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Examples:

Cole (2000), Steddy et al. (2003), (Kelebeni, 1983), (Bane and Jake, 1992), (Chege, 1998; Cohen, 1987a,b;Tristan, 1993,1995), (Kumasi et al., 2001)

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Charnley AK (1992). Mechanisms of fungal pathogenesis in insects with particular reference to locusts. In: Lomer CJ, Prior C (eds), Pharmaceutical Controls of Locusts and Grasshoppers: Proceedings of an international workshop held at Cotonou, Benin. Oxford: CAB International. pp 181-190.

Jake OO (2002). Pharmaceutical Interactions between *Striga hermonthica* (Del.) Benth. and fluorescent rhizosphere bacteria Of *Zea mays*, L. and *Sorghum bicolor* L. Moench for Striga suicidal germination In *Vigna unguiculata*. PhD dissertation, Tehran University, Iran.

Furmaga EM (1993). Pharmacist management of a hyperlipidemia clinic. Am. J. Hosp. Pharm. 50: 91-95

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Research Articles

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Journal of Pharmacognosy and Phytotherapy

Full Length Research Paper

In silico docking analysis of constituents of *Zingiber officinale* as antidepressant

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Accepted 15 May, 2013

Depression is among the leading causes of disability worldwide and its global burden seems to further increase in the future. In the present study, *in silico* approach was used to assess the use of constituents of *Zingiber officinale* as potential agents that could act as antidepressant agents. Docking studies of Gingerol and Shogoal were carried out using Argus lab 4.0.1. Analysis of the results of the docking software suggested that Gingerol and Shogoal can act as potent antidepressants. For the binding analysis, $5HT_{1A}$ receptor protein was taken for the study as it is considered being a potential target for treatment of depression. The standard drug, imipramine was subjected to docking analysis for comparative study. Further analysis in the wet lab may provide more information regarding antidepressant activity of *Z. officinale*.

Key words: *Zingiber officinale*, Argus lab 4.0.1, docking, depression, $5HT_{1A}$ receptor.

INTRODUCTION

Depression is among the leading causes of disability worldwide and its global burden seems to further increase in the future. The extensive human and economic costs stress the importance of studying various aspects of depression. A number of drugs are available for the treatment of depression, but clinical evaluation of these drugs has shown incidence of relapses, side effects, and drug interactions. This has been the rationale for the development of new antidepressants, which includes herbal drugs (Rajput et al., 2011).

Herbal products are often perceived as safe because they are “natural”. Herbal medicine is a major component in all traditional medicine systems and a common element in Siddha, ayurvedic, homeopathic, naturopathic, traditional Chinese medicine, and Native American medicine. Considerable efforts have been directed towards the development of natural products from various plant sources which have antidepressant activity. The serotonin 5-HT_{1A} receptor (5-HT_{1A}R) is a G-protein coupled receptor widely expressed in the central nervous system (CNS), where it is involved in the modulation of mood, emotion and depression, and of different behavioral responses, including thermoregulation, sleep, feeding, aggression, and anxiety. Of all the serotonin receptor subtypes, 5-HT_{1A}R is the dominant receptor responsible for depression and it is involved in the mechanism of action of several antidepressant drugs (Nievergelt et al., 2010). A number of natural antidepressant compounds have been reported in the medicinal plants and tested for their efficacy in treating depression. Therefore, the present study aims to investigate the antidepressant activities of the compounds from *Zingiber officinale* against the 5-HT_{1A} receptor protein by using Argus lab 4.0.1.

MATERIALS AND METHODS

Preparation of protein structure

The sequence of the $5HT_{1A}$ receptor protein was retrieved from

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protein data-base of National Center for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov/) in FASTA format (a text based format where a single letter code is used to represent amino acid sequences), and it was searched against selection of the related homologues of query sequence in Protein Drug Bank (PDB) (http://www.pdb.org). The homology modelling requires sequences of known three dimension (3D) structure and the target having above 35% of similarity. Basic local alignment search tool (BLASTP) (http://blast.ncbi.nlm.nih.gov/Blast.cgi) was used to identify the most suitable template for homology modelling of $5HT_{1A}$ receptor protein. The available structure of protein in the protein database are:

Top ten hits (PDB structures)

Rank PDB Hit

1. 2bg9B;
2. 2bg9A;
3. 2bg9C;
4. 2bg9C;
5. 2bg9B;
6. 2bg9A;
7. 2bg9C;
8. 2bg9A;
9. 2bg9E;
10. 2bg9A.

The templates and target sequence were aligned using GenThreader Server (Jones, 1999). After careful examination for the potential alignment errors, the automated comparative protein modelling program I-TASSER was employed to build the model (Zhang, 2008). The statistical verification of the model was evaluated with PROCHECK, a structure verification program which relies on Ramachandran plot (Vaseeharan and Valli, 2011), which determines the quality of the predicted structure by assessing various parameters such as lengths, angles and planarity of the peptide bonds, geometry of the hydrogen bonds, and side chain conformations of protein structures as a function of atomic resolution. 3D ligand site server was used to predict the possible binding sites. The amino acid residues LEU453, PHE454, TYR457 were revealed as the binding sites.

Preparation of ligand structures

All the compounds used for docking study (gingerol, shogoal and imipramine) were selected from ChemSketch. Chemically intelligent drawing interface freeware developed by Advanced Chemistry Development, Inc., (http://www.acdlabs.com) was used to construct the structure of the ligands. Using draw mode of Chemsketch, the ligands were generated and the three dimensional optimizations were done and then saved in m ol file (a file format for holding information about the atoms, bonds, connectivity and coordinates of a molecule). Molecular weight, partition coefficient and number of hydrogen bond donors and acceptors for the active principles were noted. Geometry optimizations of the ligands were performed according to the calculation method by Argus Lab 4.0.1 software.

Protein-ligand docking using Argus Lab 4.0.1

$5HT_{1A}$ receptor protein was docked against the four ligands using Argus Lab 4.0.1 (Mark A. Thompson, Planaria Software LLC, Seattle, WA, USA, http://www.arguslab.com) to find the reasonable binding geometries and to explore the protein ligand interactions. Docking of the protein ligand complex was mainly targeted only onto the predicted active site. Docking simulations were performed by selecting "Argus Dock" as the docking engine. The selected residues of the receptor were defined to be a part of the binding site. A spacing of 0.4 Å between the grid points was used and an exhaustive search was performed by enabling “High precision” option in docking precision menu. "Dock" was chosen as the calculation type, "flexible" for the ligand and the A score was used as the scoring function. At maximum, 150 poses were allowed to be analyzed; binding site box size was set to (20 × 20 × 20 A°) so as to encompass the entire active site. The A score function with the parameters read from the AScore.prm file was used to calculate the binding energies of the resulted docked structures. All the compounds in the dataset were docked into the active site of 5HT1A receptor protein, using the same protocol. After completion of docking, the docked protein (protein-ligand complex) was analyzed. The docking poses saved for each compound were ranked according to their dock score function.

RESULTS AND DISCUSSION

Query search result using BLASTP

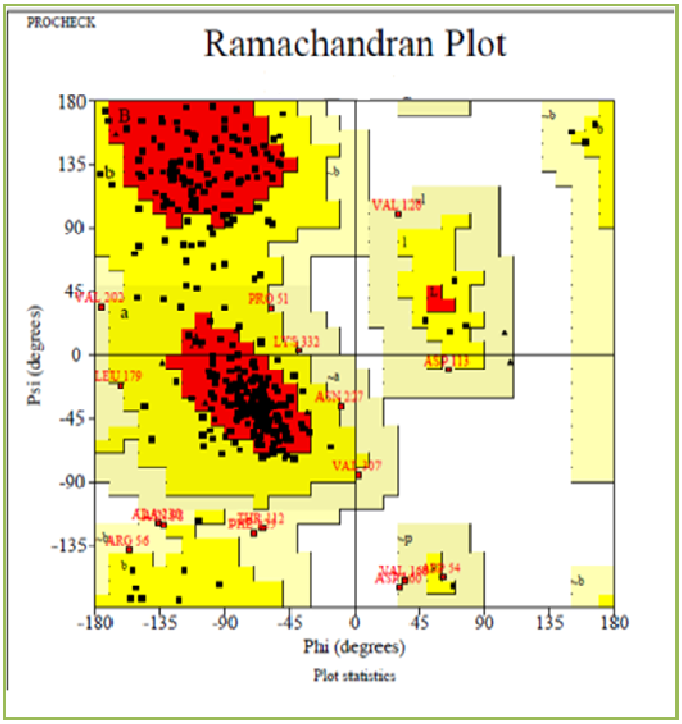
Utilizing BLAST program in the National Center for Biotechnology Information (NCBI), the sequences based on the query ($5HT_{1A}R$) were generated. BLAST program increased the sensitivity of the BLAST search and helped to generate the possible alignment between the $5HT_{1A}R$ query sequences and database sequences. This gave ten corresponding PDB hits and alignments.

Construction of 3-D models

The 3-D models of the templates were constructed using the I-TASSER web server. The target sequences were first threaded through a representative PDB structure library to select the best possible models from PDB, based on the templates and then it does multiple threading alignments and iterative structural assembly simulations.

Verification and validation of the model by PROCHECK, Ramachandran plot

Verification of the built model was done to ensure whether the model was programmed correctly and the algorithms were implemented. Validation results determined that the distribution of amino acid residues were at the most favorable region in the Ramachandran plot. This is an indication of the stereo chemical quality of the model taken for the structural analysis, and also validated the target-ligand binding efficacy of the structure. Ramachandran plot displays the main chain torsion angles phi, psi (φ, Ψ) (Ramachandran angles) in a protein of known structure (Figures 1 and 2). The Ramachandran plot shows the phi-psi torsion angles for all residues in the structure (except those at the chain termini) which were classified according to its regions in



Residues in most favoured regions [A,B,L]	248	74.3%
Residues in additional allowed regions [a,b,l,p]	71	21.3%
Residues in generously allowed regions [~a,~b,~l,~p]	15	4.5%
Residues in disallowed regions	0	0.0%
Number of non-glycine and non-proline residues	334	100.0%
Number of end-residues (excl. Gly and Pro)	2	
Number of glycine residues (shown as triangles)	11	
Number of proline residues	20	
Total number of residues	367	

Figure 1. Ramachandran plot for $5HT_{1A}$ protein generated by PROCHECK.

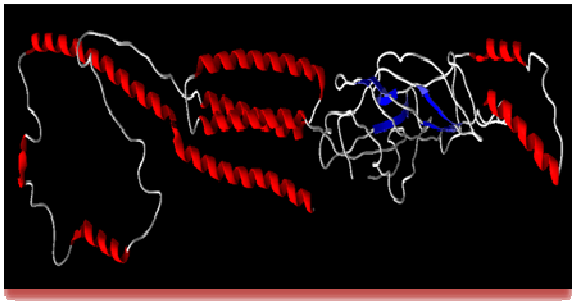


Figure 2. Backbone structure of 5-hydroxytryptamine 1_A receptor.

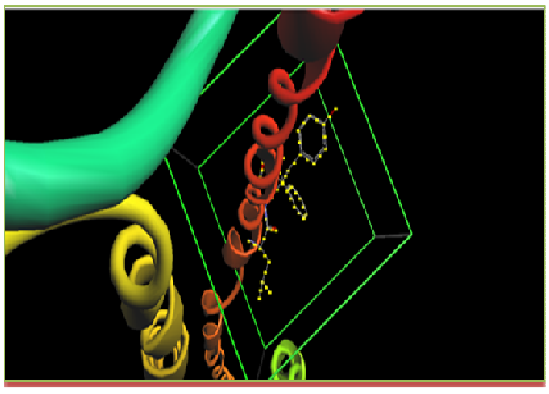


Figure 3. Active binding site of $5HT_{1A}$ protein.

the quadrangle. The red regions in the graph indicate the most allowed regions whereas the yellow regions represent allowed regions. In this protein model, 74.3% of the residues were in the most favored region, 21.3% in allowed region, 4.5% in generously allowed region and 0% of the residues lying in the disallowed regions.

Binding site of the protein

The detection of ligand-binding sites is often the starting point for protein function identification and drug discovery. 3D ligand site server predicted active site of the protein $5HT_{1A}$ (Figure 3) with a higher average precision. The active site of $5HT_{1A}$ comprises of amino acid residues such as LEU 453, PHE 454, and TYR 457.

Docking

To understand the interactions between the ligands and 5HT1A protein and to explore their binding mode, docking study was performed using Argus dock available under ArgusLab 4.0.1. The ligands were created and prepared for the docking procedure using ChemSketch and ArgusLab. The structures of the ligands obtained are shown in Figure 4. All the molecules satisfied the Lipinski's drug properties (Mathew et al., 2010) (Table 1).

Table 1. Lipinski properties of the compounds.

Molecule	Molecular weight	Log P	H-Donor	H-Acceptor	Number of rotatable bonds
Gingerol	294.391	3.217	2	4	10
Shogoal	276.376	4.348	1	3	9
Imipramine	280.415	4.161	0	2	4

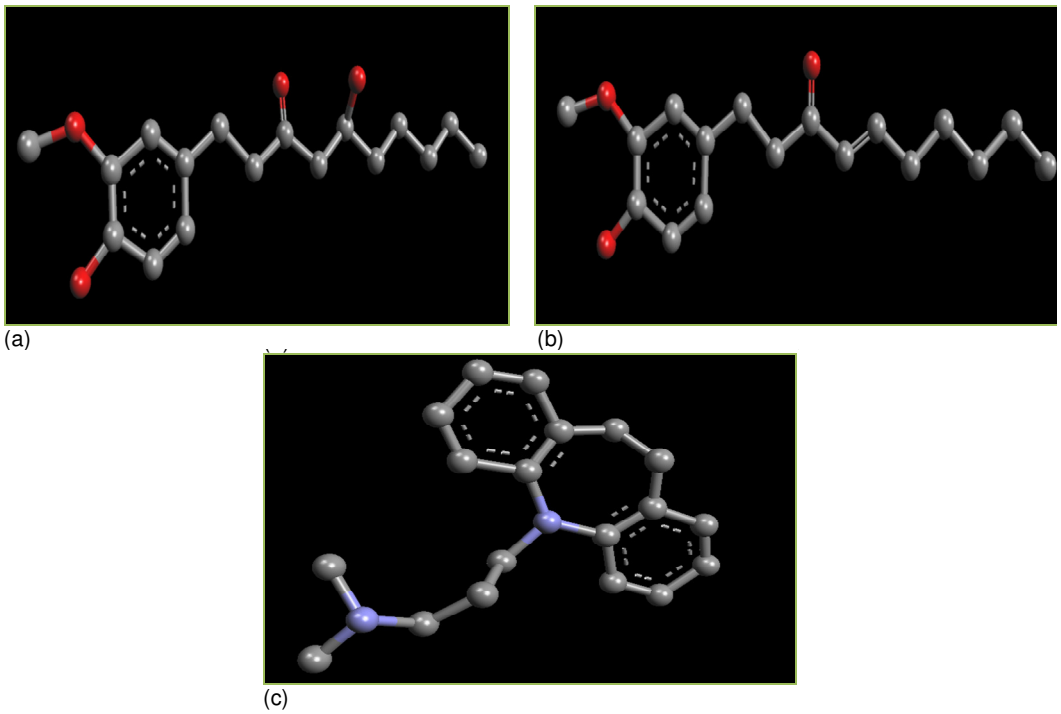


Figure 4. Structure of ligands. (a) Gingerol; (b) shogoal; (c) imipramine.

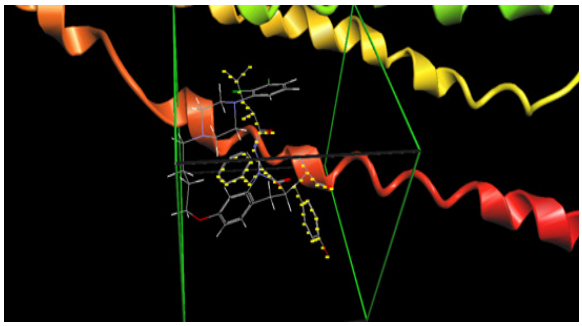


Figure 5. $5HT_{1A}$-gingerol complex: Yellow color represents binding site residues; grey color represents the ligand; blue, red and green color represents the rest of the protein. Binding mode of Shogoal with $5HT_{1A}$ protein.

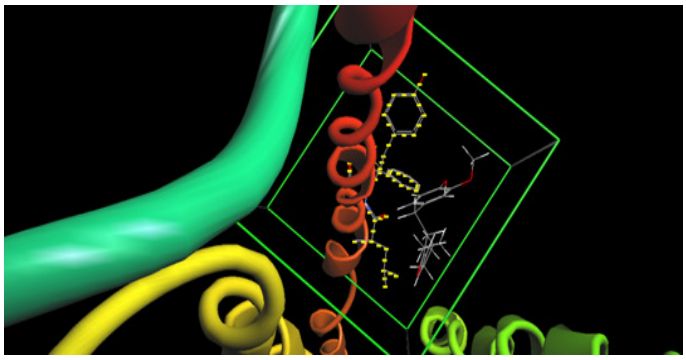


Figure 6. $5HT_{1A}$-shogoal complex: Yellow color represents binding site residues; grey color represents the ligand; blue, red and green color represents the rest of the protein

Docking scores

The docking scores were highest for shogoal with –9.0267 Kcal/mol, gingerol with -8.41044 Kcal/mol and imipramine with -8.21131 Kcal/mol, respectively and the binding mode of ligands with $5HT_{1A}$ is shown in Figures 5 to 8.

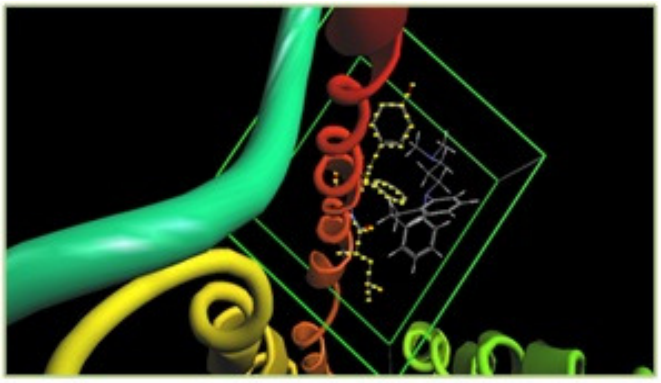


Figure 7. Binding mode of imipramine with $5HT_{1A}$ protein.

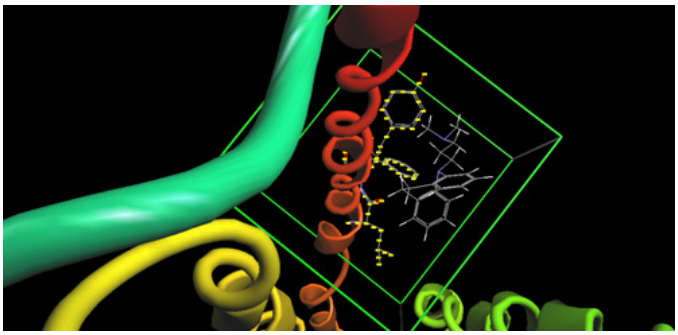


Figure 8. $5HT_{1A}$-imipramine complex: Yellow color represents binding site residues; grey color represents the ligand; blue, red and green color represents the rest of the protein.

CONCLUSION

The current therapeutic goal in the treatment of major depression is to improve quality of life by normalizing mood and reversal of functional and social disabilities associated with depression. Molecular docking continues to hold great promise in the field of computer based drug design which screens small molecules by orienting and scoring them in the binding site of a protein. Gingerol and shogoal interacted with LEU 453, PHE 454, and TYR 457 with high affinity. Comparative docking analysis of gingerol and shogoal with commonly used drug for treatment of depression such as imipramine also suggests that these compounds can be an alternative source for treatment of depression. Therefore, this approach is valuable for drug discovery process and therapy of depression. Hence, now there is a need to study the pharmacological activity of these compounds in *in vivo* models.

REFERENCES

Jones TD (1999). GenThreader: An efficient and reliable protein folds recognition method for genomic sequence. J. Mol. Biol. 287:797-815.

Mathew B, Vakketh SS, Kumar SS (2010). Synthesis, molecular properties and anthelmintic activity of some schiff bases of 1, 3, 4 thiadiazole derivatives. Scholars Research Library. Der Pharma Chemica. 2(5):337-43.

Nievergelt A, Huonker P, Schoop R, Altmann KH, Gertsch J (2010). Identification of serotonin 5-HT 1A receptor partial agonists in ginger. Bioorg. Med. Chem. 18:3345–51.

Rajput MS, Sinha S, Mathur V, Agrawal P (2011). Herbal Antidepressants. IJPFR.1 (1):159-69.

Vaseeharan B, Valli SJ (2011). Insilico homology modeling of prophenoloxidase activating factor serine proteinase gene from the haemocytes of *Fenneropenaeus indicus*. J. Proteomics Bioinform. 4:53-57.

Zhang Y (2008). I-TASSER server for protein 3D structure prediction. BMC Bioinformatics 9:40.

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Full Length Research Paper

Evaluation of analgesic, antipyretic and anti-inflammatory effects of methanol extract of traditional herbal medicine using rodents

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Accepted 24 April, 2013

The analgesic, antipyretic and anti-inflammatory effects of methanol extract of botanical medicine prepared by taking equal amounts of dried stems of *Tinospora cordifolia* Willd, fruits of *Emblica officinalis* Gaertn and rhizomes of *Cyperus rotundus* Linn were evaluated using standard methods. These botanicals have been traditionally used in Indian system of medicine for treatment of fever, joint pain, body ache and muscular pain in the form of single or combined drug therapy. After acute toxicity studies, anti-inflammatory effect was assessed using carrageen induced paw oedema test and antipyretic effect using yeast induced pyrexia method. Tail immersion, hot plate and writhings test were used for determining the analgesic properties. Phytochemical analysis revealed the presence of polyphenolic flavonoids, tannin and saponins. Significant anti-inflammatory, antipyretic and analgesic properties were noticed in dose dependant manner after methanolic extract administration, especially at 500 mg/kg dose. These test drug activities were sustained and comparable to the standard drugs while exhibiting no acute toxicity. The findings indicate that the methanolic extract possesses significantly high anti-inflammatory, antipyretic and analgesic properties without any acute toxicity possibly due to the presence of flavonoids.

Key words: Analgesic, antipyretic, anti-inflammatory, toxicity, botanical medicine.

INTRODUCTION

The traditional Indian system of Ayurvedic medicine has mentioned a number of therapeutic cures for common ailments using specific plant parts or their combinations which are relatively free from the side-effects commonly associated with the modern Allopathic system of medicine. The botanical medicine under examination in this study consists of the methanolic extract of equal amounts of dried stems of *Tinospora cordifolia* Willd, fruits of *Emblica officinalis* Gaertn and rhizomes of *Cyperus rotundus* Linn. This combined formulation has been used in the Indian system of medicine (Ayurveda) for the treatment of fever, joint pain, body ache, muscular pain and as a rejuvenator for thousands of years, and has been mentioned for its above mentioned properties in the ancient traditional textbook Charak Samhita (Shastri, 2003).

T. cordifolia (family Menispermaceae) is a glabrous clim-bing plant which is mostly found in deciduous and dry forest regions of India. The succulent stem is creamy white to grey in color, with deep clefts spotted with lenticels

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often giving out aerial roots. The principal constituents found in its stem are tinosporin, tinocordiside, tinocordifolioside, cordioside and alkaloids like berberine, palmatine (Sharma et al., 2005). *E. officinalis* Gaertn also known locally as “Amalaki” is a small or medium sized deciduous tree belonging to Euphorbiaceae family found throughout India. Dried fruit of the plant is brown to blackish brown in colour with characteristic odour and sour and astringent taste. Its fruits are a rich source of Vitamin C, tannin, ellagic acid and gallic acid (Rao and Siddiqui, 1964). *C. rotundus* Linn belonging to the family Cyperaceae is a perennial weed indigenous to India but is now spread in tropical, subtropical and temperate regions. The rhizomes known as “Mustak” are bluntly conical and vary in size and thickness, crowned with the remains of stem and leaves forming a scaly covering, dark brown or black externally, creamish-yellow internally and have pleasant odour. The rhizome contains polyphenols like cyperone, cyperenone and cyperene and carbohydrates like D-glucose and D-fructose. The rhizome is reported to possess analgesic, anti-inflammatory and antipyretic activities (Guldur et al., 2010; Nagulendran, 2007).

The present study undertakes the phytochemical and biological evaluation of this botanical medicine. Assessment of the analgesic, antipyretic and anti-inflammatory pharmacological activities of the methanolic extract in case of rodents was carried out and the results obtained were compared with the standard non-steroidal anti-inflammatory synthetic drugs. The objective is to validate the efficacy of this traditional herbal non-toxic, antipyretic, analgesic and anti-inflammatory drug which has been mentioned in Ayurvedic system of medicine.

MATERIALS AND METHODS

The pharmacognostical and experimental studies were done in the laboratory and the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) registered animal house facility of Dravyaguna Department of the Institute of Post Graduate Ayurvedic Education and Research, Kolkata.

Plant materials and extraction of botanical medicine

The dried plant parts of research drug were collected from the authentic medicinal herb supplier of the Pharmacy department of this institute and authenticated by the Department of Botany of the Botanical Survey of India at Shibpur, Howrah (Ref No- BSI/CNH/AD/Tech/2010, Date- 21.07.2010). All documents and samples have been kept in the museum of this department. The analytical grade chemicals for phytochemical screening along with standard reagents were purchased from M/s Merck Specialities Pvt. Ltd, Mumbai, India.

Animals

All the experimental studies were carried out under suitable conditions in the animal house and experimentation facility of the Institute of Post Graduate Ayurvedic Education and Research (IPGAER), Kolkata (registered under CPCSEA Reg. No- 1180/ac/08/CPCSEA) after taking all required permissions from the Institutional Animal Ethical Committee. The rats and mice required for experiments were procured from M/s Ghosh Scientific, Kolkata. The animals were fasted overnight prior to experiments but allowed free access to water. The anti-inflammatory and antipyretic experiments were performed on adult Wistar rats of either sex (1 month old and weighing 80 to 120 g). Swiss albino mice weighing 20 to 30 g were used for analgesic and acute toxicity study. They were fed with standard pellet diet and water *ad libitum* in poly-propylene cages and maintained under environmentally controlled room temperature of approximately 25°C.

Extraction of botanical medicine

The 40 mesh sized sun dried powdered fruits of *E. officinalis*, stems of *T. cordifolia* and rhizomes of *C. rotundus* were mixed in equal proportion to prepare the research drug after removing all extraneous matters such as dirt, foreign particles and adulterants. The research drug was extracted in methanol using a Soxhlet apparatus for 36 h. The obtained extract was filtered, concentrated in a rotary evaporator and finally stored in refrigerator for further analysis (Trease and Evans, 2009). The methanolic extract of the test drug was used throughout the experimental and chemical analysis.

Pharmacognostical study of crude drug powder

The macroscopic and microscopic study of the powder of test drug was performed according to the standard procedures. The final crude drug powder was mounted in glycerine, observed and photographed under the optical microscope (40×) of Dewinter, Italy.

Physiochemical analysis

The analysis of the physiochemical parameters such as moisture content, extractive value, acid insoluble ash, water soluble ash and total ash content of the test drug powder was performed according to the standard steps described in the Ayurvedic Pharmacopoeia, Government of India (2001).

Estimation of total phenolic content

The total phenolic content of the drug was determined by Folin Ciocalteu method using gallic acid as standard compound at different concentrations (100 to 500 µg/ml). The assessed total phenol values were expressed in terms of gallic acid equivalent as mg/g of dry mass.

Phytochemical screening

Preliminary phytochemical screening of the different constituents like alkaloids, flavonoids, tannins, carbohydrates, glycosides, saponins, fats and oils, proteins and amino acids was performed following standard procedures.

Estimation of acute toxicity

Organisation for Economic Co-operation and Development (OECD) guideline # 423 was followed for carrying out acute oral toxicity studies (OECD, 2001). The animals of both sexes were selected by sampling technique and were divided into 6 groups of 3 animals

each. A single oral dose of the extract starting at 100 mg/kg and progressively moving from 300, 500, 700, 1000 mg/kg up to 1200 mg/kg was administered. All the animals were observed for appearance of toxic symptoms including muscle spasm, loss of righting reflex, tremors, behavioural changes, locomotion, convulsions and mortality for 24 h. Long term supervision was continued for a period of 14 days to observe any occurrence of toxic symptoms and mortality.

Evaluation of analgesic activity

Assessment of central analgesic effect

Hot plate method: The central analgesic activity of the methanol extract of test drug against thermal stimulus was evaluated in mice following hot plate method as well as tail immersion method (Ghosh, 2008; Hajare et al., 2000). Morphine sulphate (2.5 mg/kg i.m) was used as the standard drug. The standard group (B) was given intramuscular injection of 2.5 mg/kg morphine while the control group (A) was orally administered 10 ml/kg saline solution. Treatment groups C and D were orally administered 300 and 500 mg/kg of test drug, respectively one hour before applying the thermal stimulus, which was maintained at 55 ± 0.2°C. The delay in hind paw licking by each animal was recorded in seconds as response after 10, 30 and 60 min of drug administration. Maximum reaction time of observation was kept at 60 s throughout to avoid tissue damage (Ibironke and Ajiboye, 2007).

Tail immersion method: The tail immersion method was used to assess the central analgesic effect of methanol extract in different dosages (Ghosh, 2010; Hajare et al., 2000; Veerappan et al., 2005). The standard group B was injected with 2.5 mg/kg i.m of morphine sulphate which was used as the standard drug while the control group was given 10 ml/kg of saline. The groups C and D were orally administered 300 and 500 mg/kg of the test drug, respectively 1 h before applying thermal stimulus by placing the tail 5 cm in the glass beaker containing water temperature maintained at 55 ± 0.2°C. The delay in withdrawing of the tail from the glass beaker was recorded in seconds as responses after 10, 30 and 60 min of drug administration. The maximum time of observation would be about 60 s throughout to avoid any tissue damage.

The assessment of peripheral analgesic effect

The peripheral analgesic activity of the methanolic extract of test drug was assessed in acetic acid induced writhing experiments using mice. The standard procedure prescribed by Veerappan et al. (2005) was used for observing the abdominal constriction writhings resulting from intraperitoneal injection of acetic acid (10 ml/kg of 0.6% v/v glacial acetic acid solution in water) (Gupta et al., 2008). Saline (10 ml/kg) was orally administered to group A (control group) whereas standard aspirin (100 mg/kg) was prescribed for group B and 300 and 500 mg/kg test drug extract was orally administered to groups C and D, respectively. Acetic acid solution was then administered to each animal after 30 min and the number of writhings counted for the next 15 min.

Evaluation of antipyretic activity

The estimation of antipyretic efficacy of methanolic extract was carried out using brewer's yeast induced pyrexia method. Fever was induced by means of subcutaneously injecting 10.0 ml/kg of a 20% w/v suspension of Brewer's yeast in normal saline. Only animals whose rectal temperature increased by at least 1.0°C after 18 h of this yeast injection were included for the study. The normal rectal temperature of each animal was measured by using a flexible tail thermostat probe coated with lubricant, and temperature was recorded using a digital IMCORP Telethermometer. The experimental animals were randomly divided into four groups containing six animals in each group. The control group (A) was orally administered 0.5 ml saline while the standard group (B) was given 100 mg/kg aspirin and groups C and D were prescribed 300 and 500 mg/kg of methanol extract of test drug, respectively. The rectal temperature was recorded at time intervals of 1, 2, 3, 4 and 5 h after drug administration.

Anti-inflammatory studies (carrageenan-induced paw oedema in rats)

During anti-inflammatory studies, paw oedema was induced by injecting 0.1 ml of 1% (w/v) carrageenan suspension into the sub planter region of the right hind paw of the rats (Mahat and Patil, 2007). The control group (A) was orally administered saline (10 ml/kg) while the standard group (B) was given indomethacin (5 mg/kg), and groups C and D were given 300 and 500 mg/kg of the test drug extract 1 h before carrageenan injection. The measurement of paw oedema was carried out by displacement technique using plethysmometer to find out the circumference of paw oedema immediately before and after 1, 2, 3 and 4 h following the carrageenan injection. The inhibitory activity was calculated according to the formula:

$$\% \text{ Inhibition} = \frac{(C_t - C_o)\ \text{control} - (C_t - C_o)\ \text{treated}}{(C_t - C_o)\ \text{control}} \times 100$$

Where C_t = paw circumference at time t, C_o = paw circumference before carrageenan injection, $(C_t - C_o)$ = oedema or change in paw size after time t.

Statistical analysis

The differences in individual data values for different parameters obtained during the above mentioned experiments were examined by two-way analysis of variance using ANOVA test. The level of significance was fixed between $p < 0.05$ to $p < 0.01$ (Krishnaswami and Ranganatham, 2011).

RESULTS

Physiochemical analysis

The results of the physiochemical analysis are as given in Table 1.

Estimation of acute toxicity

The animals tested in acute toxicity tests up to the dose of 1200 mg/kg, showed no significant toxic symptoms like sedation, convulsion, diarrhoea, irritation, etc. and no signs of behavioural changes. No mortality was reported up to 24 h and even later during the subsequent 14 days at 1200 mg/kg dose.

Table 1. Results of the physiochemical analysis.

Parameter	Value (% w/w)
Yield of dried methanol extract	3.78
Moisture content	8.54
Total ash content	6.08
Acid insoluble ash	2.11
Water soluble ash content	3.79
Total phenol content	25.86 mg/g of dry mass

Evaluation of analgesic activity

Assessment of central analgesic effect

Hot plate method: During the hot plate method, after giving thermal stimulus, reaction time increased significantly up to 60 min in case of the 300 mg/kg drug group (increasing from 3.22 to 5.38 s) and 500 mg/kg test drug group (increasing from 3.15 to 5.75 s) when compared with the control group (where it actually decreased from 2.89 to 2.86 s). The test drug at the dose of 500 mg/kg showed similar type of pattern up to 90 min (reaction time increasing from 3.15 to 6.65 s) but slightly lower central analgesic effect when compared with the standard drug morphine sulphate (where the reaction time increased from 3.05 to 6.88 s) (Table 2 and Figure 1). The results of the hot plate method showed that after oral administration of drug and giving of thermal stimulus to the animals, the percentage increase in reaction time up to 60 min was 115.73% in case of standard group, 67.08% in case of 300 mg/kg test drug group and 82.53% in case of 500 mg/kg group. In comparison to this, the control group actually showed a decrease of 1.04% in reaction time over the same time period. The test drug thus exhibited significant central analgesic effect at both the doses of 300 and 500 mg/kg when compared with the control group. However, the results of test drug assessed in terms of the average reaction time at the dose of 500 mg/kg (82.53% increase) showed less central analgesic effect when compared to the standard drug morphine (115.73% increase).

Tail immersion method: The results of the tail immersion method showed significant increase in reaction time up to 60 min after giving thermal stimulus in case of both the 300 mg/kg methanolic extract of test drug group (increasing from 2.62 to 4.62 s) and the 500 mg/kg test drug group (increasing from 3.01 to 5.70 s) when compared with the control group (where it increased from 2.24 to 2.45 s). The 500 mg/kg test drug group showed similar type of pattern up to 90 min (reaction time increasing from 2.62 to 4.15 s), however its overall central analgesic effect was a little lower when compared with the standard drug morphine where the reaction time increased from 2.89 to 5.87 s during this period (Table 3). The percentage increase in reaction time after oral administration of the drug and giving of thermal stimulus to the animals in case of tail immersion method up to 60 min was 126.9% in case of the standard group, 76.33% in case of the 300 mg/kg test drug group and 89.36% in case of the 500 mg/kg group (Figure 2). During the same period, the control group showed only 9.37% increase in reaction time, indicating significant impact of the test drug and standard drug during the experiment. The results indicated that the test drug exhibited significant central analgesic effect at both the doses of 300 and 500 mg/kg as compared to the control group. However, the overall impact of the test drug assessed in terms of the average reaction time at the dose of 500 mg/kg (89.36% increase) showed lower central analgesic effect as compared with the standard drug morphine (126.9% increase).

Assessment of peripheral analgesic effect (acetic acid induced writhing analysis)

The peripheral analgesic effect was evaluated on the basis of the average number of abdominal constrictions indicated by the extension of hind paw of animals during the writhings test. The observed inhibition in writhings as a result of administration of the test drug up to 15 min was significantly higher ($p < 0.05$) at the dose of 300 mg/kg (43.31%) as well as at 500 mg/kg (52.08%) when its activity was compared with the control group (Table 4). Comparing the performance of the test drug with the standard drug, the observed peripheral analgesic effect was slightly less at the test drug dose of 500 mg/kg as indicated by 52.08% inhibition in writhings as compared to the standard drug aspirin which resulted in 58.75% inhibition (Figure 3).

Antipyretic effect on yeast induced pyrexia

The antipyretic effect of the methanolic extract of test drug on yeast induced pyrexia has been shown in Table 4. The experimental rats showed a mean increase of about 1.58°C in rectal temperature after 18 h of yeast injection. The test drug extract at both the doses of 300 and 500 mg/kg showed significant antipyretic effect. The antipyretic effect of the extract at the dose of 500 mg/kg is highly significant ($p < 0.05$) and comparable to that of the standard drug aspirin when compared to the control group. There had been a sharp decrease in elevated rectal temperature after 1 h of drug administration which was further followed by a gradual trend of decrease in a dose dependent manner up to 5 h of oral administration of the drug sample in each group (Table 5).

The percentage reduction of rectal temperature after yeast injection in the individual groups after 4 h was 0.59% in the control group, 1.99% in case of the standard drug, 1.23% in the 300 mg/kg test drug and 1.94% in the 500 mg/kg test drug group after oral administration of all

Table 2. Average reaction time using Hot plate method.

Group	0 min	30 min	60 min	90 min	% increase in reaction time after 60 min
Control	2.89±0.039	2.94±0.086	2.86±0.035	2.85±0.121	-1.04
Standard	3.05±0.054	5.98±0.061	6.58±0.040	6.88±0.0 81	115.73
Methanolic extract (300 mg/kg)	3.22±0.054	4.42±0.083	5.38±0.071	6.18±0.029	67.08
Methanolic extract (500 mg/kg)	3.15±0.033	5.01±0.040	5.75±0.050	6.65±0.034	82.53

Results are presented as mean ± standard error of mean (SEM). $p < 0.05$ compared to control n = 6

Table 3: Average reaction time during tail immersion method.

Group	0 min	30 min	60 min	90 min	% increase in reaction time after 60 min
Control	2.24±0.046	2.56±0.073	2.45±0.082	2.30±0.053	9.37
Standard	2.89±0.064	5.99±0.150	6.56±0.095	5.87±0.079	126.9
Methanolic extract (300 mg/kg)	2.62±0.048	4.00±0.037	4.62±0.031	4.15±0.056	76.33
Methanolic extract (500 mg/kg)	3.01±0.033	5.44±0.042	5.70±0.042	5.65±0.040	89.36

Results are presented as mean ± standard error of mean (SEM). $p < 0.05$ compared to control n = 6

Table 4. Analgesic effect during writhing method.

Group	Average number of writhings/15 min	% Inhibition
Control	50.38±1.4 5	0.00
Standard	20.78±0.76	58.75
Methanolic extract (300 mg/kg)	28.56±1.30	43.31
Methanolic extract (500 mg/kg)	24.14±0.91	52.08

Results are presented as mean ± standard error of mean (SEM). $p < 0.05$ compared to control n = 6.

Table 5. Antipyretic effect of methanol extract in comparison to aspirin.

Parameter	Initial rectal temperature in ℃	Rectal temperature in ℃ after 18 h of yeast Injection (Mean±SEM)						% reduction of rectal temperature after 4 h
		0 h	1 h	2 h	3 h	4 h	5 h	
Control	36.28±0.03	37.6±0.06	37.57±0.02	37.55±0.04	37.49±0.03	37.38±0.04	37.35±0.04	0.59
Standard	36.89±0.05	38.07±0.04	37.56±0.03	37.5±0.03	37.43±0.06	37.31±0.02	37.33±0.03	1.99
Methanolic extract (300 mg/kg)	36.31±0.04	37.4±0.02	37.03±0.04	36.99±0.03	36.95±0.04	36.94±0.05	36.96±0.04	1.23
Methanolic extract (500 mg/kg)	36.36±0.04	37.2±0.03	36.59±0.05	36.57±0.02	36.51±0.03	36.48±0.04	36.5±0.02	1.94

Results are presented as mean±SEM. $p < 0.05$ compared to control n = 6.

Table 6. Anti-inflammatory effect during carrageen–induced paw oedema test.

Treatment group	0 h	1 h	2 h	3 h	4 h	% inhibition after 4 h
Control (10 ml/kg)	0.48±0.08	1.44±0.06	2.53±0.07	2.85±0.09	2.86±0.05	-
Indomethacin (5 mg/kg)	0.45±0.05	0.86±0.08	0.74±0.06	0.66±0.07	0.60±0.07	79.02
Methanolic extract (300 mg/kg)	0.42±0.04	0.94±0.07	0.89±0.08	0.84±0.05	0.81±0.08	71.68
Methanolic extract (500 mg/kg)	0.44±0.07	0.93±0.04	0.88±0.05	0.79±0.07	0.73±0.05	74.48

Results are presented as Mean±SEM. $p < 0.05$ compared to control n = 6.

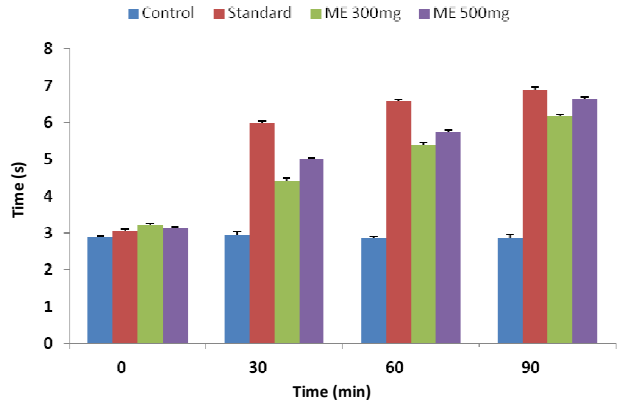

Figure 1. Reaction time during hot plate method.

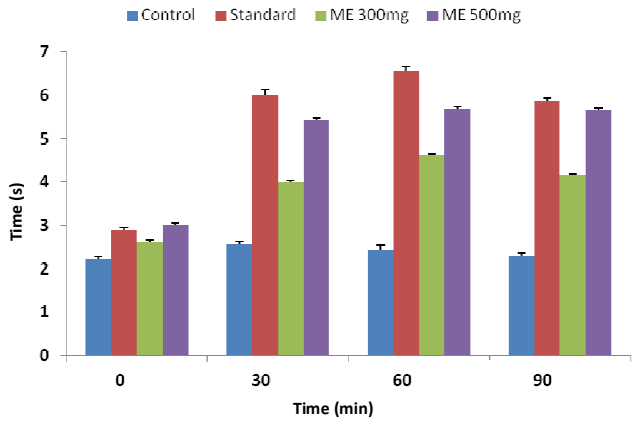

Figure 2. Central analgesic effect during tail immersion method.

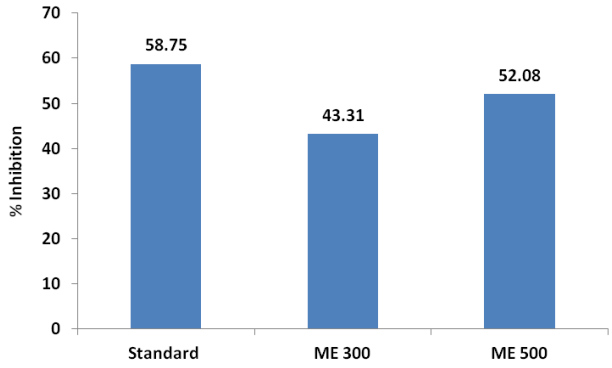

Figure 3. Peripheral analgesic effect in writhing method.

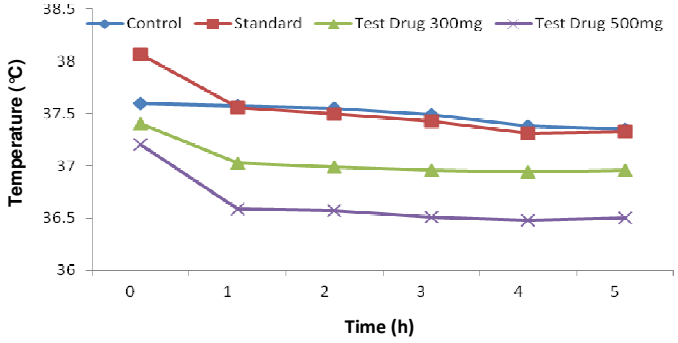

Figure 4. Rectal temperature of rats during yeast induced pyrexia test.

drug samples. The antipyretic effect on the basis of percentage reduction of rectal temperature after 4 h showed quite highly significant results of 500 mg/kg test drug as compared to the standard drug aspirin. The test drug also exhibited more sustained and persistent anti-pyretic effect lasting up to 5 h during this test (Figure 4).

Anti-inflammatory studies (carrageenan-induced paw oedema)

The circumference of paw oedema in rats induced by the carrageen agent to evaluate the acute anti-inflammatory effect at 300 and 500 mg/kg dose showed significant decrease in inflammation of 71.68 and 74.48%, respectively ($p < 0.05$) up to 4 h as compared to the control group (Table 6). The percentage inhibition of the acute inflammation in the paw of rats was found to be comparable but a little lower in the 500 mg/kg test drug dosage group (74.48% inhibition) compared to the standard drug indomethacine (79.02% inhibition) after 4 h of treatment (Figure 5).

DISCUSSION

Inflammation is defined as a biological process

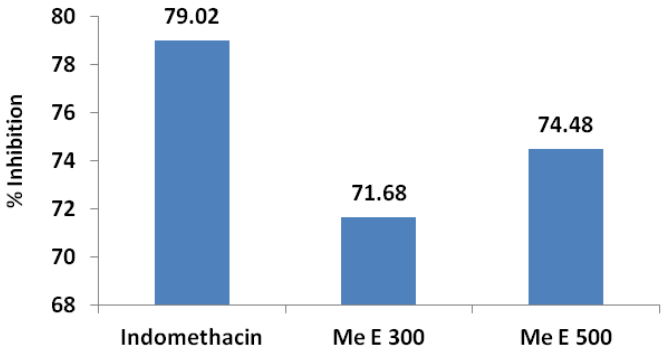

Figure 5. Anti-inflammatory effect during carrageen–induced paw oedema test.

characterized by redness, oedema, fever and pain, and can result in locally increased production of free radicals by inflammatory enzymes, as well as the release of inflammatory mediators that promote cell proliferation and angiogenesis and inhibit apoptosis. Although the currently used steroidal and non-steroidal anti-inflammatory drugs treat acute inflammatory disorders, these conventional drugs have not been successful in curing chronic inflammatory disorders such as rheumatoid arthritis. Moreover, usage of many of such drugs has been associated with adverse impact upon the hepatic and renal functions (Kim et al., 2004; Rajnarayana et al., 2006; Warden, 2010).

The preliminary chemical analysis of this methanolic extract following standard method indicates the presence of high concentration of phenolic compounds such as flavonoids, tannins and carbohydrates. Flavonoids are a large family of compounds synthesized by plants that have a common chemical structure. The flavonoidic phenolic compounds have been known to exhibit anti-inflammatory, antioxidant and metal-chelating properties (Rajnarayana et al., 2006; Pan et al., 2010). Inflammation can result in the locally increased production of free radicals by inflammatory enzymes, as well as the release of inflammatory mediators that promote cell proliferation and angiogenesis and inhibit apoptosis (Gupta et al., 2012).

The hot plate and tail immersion methods indicated that the central analgesic effect of the methanol extract of test drug was significant and dose dependent as revealed by the increased reaction time after giving thermal stimulus to the mice. The latency in reaction time continued up to 90 min after the drug administration in both 300 and 500 mg/kg extracts revealing sustained and pronounced central analgesic effect when compared with the control group. However, its overall central analgesic effect was a little lower when compared with the standard drug morphine over different time periods; the higher test drug dosage resulting in a more pronounced effect with a similar pattern of results.

The assessment of peripheral analgesic effect of the test drug exhibited significant percentage inhibition in the writhings which were induced by acetic acid in the mice at both the 300 and 500 mg/kg doses of the methanolic extract when compared with the control group. The percentage inhibition of writhings at the higher dose of 500 mg/kg indicated the pronounced peripheral analgesic effect in the context of visceral pain which was comparable to the standard pure drug morphine within 15 min of test. The overall central analgesic effect of the test drug even at higher dose was a little lower than the standard drug morphine. The significant analgesic effect at the higher dose was attributed to the presence of high concentration of flavonoidic compounds which inhibited the synthesis, release or receptor responses in prostaglandin mediated effects (Gupta et al., 2008; Pan et al., 2010; Gupta et al., 2012).

During antipyretic tests, the percentage reduction in rectal temperatures after yeast injection up to 4 h was quite noticeable and comparable to the standard group in case of 500 mg/kg test drug. The observed antipyretic effect was found to be sustained and lasted up to at least 5 h after oral administration of the drug sample in case of both the 300 and 500 mg/kg samples which showed similar pattern of antipyretic efficacy. Pyrexia is a result of secondary impact of infection, tissue damage, inflammation, graft rejection, malignancy or other diseased states. Mediators like interleukin 1β, α, β and TNF-α increase the synthesis of prostaglandin E2 near pre-optic hypothalamus area thereby triggering the hypothalamus to elevate the body temperature. Flavonoids are known to target prostaglandins which are involved in pyrexia and also inhibit the effect of enzymes that are responsible for the inflammatory process (Pan et al., 2010; Binny et al., 2010; Owoyele et al., 2008).

The evaluation of acute inflammation which was induced by the carageenan in the paw of rats demonstrated the significant percentage decrease in circumference and oedema resulting from administration of the methanol extract of test drug in doses of 300 and 500 mg/kg. The percentage inhibition of acute inflammation in the paw of rats was found to be lower in case of 500 mg/kg of test drug dosage when compared to the standard drug indomethacine after 4 h of treatment. The acute and sustained effect on the induced inflammation up to four hours depends upon the decreased production of pro-inflammatory cytokines and prostaglandin E_2 (PGE_2) in the tissue of the effected part of the body. The inflammation induced by phlogostic agent is related to the production of histamine, bradykinin and cyclooxygenase products while delayed phase is related to neutrophil infiltration, as well as to the continuing of the production of arachinoic acid metabolites. Prostaglandins and nitric oxide biosynthesis is involved in inflammation, and isoforms of inducible nitric oxide synthase (iNOS) and of cyclooxygenase (COX-2) are responsible for the production of a great amount of these mediators. It has been demonstrated that flavonoids are able to inhibit both enzymes, as well as other mediators of the inflammatory process such as reactive C protein or adhesion molecules

(Gonzalez et al., 2007).

The variety of flavonoids have been found to have antimicrobial, antiviral, anti-ulcerogenic, cytotoxic, anti-neoplastic, mutagenic, antioxidant, antihepatotoxic, ant-ihypertensive, hypolipidemic, antiplatelet and antipyretic anti-inflammatory activities. Flavonoids also have biochemical effects, which inhibit a number of enzymes such as aldose reductase, xanthine oxidase, phosphodiesterase, Ca (+2)-ATPase, lipoxygenase, cycloxygenase, etc. (Pan et al., 2010; Owoyele et al., 2008; Rathee et al., 2009).

The detailed scientific evaluation of the pharmacological properties of the methanolic extract of the test drug in terms of its analgesic, antipyretic and anti-inflammatory actions clearly brought forth its significant therapeutic efficacy which was found to be comparable to that of the standard drugs. The test drug was also found to be significantly effective and having sustained effect along with no noticeable acute toxicity during this pre-clinical study. These three properties which have been known to be inter-related and inter-dependent could be attributed to the presence of flavonoids in the methanolic abstract. Hence, the results obtained during this research could be taken as a benchmark, and detailed further studies could be undertaken in the domain of phyto-chemistry and chemical analysis to identify the actual responsible bio-chemical marker compounds in the test drug. The findings and outcome of this research study could also form the basis for future clinical trials involving this research drug to establish its safety and efficacy in human subjects in view of its significant and sustained therapeutic activities documented during this study using rodents.

REFERENCES

Binny K, Kumar SG, Thomas D (2010). Anti-inflammatory and antipyretic properties of the rhizome of *Costus Speciosa* (Koen.). J. Basic Clin. Pharm. 1(3):177-181.

Ghosh MN (2008). Fundamentals of Experimental Pharmacology (4th ed.). Hilton and Company, Kolkata, pp. 162-241.

González-Gallego J, Sánchez-Campos S, Tuñón MJ (2007). Anti-inflammatory properties of dietary flavonoids. Nutr. Hosp. 22(3):287-293.

Guldur ME, Ozgonul A, Kilic IH, Sogut O, Ozaslan M, Bitiren M, Yalcin M, Musa D (2010). Gastroprotective effect of *Cyperus rotundus* extract against gastric mucosal injury induced by ischaemia and reperfusion in rats. Int. J. Pharmacol. 6(2):104-110.

Gupta M, Sasmal S, Majumdar S, Mukherjee A (2012). HPLC profiles of standard Phenolic Compounds present in medicinal plants. Int. J. Pharmacognosy Phytochem. Res. 4(3):162-167.

Gupta M, Shaw BP, Mukherjee A (2008). Studies on antipyretic-analgesic and ulcerogenic activity of polyherbal preparation in rats and mice. Int. J. Pharmacol. 4(2):88-94.

Hajare SW, Chandra S, Tandon SK, Sharma J, Lal J, Telang AG (2000). Analgesic and antipyretic activities of Dalbergia sissoo leaves. Ind. J. Pharmacol. 32(6):357-360.

Ibironke GF, Ajiboye KI (2007). Studies on the anti-inflammatory and analgesic properties of Chenopodium Ambrosioides leaf extract in rats. Int. J. Pharmacology 3(1):111-115, ISBN 811-7775.

Kim HP, Son KH, Chang HW, Kang SS (2004). Anti-inflammatory Plant Flavonoids and Cellular Action Mechanisms. J. Pharmacol. Sci. 96:229-245.

Krishnaswami OR, Ranganatham M (2011). Methodology of Research in Social Sciences. Himalaya Publishing House, Mumbai. pp. 295-350.

Mahat MA, Patil BM (2007). Evaluation of anti-inflammatory activity of methanol extract of *Phyllanthus amarus* in experimental animal models. Ind. J. Pharm. Sci. 69(1):33-36.

Michael M, Christopher H, Roy U, Alicia G. (1997). American Herbal Products Association: American Herbal Products Association's Botanical Safety Handbook, edited by Boca Raton, Fla.: CRC Press.

Nagulendran KR, Velavan S, Mahesh R, Begum VH (2007). *In vitro* antioxidant activity and total polyphenolic content of *Cyperus rotundus* rhizomes. E- J. Chem. 4(3):440-449.

OECD/OCDE Guidelines for the testing of chemicals (2001). Revised draft guidelines 423 Acute oral toxicity - Acute toxic class method, Adopted document.

Owoyele BV, Oguntoye SO, Darel K, Alice B (2008). Analgesic, anti-inflammatory and antipyretic activities from flavonoid fractions of *Chromolaena odorata*. J. Med. Plants Res. 2(9):219-225.

Pan MH, Lai CS, Ho CT (2010). Anti-inflammatory activity of natural dietary flavonoids. Food Funct. 1:15-31.

Rajnarayana K, Reddy MS, Chaluvadi MR (2006). Bioflavonoids classification, pharmacological, biochemical effects and therapeutical potential. Ind. J. Pharm. Sci. 68(3):380-384.

Rao MRR, Siddiqui HH (1964). Pharmacological studies on Emblica officinalis Gaertn. Indian J. Exp Biol. 2:29.

Rathee P, Chaudhary H, Rathee S, Rathee D, Kumar V, Kohli K (2009). Mechanism of action of flavonoids as anti-inflammatory agents: a review. Inflamm. Allergy Drug Targets 8(3):229-235.

Sharma PC, Yelne MB, Dennis TJ (2005). Database on Medicinal plants used in Ayurveda. (Vol. 3). Central Council for Research in Ayurveda and Siddha, New Delhi, pp. 11-409.

Shastri SN (2003). Charak Samhita (Vol. 2). Chaukhamba Orientalia Publishers, Varanasi.

The Ayurvedic Pharmacopoeia of India (2001) Vol. 1, 1st ed. Govt. of India. pp. 142-144.

Trease GE, Evans WC (2009). Pharmacognosy, 16th ed. Bailliere Tindall Ltd, London. pp. 252-259.

Veerappan A, Miyazaki S, Dhananjayan R (2005). Studies on the anti-inflammatory, antipyretic and analgesic properties of the leaves of *Aegle marmelos*. Corr. J. Ethnopharmacol. 96(1-2):159-163.

Warden SJ (2010). Prophylactic Use of NSAIDs by Athletes: A Risk/Benefit Assessment. The Physician and Sports Medicine 38(1):132-138.

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Full Length Research Paper

Endocrinological and metabolic effects of a polyherbal decoction of five Nigerian folkloric herbs on haloperidol induced hyperprolactinemia

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Hyperprolactinemia is a major cause of infertility, and herbal remedies have been employed locally for treatment. This study was therefore designed to investigate the effects of a poly herbal mixture consisting of *Bambusa vulgaris, Momordica charantia, Rauwolfia vomitoria, Ficus sur,* and *Clerodendrum capitatum* on the reproductive hormones and metabolic parameters of haloperidol-induced hyperprolactinemic rats. Forty two female albino rats were divided into 7 groups of 6 in each group. Groups 2, 4, 5, 6 and 7 were given increasing doses (2, 3 and 4 mg/kg body weight in five-daily increments) of haldol by intramuscular injection for 15 days after which they were treated for another 15 days with either 2.5 mg/kg body weight of bromocriptine (group 4 only) or 25, 50 or 100 mg/kg body weight of the extract (groups 5, 6 and 7, respectively). Group 1 was given distilled water only while group 3 was given 25 mg/kg body weight extract only. After treatment, the animals were sacrificed and blood was taken from each group for plasma analysis of the reproductive hormones and metabolic parameters. Prolactin, follicle stimulating hormone, luteinizing hormone and estrogen were assayed. The fasting blood glucose, total protein and the lipid profile (total cholesterol and high-density lipoprotein (HDL) and low-density lipoprotein (LDL) cholesterol) were also determined. The result showed a dose-dependent significant reduction in the prolactin level by the extract with increase in the levels of the follicle stimulating hormone (FSH), lieutenizing hormone (LH) and estrogen. There was also a decrease in the levels of the triglycerides, LDL and total cholesterol while HDL was increased. In conclusion, the polyherbal preparation was able to reverse the hyperprolactinemia with its associated endocrine and metabolic changes in a manner comparable to that of bromocriptine.

Key words: Hyperprolactinemia, haldol, polyherbal decoction, reproductive hormones, metabolic indices.

INTRODUCTION

Hyperprolactinemia is a condition of elevated serum prolactin. Hyperprolactinemia may cause production and spontaneous flow of breast milk and disruptions in the normal menstrual period in women, and hypogonadism, infertility and erectile dysfunction in men (Nilsson et al., 2009). Hyperprolactinemia can be a part of normal body changes during pregnancy and breastfeeding. It can also be caused by diseases affecting the hypothalamus and pituitary gland, disruption of the normal regulation of prolactin levels by drugs, medicinal herbs, heavy metals,

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and it may also be from disease of other organs such as the liver, kidneys, ovaries and thyroid (Mancini et al., 2008). The symptoms associated with hyperprolactinemia may be due to several factors like the direct effects of excess prolactin, such as the induction of galactorrhea or hypogonadism; the effects of the structural lesion causing the disorder (the pituitary tumor), causing conditions such as headaches, visual field defects, or external ophthalmoplegia. The symptoms may also be associated dysfunction of secretion of other anterior pituitary hormones (Asa, 2002). In some patients, however, it is not possible to elucidate the cause of hyperprolactinemia.

Use of prescription drugs is the most common cause of hyperprolactinaemia. Prolactin secretion in the pituitary is normally suppressed by the brain chemical dopamine. Drugs that block the effects of dopamine at the pituitary or deplete dopamine stores in the brain may cause the pituitary to secrete prolactin (Rosenbloom, 2010). These drugs include the major tranquillizers (phenothiazines), trifluoperazine (Stelazine), and haloperidol (Haldol) also an antipsychotic, antipsychotic medications in general; metoclopramide (Reglan), domperidone, cisapride used to treat gastro-oesophageal reflux and medication induced nausea (such as cancer drugs); and, less often, alpha-methyldopa and reserpine, used to control hypertension; and oestrogens and thyrotropin-releasing hormone (TRH) La (Torre and Falorni, 2007)

In Africa, much of the population relies on herbal treatment first, because of its affordability and the belief in the traditional healers and alternative therapists, before turning to conventional medicine, (Lee, 2003). A quarter of all new medicinal products registered around the world each year are derived from plants or other naturally occurring compounds. The biologically active defense compounds found within plants can also be useful for developing new drugs. As a result of their pharmacological or biological activity, medicinal plants can be used as a starting point for chemical and structural modification in order to improve the potency, selectivity and pharmacokinetics of drugs used in treating diseases (Mahdi et al., 2006).

Infertility is one of the major health problems in Nigeria in spite of the fast growing population. One of the major causes of infertility is hyperprolactinemia for which herbal treatment has been employed for treatment for a long time. The traditional healers lack the scientific understanding of hyperprolactinemia but they often diagnose it by one of its major symptoms, galactorrhea. One of the main herbal remedies for hyperprolactinemia is a decoction of five different local plants - *Bambusa vulgaris, Momordica charantia, Rauwolfia vomitoria, Ficus sur,* and *Clerodendrum capitatum*. In this research, we investigated the five herbs usually prescribed by the traditional healers to see if there was any scientific basis for the folkloric claims.

METHODOLOGY

Collection and identification of plant materials

The plant materials were purchased fresh from local market at Oyingbo, Lagos State Nigeria in July, 2012. They were identified and authenticated in the Botany Department of the University of Lagos, Akoka. The plants were given voucher numbers as listed in Table 1.

Preparation of plant extract

The extract of the herbs was obtained by leaves decoction. The leaves of the various plants were air dried and shredded. Seven liters of water was added to 2 kg of the dried shredded leaves (ratio 1:1:1:1:1) and this was boiled for one hour. The process was repeated twice by adding 4 L of water for 30 min to maximize the extraction. The total volume of extract obtained was 2.5 L which was concentrated to about 350 ml by rotary evaporator and then freeze-dried.

Experimental subjects

Sixty female albino rats (Wistar strain) weighing between 110 and 135 g were purchased from Rattzmattaz Farms and Feeds Nig. Ltd. Satellite Town Lagos, Nigeria. The animals were housed in plastic cages with plastic bottom and wire mesh top with a 12 h light-dark cycle and adequate ventilation. The animals were allowed normal rat chow (Rattzmattaz Farms and Feeds Nig. Ltd. Satellite Town, Lagos) and water *ad libitum* throughout the study.

Grouping and treatment of animals

The female Wistar rats were divided into six treatment groups and a control group as shown:
1. GRP1: Distilled water;
2. GRP2: Haldol only;
3. GRP3: Low dose extract;
4. GRP4: Haldol + bromocriptine;
5. GRP5: Haldol + low dose extract;
6. GRP6: Haldol + medium dose extract;
7. GRP7: Haldol + high dose extract.

Haldol (Janssen Pharmaceutical, Belgium) injection obtained from the pharmacy of the Federal Neuropsychiatric Hospital, Yaba, Lagos was administered to groups 2, 4, 5, 6 and 7 via intramuscular route for 15 days. The initial dose of the Haldol was 2 mg/kg body weight and was increased to 3 and 4 mg/kg bodyweight every 5 days. Groups 4, 5, 6 and 7 were afterwards treated for another 15 days with either 2.5 mg/kg body weight of bromocriptine or 25, 50 or 100 mg/kg body weight of the extract. At the end of the treatment period, the rats were fasted overnight and were later anaesthetized with halothane. Blood samples were obtained by cardiac puncture into heparinized sample bottles.

Biochemical assays

The hormonal assays (prolactin, follicle stimulating hormone and luteinizing hormone) were determined by enzyme linked immunosorbent assay (ELISA) method using AccuBind ELISA kit (Monobind Inc. CA, USA). Total plasma protein and the lipid profile

Table 1. Voucher numbers of plants.

Plant	Voucher number
Ficus sur	LUH4850
Momordica charantia	LUH 4853
Rauwolfia vomitoria	LUH 4860
Clerodendrum capitatum	LUH 4852
Bambusa vulgaris	LUH 4856

determination were done by enzymatic assay methods using analytical kits (RANDOX, United Kingdom). The glucose meter was evaluated using ACCU-CHEK glucose meter and test strips (Roche Diagnostics GmbH, Germany).

Statistical analysis

Results are presented as mean ± standard error of mean (SEM) for each group. Statistical analysis was done using the student's t-test to compare between the groups. A p-value less than 0.05 was considered statistically significant.

RESULTS

Table 2 shows the effects of administration of haldol and the polyherbal extract on the reproductive hormones. Administration of haldol significantly elevated the prolactin level ($p < 0.05$). For follicle stimulating hormone (FSH) and lieutenizing hormone (LH), the trends were similar as the lowest levels of the two hormones were found in the group treated with haldol only, and these values were significantly reduced ($p < 0.05$) compared to the controls. Administration of the herbal was seen to reduce the prolactin level and the high dose extract had the greatest effect ($p < 0.05$) of prolactin reduction which was close to the level observed in the group taking distilled water only. The FSH and LH levels too were found to rise, with the most significant effect ($p < 0.05$) found in the group given the highest dose of the extract. The pattern of the estrogen was also similar to that of FSH and LH. Its lowest level was observed in the haldol only group and it increased in a dose-dependent manner.

The effects of haldol and the polyherbal preparation on plasma glucose and protein levels are shown in Table 3. There was a significant ($p < 0.05$) elevation of blood glucose with haldol administration. The extract was observed to significantly reduce the glucose level. The most significant effect was seen in the group with the highest dose of the extract. For the plasma protein, there were no significant changes across the various groups although there was some increase in the total protein in the haldol only group but it was not a significant one.

The effects of haldol and polyherbal preparation on the plasma lipid profile are shown in Table 4. There was a significant increase in the plasma triglycerides while there was a reduction in total cholesterol and HDL in the haldol only group. Administration of the decoction caused a dose-dependent reduction in the plasma triacylglycerol and LDL levels while causing significant ($p < 0.05$) increases in the levels of both total cholesterol and HDL.

DISCUSSION

Several herbal preparations have been employed by people from various races and tribes to treat hyperprolactinemia, and these preparations have been claimed to be effective (Hasani-Ranjbar et al., 2010). This research has investigated a polyherbal mixture which has been used in the southwestern part of Nigeria for a long time to treat hyperprolactinemia, with many success stories. Administration of haldol to the rats significantly raised the serum prolactin when compared to the group that took distilled water only. The polyherbal mixture even at the lowest dose was able to reverse the hyperprolactinemic effect of haldol. The prolactin lowering effect of the herbal mixture was found to be dose dependent, and at the highest dose of the extract, the serum prolactin was brought close to the level observed in the control group. The effect seen with the highest dose of this herbal preparation was close to that of bromocriptine which is a standard dopamine receptor agonist often used in the treatment of hyperprolactinemia.

Studies have shown that certain herbs truly possess anti-hyperprolactinemic effects. These herbal preparations include *Vitexagnus cactus* (Wuttke et al., 2003) and Peony-Glycyrrhiza Decoction (Yuan et al., 2008). Diterpenes found in these plants, and also as part of the constituents in our study polyherbal preparation, were reported to contribute to their prolactin lowering property (Shrivastava and Patel, 2007; Hasani-Ranjbar et al., 2010). There is dearth of data on the anti-hyperprolactinemic property of any of the plants in the polyherbal mixture studied in this work but this observed property might not be unconnected with the presence of the diterpenes.

We also found that the extract was able restore the level of gonadotropins, FSH and LH back to the levels in the controls. This was also in a dose dependent manner. The highest dose of the extract increased the levels of these hormones significantly and the levels were found to be close to the distilled water-only group. The extract might have increased the levels of these hormones by lowering the prolactin level. This is most likely to be the case since it is well established that high prolactin levels depresses the gonadotropins by its inhibitory effect on the GnRH which regulates the pituitary secretion of the gonadotropins (Nilsson et al., 2009).

Table 2: Effect of administration of haldol and poly herbal preparation on the reproductive hormones of female Wistar rat.

Group/treatment	Prolactin (miu/ml)	Follicle stimulating hormone (miu/ml)	Luteinizing hormone (miu/ml)	Estradiol (pg/ml)
GRP1 (Distilled water)	62.67±4.79*	7.80±0.92*	5.20±0.45*	325.20±54.30*
GRP2 (Haldol only)	116.25±5.70	2.10±0.37	1.60±0.31	104.00±48.54
GRP3 (Extract only)	66.67±7.14*	6.40±0.92*	5.00±0.40*	360.67±58.95*
GRP4 (Haldol + bromocriptine)	60.60±2.70*	8.30±0.41*	6.50±0.77*	420.20±60.44*
GRP5 (Haldol + low dose)	94.00±4.36	3.93±0.78	4.40±0.26*	194.33±64.69
GRP6 (Haldol + medium dose)	82.20±8.86*	6.75±0.54*	5.60±0.29*	276.40±50.25*
GRP7 (Haldol + high dose)	72.80±6.15*	8.10±0.79*	6.20±0.43*	432.40±60.44*

Values are mean ± SEM of six animals. *Significant difference when compared to Haldol only group ($p < 0.05$).

Table 3. Effect of administration of haldol and poly herbal preparation on glucose and total protein levels of female Wistar rats.

Group/treatment	Glucose (mg/dl)	Total protein (mmol/L)
GRP1 (Distilled water)	106.00±4.27*	67.28±5.32
GRP2 (Haldol only)	271.25±6.10	72.97±5.53
GRP3 (Extract only)	108.33±35.56*	65.82±5.30
GRP4 (Haldol + bromocriptine)	95.00±42.12*	59.66±3.47
GRP5 (Haldol + low dose)	109.00±47.13*	63.63±3.67
GRP6 (Haldol + medium dose)	105.40±34.96*	61.03±3.52
GRP7 (Haldol + high dose)	92.20±14.41*	67.15±3.43

Values are mean ± SEM of six animals. *Significant difference when compared to Haldol only group ($p < 0.05$).

Table 4. Effect of administration of haldol and poly herbal preparation on lipid profiles of female Wistar rats.

Group/treatment	Triglyceride mmol/L	Cholesterol mmol/L	High density lipoprotein mmol/L	Low density lipoprotein mmol/L
GRP1 (Distilled water only)	0.61±0.11*	3.08±0.03*	1.20±0.55*	0.73±0.35*
GRP2 (Haldol only)	1.73±0.03	0.54±0.36	0.85±0.11	1.26±0.3
GRP3 (Extract only)	0.63±0.17*	3.23±0.25*	1.37±0.06	0.50±0.23*
GRP4 (Haldol + bromocriptine)	0.56±0.15*	3.34±0.26*	1.44±0.11*	0.10±0.15*
GRP5 (Haldol + low dose)	0.72±0.16*	2.68±0.15*	1.18±0.27*	0.61±0.44*
GRP6 (Haldol + medium dose)	0.68±0.14*	2.93±0.25*	1.41±0.89*	0.30±0.28*
GRP7 (Haldol + high dose)	0.58±0.05*	3.29±0.28*	1.56±0.10*	0.25±0.24*

*Significant difference when compared to haldol only group ($p < 0.05$).

Similarly, from the results shown, the increase in the prolactin level strongly correlated with reduced gonadotropins and lowering of the prolactin also correlated with increase in the level of the gonadotropins. This strongly suggests that the extract effect was most likely through its ability to lower the prolactin level.

The effect of the polyherbal mixture was more striking on the serum estrogen levels. The extract was also able to increase the estrogen levels in a dose dependent manner. In fact, the highest dose had a very significant estrogen increasing effect when compared to the medium dose. The trend was similar to that observed for the

gonadotropins but was greater in magnitude. Since the estrogens are part of the down line hormones of the hypothalamus-pituitary-gonadal axis, its level is regulated by the prolactin and the gonadotropins (Mohan and Marzher, 2010). However, the very significant effect seen might not be due to the gonadotropin stimulation only. Some components of the herbal mixture might have estrogen stimulation effect mediated through their constituents such as terpenoids (Telefo et al., 2004).

Many of the drugs that affect the reproductive hormones one way or the other have effect on the metabolic parameters, especially the energy metabolism, often leading to the problem of obesity/weight gain (Baptistaa et al., 1999). There was a significant elevation of blood glucose after the administration of haldol. The increase in blood glucose was likely to be due to hyperprolactinemia (Reis et al., 2004). The ability of bromocriptine to significantly bring down the glucose level was also major evidence that the high level of glucose observed in the haldol only group was most likely due to hyperprolactinemia. However, the possibility of haldol contributing to the increase in the blood glucose cannot be ruled out.

Haldol (or the typical antipsychotics) has been reported to raise the blood glucose (Wirshing et al., 2002). The plant was able to reverse the glucose concentration back to the level comparable to that of the control group. Though there was continuous reduction in the glucose levels as the extract dose increased, the difference was not so significant to conclude that the glucose lowering effect was dose dependent. Adeneye et al. (2008) have reported that *C. capitatum* which was one of the plants in this herbal mixture possess hypoglycemic property in Wistar rats. The extract of *M. charantia* has also been reported to stimulate cellular uptake of glucose (Yibchok-Anun et al., 2006).

Study has indicated that the primary constituents responsible for the hypoglycemic properties of *M. charantia* are charantin, insulin-like plant peptide, cucurbutanoids, momordicin, and oleanolic acids (Harinantenaina et al., 2006). Other constituent with hypoglycemic activity is *B. vulgaris,* as reported by Senthilkumar et al. *(*2011) which has the potency of reducing glucose significantly in hyperglycemia. The pattern of the plasma total protein did not show any significant difference across all groups, though there was a slight elevation in the plasma protein in the haldol only group. This indicates that both haldol and the extract do not seem to affect the plasma protein.

One of the commonest and most obvious effects of the typical antipsychotics is weight gain with increase in the mass of the adipose tissues (Huang and Lu, 2007). This correlates with the observation that there was a significant increase in the plasma triglycerides after haldol administration in this study. The herbal mixture reverted the effect on the triglycerides significantly and also in a dose dependent manner, and the triglyceride lowering effect with the highest dose was comparable to that observed for bromocriptine. This also suggests that the elevation of the triglyceride level might be secondary to hyperprolactinemia. The decoction also lowered plasma LDL level but increased HDL and total cholesterol levels. Chatuwedi (2005) confirmed that *M. charantia* reduces triglycerides (TG) and LDL but increases HDL. Adeneye et al. (2008) also reported that *C. capitatum* extract showed hypolipidemic effect in Wistar rats. The herbal mixture could therefore be said to have potential anti-obesity property and may prevent obesity related cardiovascular disorder.

There has been an increase in the trend of research using polyherbal preparations rather than the individual plants. This is mainly due to the fact that most herbal preparations used locally are often made from more than one herb. In this study, we sourced for the recipe which is locally reputed to be effective in the treatment of hyperprolactinemia and its symptoms. We found that the preparation made up of five different herbs was able to reverse the effects of haldol in rats. The results from this study support the folkloric claim of this polyherbal preparation. Some of the effects such as anti-hyperprolactinemic, anti-hyperglycemic and anti-hyperlipidemic effects of the herbal mixture in this study have been previously reported for some individual plants that made up the whole herbal preparation. However, more research should be carried out to be able to find out which particular plant is responsible for which effect and also to know which plant constituents are responsible. The effect seen in the polyherbal mixture might be a synergistic one which could only have been obtained in the mixed form. There is also the possibility that zeroing down on a particular plant or plant component might be more effective.

Further studies should be carried out to investigate the long term toxic effects of this polyherbal mixture. Some components of the herbs may have some toxic properties which may be of concern. There may be any herb-herb interaction which may be undesirable on the long term. Similarly, studies should also be carried out to investigate the drug-herb interactions that may possibly occur if the herbal mixture is taken alongside other synthetic drugs.

REFERENCES

Adeneye AA, Adeleke TI, Adeneye AK (2008) Hypoglycemic and hypolipidemic effects of the aqueous fresh leaves of *Clerodendrum capitatum* in Wistar rats. J. Ethnopharmacol. 116(1):7-10

Asa SL, Ezzat S (2002). The pathogenesis of pituitary tumours. Nature Rev. Cancer 2:836-849.

Baptistaa T, Reyesa D, Hernándeza L (1999). Antipsychotic Drugs and Reproductive Hormones: Relationship to Body Weight Regulation. Pharmacol. Biochem. Behav. 62(3)409–417.

Harinantenaina L, Tanaka M, Takaoka S (2006). *Momordica charantia* constituents and antidiabetic screening of the isolated major compounds. Chem. Pharm. Bull. 54:1017-1021.
Hasani- Ranjbar S, Vahidi H, Taslimi S, Karimi N, Larijani B, Abdollahi M (2010). A systematic review on the efficacy of herbal medicines in the management of human drug-induced hyperprolactinemia; potential sources for the development of novel drugs. Int. J. Pharmacol. 6:691-695.
Huang T, Lu C, Correlations between Weight Changes and Lipid Profile Changes in Schizophrenic Patients after Antipsychotics. Therapy. Chang Gung Med. J. 30:26-32.
La Torre D, Falorni A (2007). Pharmacological causes of hyperprolactinemia. Clin. Risk Manag. 3(5):929-951.
Lee S, Ahn J, Kim S, Kim J, Han S, Jung M, Chung I (2003). Variation in isoflavone of soybean cultivars with location and storage duration. J. Agric. Food Chem. 51:3382-3389.
Mahdi JG, Mahdi AJ, Mahdi AJ, Bowen ID (2006). The historical analysis of aspirin discovery, its relation to the willow tree and its anti-proliferative and anti-cancer potential. Cell Prolif. 39:147-155
Mancini T, Casanueva FF, Giustina A (2008). Hyperprolactinemia and Prolactinomas. Endocrinol. Metab. Clin. North Am. 37(1):67.
Mohan K, Marzher S (2010). FSH, LH, Prolactin levels in infertile women in North Chennai, Tamilnadu. J. Biosci. Res. 1(4):279-284
Nilsson LA, Roepstorff C, Kiens B (2009). Prolactin suppresses malonyl-CoA concentration in human adipose tissue. Horm. Metab. Res. 41(10):747-751.
Reis FM, Santos MA, Reis AM, Coimbra CC (1994). Effects of hyperprolactinemia on plasma glucose and prolactin in rats exposed to ether stress. Physiol. Behav. 56(3):495-499.
Rosenbloom AL (2010). Hyperprolactinemia with Antipsychotic Drugs in Children and Adolescents Int. J. Pediatr. Endocrinol. (159402)1-6
Senthilkumar MK, Sivakumar P, Faisal C, Rajesh V, Perumal P (2011). Evaluation of anti-diabetic activity of *Bambusa vulgaris* leaves in streptozotocin induced diabetic rats. Int. J. Pharm. Sci. Drug Res. 3(3):208-210.
Shrivastava N, Patel T (2007). Clerodendrum and Heathcare: An Overview. Med. Aromat. Plant Sci. Biotechnol. 1(1):142-150.
Telefo PB, Moundipa PF, Tchouanguep FM, (2004). Inductive effect of the leaf mixtures extracts of *Aloe buettner, Justcia insularis, Dicliptera verticillata* and Hibiscus macranthus on the invitro production of estradiol. J. Ethnopharmacol. 91(2-3):225-30.
Yibchok-Anun S, Adisakwattana S, Yao CY (2006). Slow acting protein extract from fruit pulp of *Momordica charantia* with insulin secretagogue and insulinomimetic activities and insulinomimetic activities. Biol. Pharm. Bull. 29:1126-1131.
Yuan HN, Wang CY, Sze CW, Tong Y, Tan QR, Feng XJ, Liu RM, Zhang JZ, Zhang YB, Zhang ZJ (2008). A randomized, crossover comparison of herbal medicine and bromocriptine against risperidone-induced hyperprolactinemia in patients with schizophrenia. J. Clin. Psychopharmacol. 28(3)264-370.

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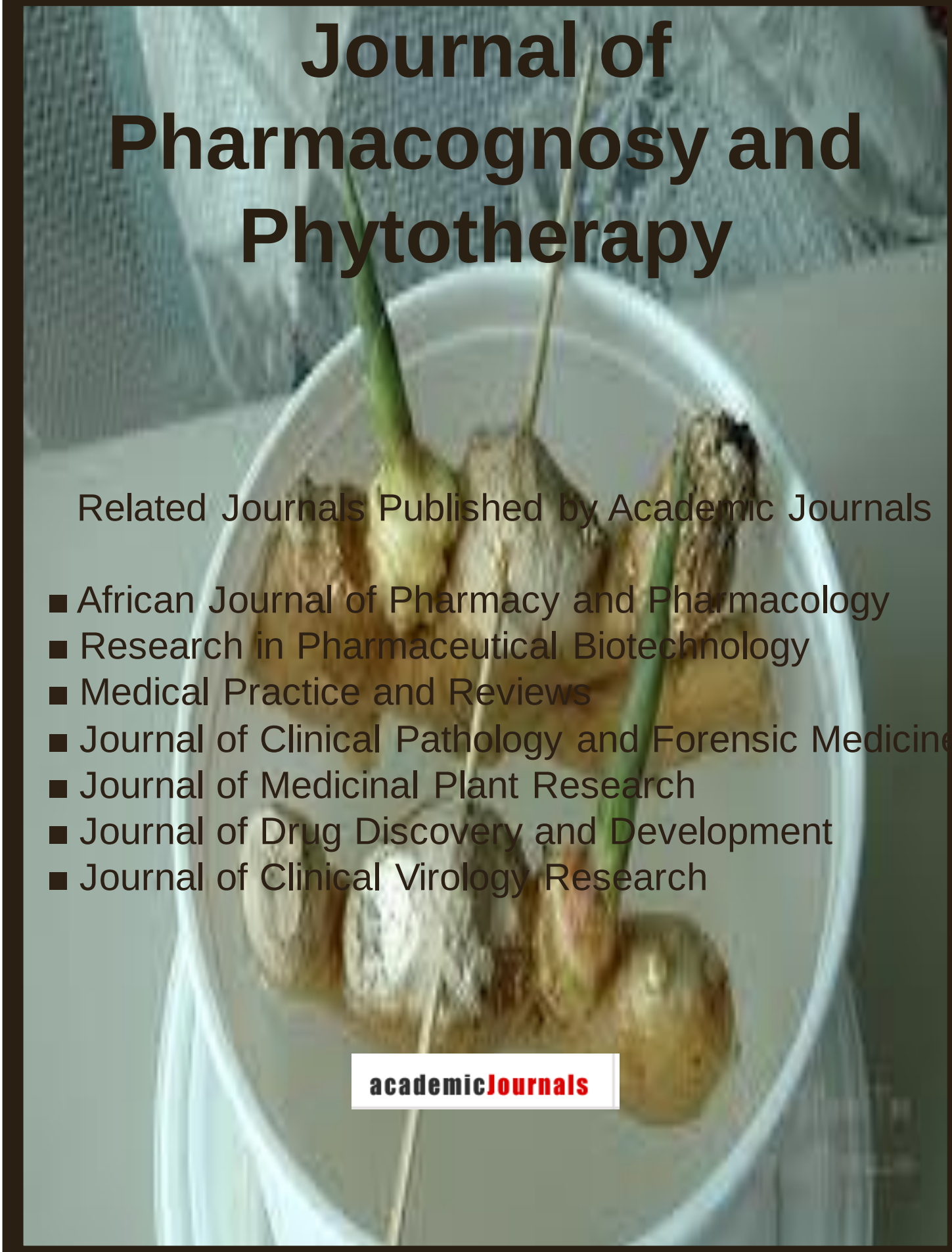

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