group V
L-Hydroxy proline	0.126± 0.0048	0.156 ± 0.0013	0.183 ± 0.0045	0.207 ± 0.0017*	0.224 ± 0.0021
Hexosamine	0.0413 ± 0.0017	0.0672 ± 0.0021	0.0972 ± 0.0031	0.1132 ± 0.0078*	0.1242± 0.008
Malondialdehyde	6.429 ± 0.0039	5.321 ± 0.0014	4.45 ± 0.0021	3.321 ± 0.0034*	2.231 ± 0.0056
Ascorbic acid	5.34 ± 0.013	4.2 ± 0.104	5.19 ± 0.104	8.3 ± 0.104*	9.2 ± 0.054

Values are mean ± S.E.M. (n = 4)
*P<0.05 statistically significant when compared with control group.

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