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Full Length Research Paper

Insilico docking studies and evaluation of volatile oil of *Zingiber officinale* Rosc. and Aripiprazole for antidepressant activity

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The objective of the study was to evaluate the volatile oil of *Zingiber officinale* Rosc. and Aripiprazole for antidepressant activity and to carry out the docking studies for their respective binding sites. Argus Lab 4.0.1 software (Mark A. Thompson, Planaria Software LLC, and Seattle, WA, USA) was used to perform the docking studies and the elevated plus maze, forced swim test, learned helplessness and open field tests were used for assessing the antidepressant activity. Imipramine (20 mg/kg, orally) was administered to Albino wistar rats as reference drug. Docking results reveal that the gingerol, aripiprazole and imipramine actively interacted with $5HT_{1A}$ receptor with high docking score (shogoal: -9.0267, gingerol: -8.41044, aripiprazole: -8.73428 and imipramine: -8.21131 Kcal/mol).The volatile oil of *Z. officinale* Rosc. (14 mg/kg, orally) and aripiprazole (10 mg/kg, orally), was able to increase the time and number of entries in the open arm in elevated plus maze, as well as to decrease the immobility time of rats subjected to forced swimming test, and to decrease the number of failures to escape when subjected to learned helplessness test. Volatile oil of *Z. officinale* Rosc. and aripiprazole showed a significant increase in the locomotor and exploratory behavior in open field test. The volatile oil of *Z. officinale* Rosc. and aripiprazole showed significant antidepressant activity compared to that of standard drug.

Key words: *Zingiber officinale* Rosc., Aripiprazole, Docking, Antidepressant, $5HT_{1A}R$.

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. Tricyclic antidepressants routinely produce adverse autonomic responses, in part related to their relatively potent antimuscarinic effects. These include dry mouth and a sour or metallic taste, epigastric distress, constipation, dizziness, tachycardia, palpitations, blurred vision and urinary retention. MAO inhibitors can induce sedation or behavioral excitation and have a high risk of inducing postural hypotension, sometimes with sustained mild elevations of diastolic blood pressure. As a group, the SSRIs and SNRIs have a high risk of nausea and vomiting, headache, and sexual dysfunction. Newer antidepressants generally present lesser or different side effects and toxic risks than older tricyclics and MAO inhibitors (Rajput et al., 2011).

Due to the shortcomings of the available antidepressant

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drugs, attempts are underway to explore newer antidepressants and plants with antidepressant activity.

Computational (*In silico*) methods have been developed and widely applied to pharmacology hypothesis development and testing. These *in silico* methods include database searching, quantitative structure-activity relationships, similarity searching, pharmacophore identification, computational modeling and docking. Such methods have seen frequent use in the discovery and optimization of novel molecules with affinity to a target, the clarification of absorption, distribution, metabolism, excretion and toxicity properties.

The aim of the current study was to evaluate the volatile oil of *Z. officinale* and Aripiprazole in animal models of depression, and to scientifically validate the claim by *insilico* docking studies for their respective binding sites.

MATERIALS AND METHODS

Drugs

Imipramine tablet (Depsonil 25mg/kg), Aripiprazole tablet (Arpizol 10 mg/kg), Ginger rhizome [rhizomes of *Zingiber officinale* Roscoe (Zingiberaceae)] was obtained locally from Ettumanoor, botanically authenticated and volatile oil of *Z. officinale* (14 mg/kg) was collected (Sayyad et al., 2010) and administered to rats. The imipramine at a dose of 20 mg/kg and Aripiprazole at a dose of 10 mg/kg were suspended in 0.5% Carboxymethylcellulose sodium and administered.

Softwares

The softwares used included, ACD Labs Chemsketch 10.00 software, Argus Lab 4.0.1 software (Mark A. Thompson, Planaria Software LLC, Seattle, WA, USA).

Animals

Adult male Albino wistar rats weighing 200-250 g were procured from animal house of University college of Pharmacy, Cheruvandoor, Ettumanoor, Kottayam for experimental purpose. Then all animals were acclimatized under standard husbandry conditions at room temperature of 24 ± 1ºC, relative humidity of 45-55% and a 12:12 h light and dark cycle. The animals were kept in groups of 6 in polypropylene cages and had free access to standard rat pellet, with water supplied *ad libitum* under strict hygienic conditions. All experimental protocols were designed to minimize the number of animals and sufferings, and were approved by the Institutional Animal Ethics Committee (IAEC No: 017/MPH/UCP/CVR/12) of the University college of Pharmacy, Cheruvandoor, Ettumanoor, Kottayam.

Computational details-docking

Docking studies were performed for natural compounds such as Gingerol and Shogoal, and synthetic compounds such as Aripiprazole and Imipramine (ligands) with $5HT_{1A}$ receptor protein by using Argus Lab software.

Preparation of protein structure

The sequence of the $5HT_{1A}$ receptor protein was retrieved from protein data-base of NCBI (http://www.ncbi.nlm.nih.gov/) in FASTA format and it was searched against selection of the related homologues of query sequence in PDB. The homology modelling requires sequences of known 3D structure and the target having above 35% of similarity. BLASTP was used to identify the most suitable template for homology modeling of $5HT_{1A}$ receptor protein. The available structures of protein in the Protein Database were: 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 et al., 1999). After careful examination for the potential alignment errors, the automated comparative protein modeling program I-TASSER was employed to build the model (Zhang et al., 2008). The statistical verification of the model was evaluated with PROCHECK; a structure verification program relies on Ramachandran plot which determines the quality of the predicted structure (Vaseeharan et al., 2011). 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, Aripiprazole and Imipramine) were selected from ChemSketch, and chemically intelligent drawing interface freeware developed by Advanced Chemistry Development, Inc., (http://www.acdlabs.com) was used to construct the structure of the ligands. Geometry optimizations of the ligands were performed according to the calculation method by Argus Lab 4.0.1 software.

Protein-ligand docking

$5HT_{1A}$ 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 on to the predicted active 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 AScore was used as the scoring function. At maximum, 150 poses were allowed to be analyzed, binding site box size was set to 20 x 20 x 20 angstroms so as to encompass the entire active site. The AScore 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 datasetwere 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.

Experimental laboratory *in vivo* studies

Elevated plus maze (EPM) test (Ruiz et al., 2006; Bajaj et al., 2000; Mineur et al., 2006)

The plus-maze consists of two open arms, 50 × 10 × 40 cm, and two enclosed arms, 50 × 10 × 40 cm, with an open roof, arranged so that the two open arms are opposite to each other. The maze is elevated to a height of 50 cm. The rats (200-250 g body weight) were housed in pairs for 10 days prior to testing in the apparatus. Groups consisted of six rats for each dose. 30 min after administration of the test drug or the standard, the rat was placed in the center of the maze, facing one of the enclosed arms. Entry into an arm was defined as the animal placing all four paws onto the arm. During a 5 min test period the following measures are taken: the number of entries into and time spent in the open and enclosed arms.

Forced swim test (FST) (Mineur et al, 2006; Burda et al, 2011, Yadav et al, 2010)

The FST is the most widely used pharmacological *in vivo* model assessing anti depressant activity. Male Albino wistar rats weighing 160-180 g weare used. They were brought to the laboratory at least one day before the experiment and were housed separately in cages with free access to food and water. Rats are individually forced to swim inside a vertical Plexiglas cylinder (height: 40 cm; diameter: 18 cm, containing 15 cm of water maintained at 25°C). Rats placed in the cylinders for the first time were initially highly active, vigorously swimming in circles, trying to climb the wall or diving to the bottom. After 2-3 min, activity begins to subside and to be interspersed with phases of immobility or floating of increasing length. After 5-6 min, immobility reached a plateau where the rats remained immobile for approximately 80% of the time. After 15 min in the water, the rats were removed and allowed to dry in a heated enclosure (32°C) before being returned to their home cages. They were again placed in the cylinder 24 h later and the total duration of immobility was measured during a 6 min test. Test drugs or standard were administered 1 h prior testing.

Open field test (OFT) (Bhattamisra et al., 2007; Ruiz et al., 2006)

The open field apparatus was made of plywood and consisted of squares (61×61 cm). Blue lines were drawn on the floor with a marker. The lines divided the floor into sixteen squares. A central square was drawn in the middle of the open field. Each animal was centrally placed in the test apparatus for 5 min and the following behavioral aspects were noted: i) ambulation; ii) rearing; iii) self grooming, and iv) activity in center. To assess the process of habituation to the novelty of the arena, animals were exposed to the apparatus for 5 min on two consecutive days. The number of line crosses and the frequency of rearing are usually used as measures of locomotor activity.

Learned helplessness test (Bajaj et al., 2000; Vogel et al., 2002)

Animals exposed to inescapable and unavoidable electric shocks in one situation later failed to escape shock in a different situation when escape is possible. This phenomenon was evaluated as a potential animal model of depression. Learned helplessness is produced in male Albino wistar rats by exposure to electric shock (0.8 mA) for 1 h on a schedule of 15 s of shock/min. The apparatus is a 30 × 45 × 30 cm box with a grid floor. At a height of 20 cm above the floor, a platform (7.5 × 7.5 cm) can be inserted through one side wall to allow a jump-up escape response. The platform is not available during training.

During this avoidance training, the rats were placed in the electrified chamber, allowed to acclimatize for 5 min. After the appropriate treatment, the animals are tested for acquisition of a jump up escape in the same apparatus.

At the beginning of a trial, the platform is pushed into the box and shock initiated. Shock is terminated in 10 s if the animal has not escaped onto the platform by this time. If an escape response occurred, the animal is allowed to remain on the platform for the duration of 10 s, and then returned to the grid floor. 30 such trials with an intertrial interval of 30 s are given. Drugs were given before the training and the test period. The avoidance response characterized by escape to the adjoining “safe” chamber during conditioned stimulus, was noted and failure to escape within 15 s was assessed as “escape failure”.

Statistical analysis

The values were expressed as mean ± standard error of mean (SEM) from six animals. The results were subjected to statistical analysis by using one-way analysis of variance (ANOVA) followed by Dunnet test to calculate the significance difference if among the groups. $P < 0.05$ was considered as significant.

RESULTS

Homology modeling

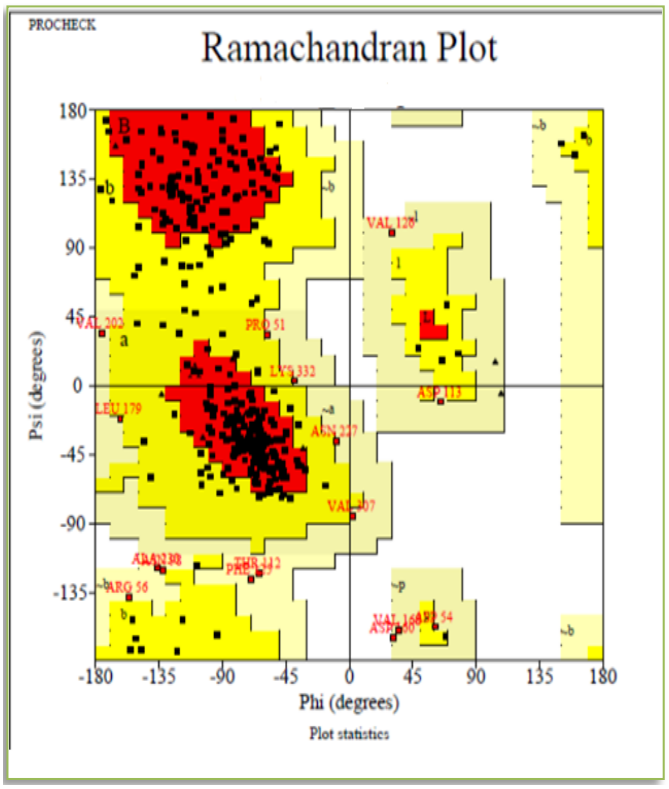
The 5-HT_{1A} protein was modeled using the templates for homology modeling using I-TASSER. The stereo chemical quality of the protein model was assessed using Ramachandran plot analysis (Figure 1). The red regions in the graph indicate the most allowed regions whereas the yellow regions represent allowed 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 5HT1A with a higher average precision. The active site of $5HT_{1A}$ comprised of amino acid residues such as LEU 453, PHE 454, and TYR 457 (Figure 2).

Docking

To understand the interactions between the ligands and $5HT_{1A}$ protein and to explore their binding mode, docking study was performed using ArgusDock available under



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.

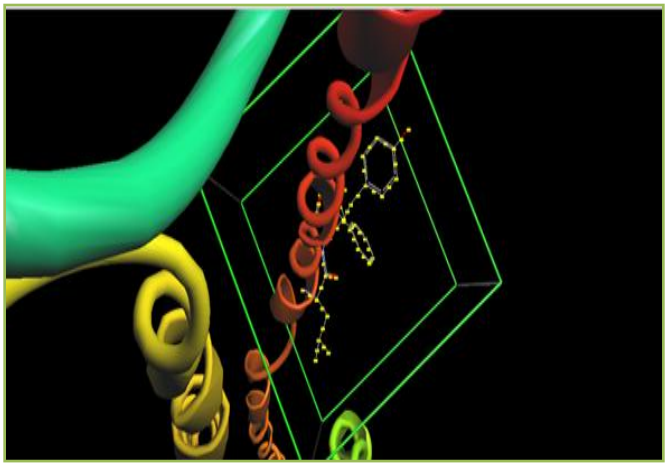


Figure 2. Active binding site of $5HT_{1A}$ protein.

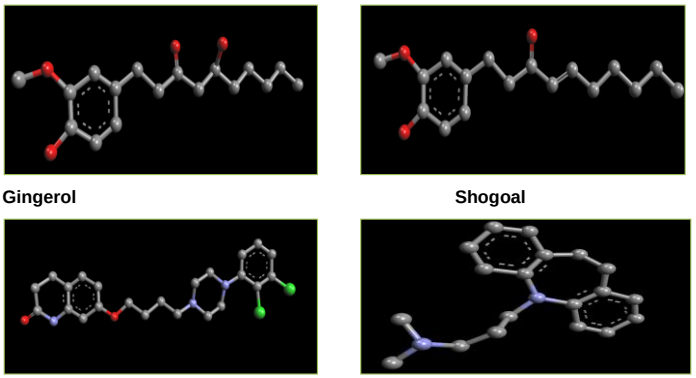


Figure 3. Structure of ligands.

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 3.

Docking scores

The docking scores were highest for Shogoal with -9.0267 Kcal/mol, Aripiprazole with -8.73428 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 4 to 7. Binding mode of Gingerol with $5HT_{1A}$ Protein

In vivo studies

Elevated plus maze test (EPM)

The volatile oil of *Z. officinale* and Aripiprazole were

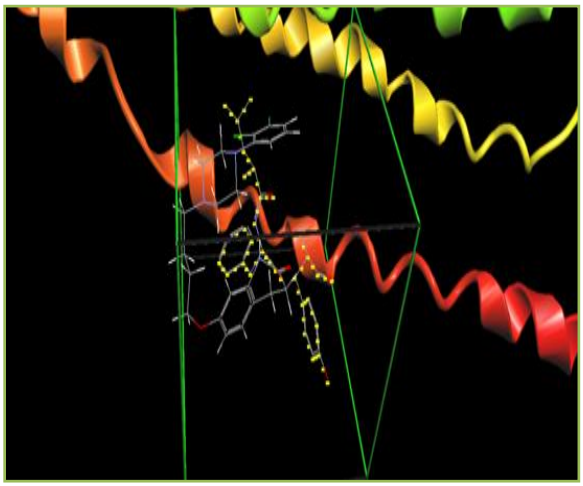


Figure 4. $5HT_{1A}$-Gingerol complex: Yellow color represents binding site residues; grey color represents the ligand; blue, red and green color represents the rest of the protein.

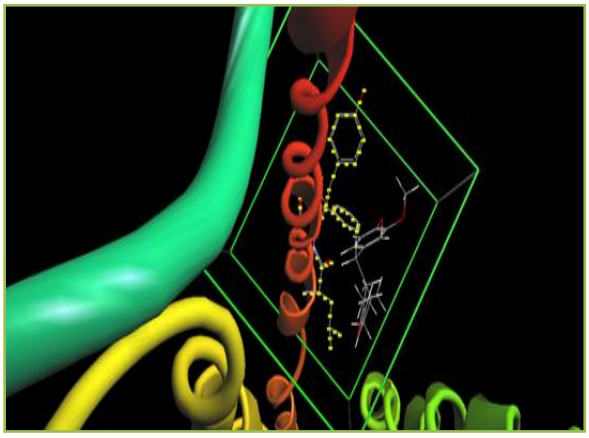


Figure 5. $5HT_{1A}$- Shogoal complex: Yellow color represents binding site residues; grey color represents the ligand; blue, red and green color represents the rest of the protein.

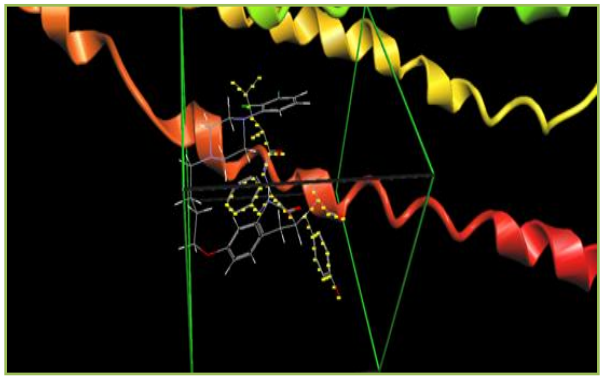


Figure 6. $5HT_{1A}$- Aripiprazole complex: Yellow color represents binding site residues; grey color represents the ligand; blue, red and green color represents the rest of the protein.

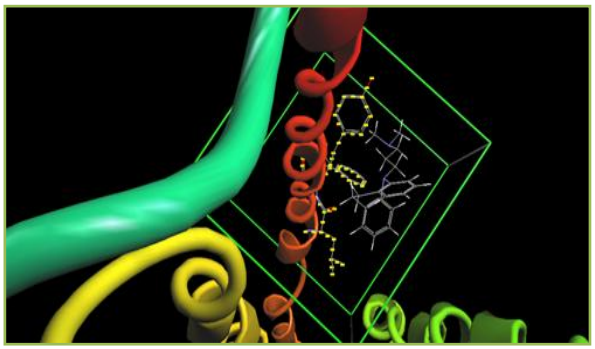


Figure 7. $5HT_{1A}$- Imipramine complex: Yellow color represents binding site residues; grey color represents the ligand; blue, red and green color represents the rest of the protein.

administered orally to male Albino wistar rats and the number of entries and time spent in open arms and closed arms were measured when exposed to elevated plus maze test. The animals showed more number of entries and time spent in open arms compared to the negative control (Table 1, Figures 8 and 9).

Forced swim test (FST)

The volatile oil of *Z. officinale* and Aripiprazole were administered orally to male Albino *wistar* rats and the duration of immobility was measured. The immobility period was shortened during the forced swimming test in comparison with the control. The results are summarized in Table 2 and Figure 10.

Open field test (OFT)

The open field test was done in order to determine the effect of the administration of the volatile oil of *Zingiber officinale* and Aripiprazole upon spontaneous motor activity. The volatile oil of *Z. officinale* (14 mg/kg) showed an increase in all exploratory and locomotor parameters in open field test compared to control group. Animals treated with Imipramine have shown significant antidepressant activity in the open field test (Table 3 and Figure 11).

Learned helplessness test

The volatile oil of *Z. officinale* and Aripiprazole was administered orally to male Albino *wistar* rats and number of escape failure was measured when subjected to learned helplessness test. The treatment of test drugs caused reduction in escape failure when compared to control animals subjected to shock. The effects were

Table 1. Antidepressant effects of volatile oil of *Zingiber officinale* and Aripiprazole using Elevated plus maze test in rats.

Treatment group	Number of entries in		Time spent in seconds in	
	Closed arms	Open arms	Closed arms	Open arms
Control	8.16 ± 0.70	4.33 ± 0.95	205.83 ± 3.27	94.16 ± 3.27
Imipramine	5.66 ± 0.42	7.33 ± 0.55*	140.00 ± 10.40**	160.00 ± 10.40**
Aripiprazole	6.16 ± 0.47	7.5 ± 0.56	145.83 ± 10.23*	154.16 ± 10.23*
Volatile oil of *Z. officinale*	4.83 ± 0.60*	8.16 ± 0.47*	132.33 ± 3.31**	167.66 ± 3.31**
F	5.142	5.730	18.78	19.83
DF	3. 5	3. 5	3. 5	3. 5
P value	0.0244	0.0333	0.0025	0.0008

Values are mean ± SEM. *P< 0.05, **P< 0.01, ***P< 0.001 when compared to control group, n= 6, on the ANOVA followed by post-hoc Dunnet test.

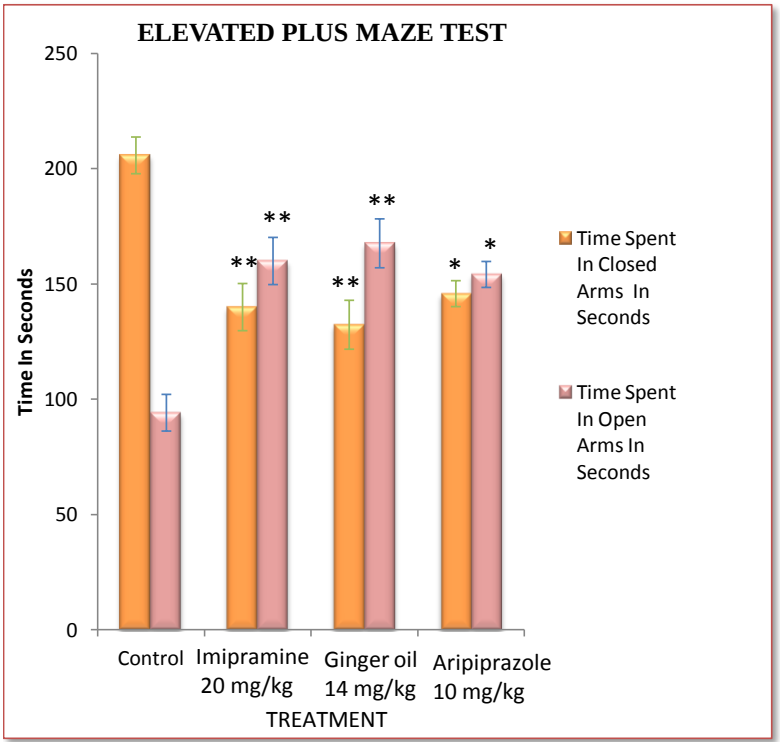


Figure 8. Antidepressant effect produced by the oral administration of volatile oil of *Zingiber officinale* and Aripiprazole on the time spend into the open arms by rats exposed to the EPM test. *P< 0.05, **P< 0.01, ***P< 0.001 when compared to control group, n= 6, on the ANOVA followed by post- hoc Dunnet test (mean ± SEM).

comparable to Imipramine. The test drugs and Imipramine also caused significant increase in avoidance response.

Analysis of the results of the docking software suggested that Gingerol and Shogoal can act as potent antidepressants (Table 4). For the binding analysis, 5HT1A 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.

DISCUSSION

The current therapeutic goal in the treatment of major depression is to improve quality of life by normalizing

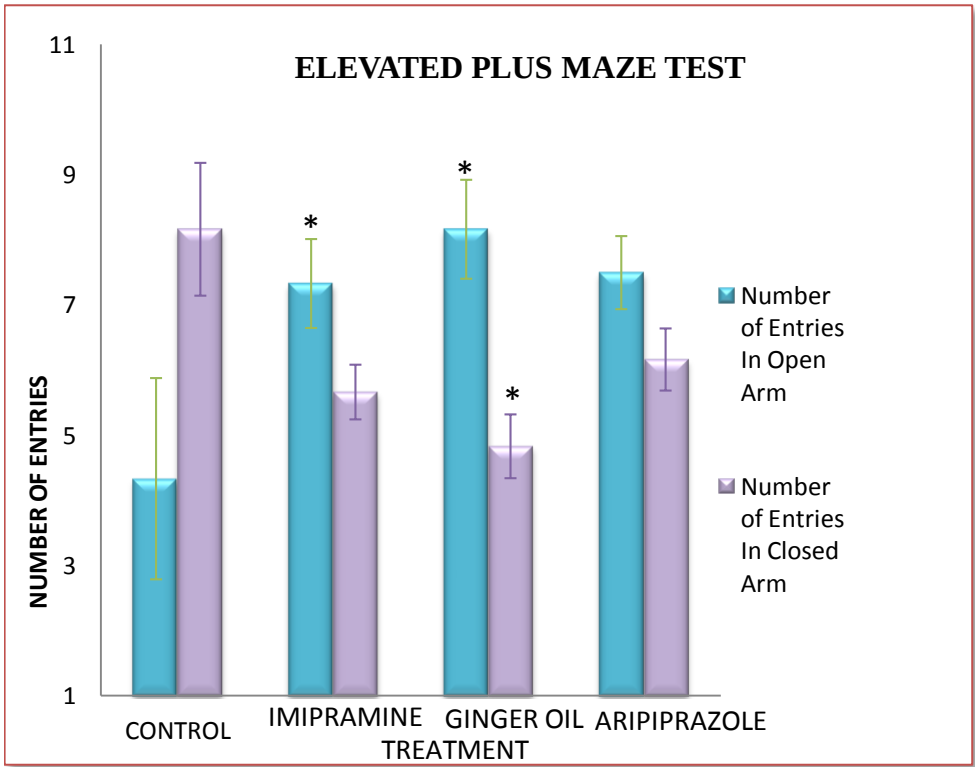


Figure 9. Antidepressant effect produced by the oral administration of volatile oil of *Zingiber officinale* and Aripiprazole on the number of entries into the open arms by rats exposed to the EPM test. *P< 0.05, ** P< 0.01, *** P< 0.001 when compared to control group, n= 6, on the ANOVA followed by post-hoc Dunnet test (mean ±SEM).

Table 2. Antidepressant effect of volatile oil of *Zingiber officinale* and Aripiprazole on duration of immobility in forced swim test using rats.

Treatment group	Latency period (s)	Duration of immobility (s)
Control	101.66 ± 4.77	263.33±5.57
Imipramine	193.33 ± 8.02**	166.66±8.02**
Aripiprazole	160.83 ± 8.98*	199.16±8.98*
Volatile oil of *Zingiber officinale*	208.33±10.77**	151.66±10.77**
F	33.16	30.74
DF	3.5	3. 5
P value	<0.0001	<0.0001

Values are mean ± SEM. *P< 0.05,** P< 0.01,*** P< 0.001 when compared to control group, n= 6.

mood and reversal of functional and social disabilities associated with depression. Molecular docking continues to holds 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.

The 5-HT_{1A} protein was modelled and the quality of the 3D model was evaluated using the PROCHECK program and assessed using the Ramachandran plot. It is evident from the Ramachandran plot that the predicted models have most favorable regions, the allowed regions, the generic regions and the disallowed regions. Such a percentage distribution of the protein residues determined by Ramachandran plot shows that the predicted models are of good quality. The models show all the main chain and side chain parameters to be in the 'better' region.

The docking analysis of Gingerol, Shogoal, Aripiprazole and Imipramine was carried out using Argus lab and the

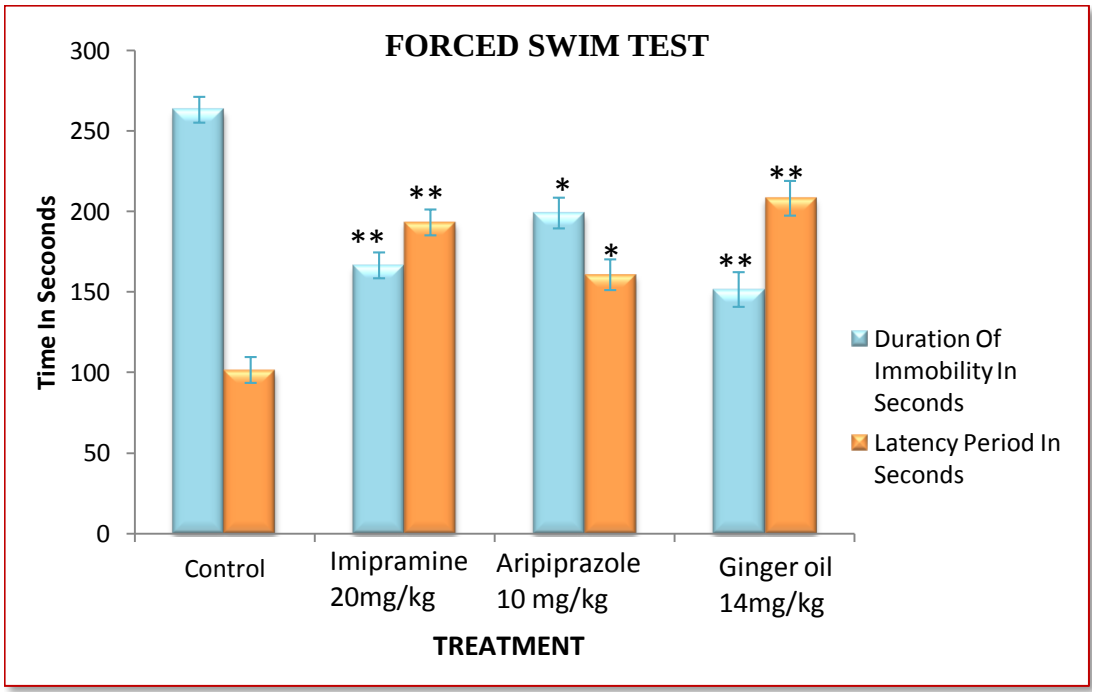


Figure 10. Antidepressant effect produced by the oral administration of volatile oil of *Zingiber officinale* and Aripiprazole on the immobility time of male Albino wistar rats exposed to the forced swimming paradigm. *P< 0.05, ** P< 0.01, ***P < 0.001 with ANOVA followed by post- hoc Dunnet test (mean ±SEM).

Table 3. Antidepressant effect of volatile oil of *Zingiber officinale* and Aripiprazole on rats in open field test.

Treatment group	Ambulation (N)	Rearing (N)	Self grooming (N)	Activity in the center (N)
Control	30.66 ± 2.53	17.33 ± 1.20	3.16 ± 0.54	2.33 ± 0.47
Imipramine	61.33 ± 3.19***	29.33 ±0.66***	6.66 ± 0.66***	4.83 ± 0.79*
Aripiprazole	41.33 ± 2.33*	17.66 ± 0.84	5.00 ± 0.44	3.00 ± 0.57
Volatile oil of *Zingiber officinale*	54.00 ± 2.26***	21.00 ± 0.73*	5.33 ± 0.49*	3.5 ± 0.42
F	26.44	46.65	6.183	4.515
DF	3.5	3.5	3.5	3.5
P Value	0.0001	<0.0001	0.0143	0.0145

Values are mean ±SEM. *P< 0.05, ** P< 0.01, ***P< 0.001 when compared to control group, n= 6, on the ANOVA followed by post-hoc Dunnet test (mean ±SEM).

binding energies obtained ranged from -8.21131 to -9.0267 Kcal/mol (Figure 4 to 7) A higher score indicates a stronger receptor -ligand binding affinity (Daisy et al., 2009). The docking result indicates that these ligands possess antidepressant activity and further evaluated by *in vivo* studies.

In the present investigation, volatile oil of *Z. officinale* (14 mg/kg) and Aripiprazole (10 mg/kg) exhibited significant (P<0.01 and P<0.05 respectively) antidepressant effects in FST in stressed rats. (Figure10) This model of depression is widely used for the screening of novel antidepressant drugs. The test is quite sensitive and relatively specific to all major classes of antidepressants drugs like tricyclics, selective serotonin reuptake inhibitors (SSRIs) and monoamine oxidase inhibitors (MAOIs).

In the present study, rats were forced to swim in restricted space from which the escape is not possible and their struggling time of 5-min session ends at a point when the helpless despair syndrome produced. It has been shown that stress induces a state of helpless despair condition in animals, which is equivalent and claimed to a condition similar to human depression. This attribution of animals' response to the development of depression

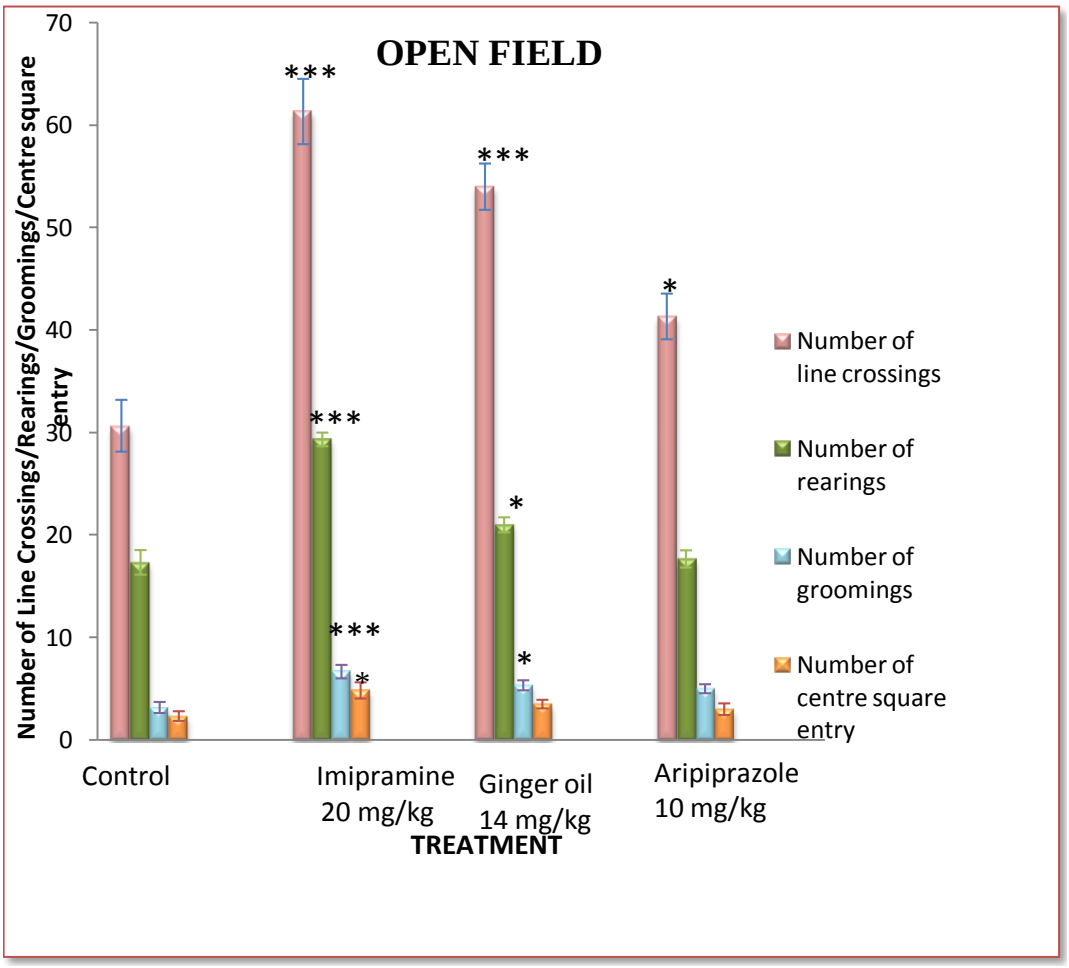


Figure 11. Antidepressant effect produced by the oral administration of the volatile oil of *Zingiber officinale* and Aripiprazole on the total number of line crossings, rearings, grooming and center square entry in male Albino *wistar* rats exposed to the open field paradigm. *P< 0.05, **P< 0.01, ***P< 0.001 when compared to control group, n=6, on the ANOVA followed by Dunnet test (mean ±SEM).

Table 4. Antidepressant effect of volatile oil of *Zingiber officinale* and Aripiprazole on learned helplessness using rats.

Treatment group	Number of escape failure
Control	9.16 ± 0.30
Imipramine	6.33 ± 0.55**
Aripiprazole	6.50± 0.22**
Volatile oil of *Zingiber officinale*	4.33 ± 0.42**
F	20.63
DF	3.5
P value	0.0018

Values are mean ±SEM. *P< 0.05, **P< 0.01, ***P< 0.001 when compared to control group, n= 6, on the ANOVA followed by post- hoc Dunnet test (mean ± SEM).

process can be managed by the treatment with antidepressants. In this study, a significant reduction in the immobility (by increasing mainly swimming behaviors and partially climbing) was observed following the oral

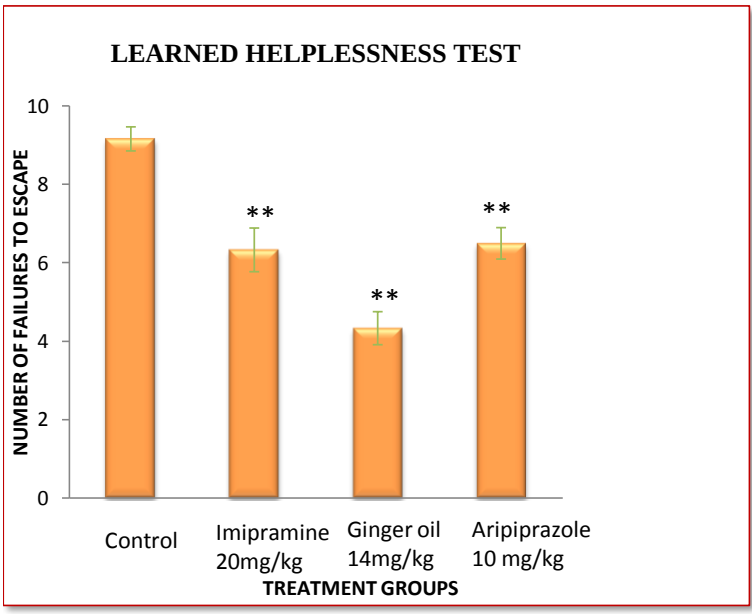


Figure 12. Antidepressant effect produced by the oral administration of volatile oil of *Zingiber officinale* and Aripiprazole on the escape response of male Albino wistar rats exposed to the learned helplessness paradigm. *P< 0.05, **P< 0.01, ***P < 0.001 with ANOVA followed by post- hoc Dunnet test (mean ±SEM).

administration of the volatile oil of *Z. officinale* and Aripiprazole (Figure 10). Antidepressant drugs with predominant noradrenergic or dopaminergic elevating effects reduce immobility by increasing climbing behavior. Conversely, antidepressant drugs with predominant serotonin-elevating effects reduce immobility by increasing swimming behavior (Suzan *et al*, 2011). Hence one of the possible mechanisms of action of volatile oil of *Z.officinale* and Aripiprazole to decrease the immobility time may be due to the inhibition of monoamine uptake.

Open field behavioral model was used to study exploratory and locomotor activity of rats. It was reported that stress factors account for the decrease in mobility and functional responses against novel environment (Batool et al., 2011). The results observed in the open field test showed that oral administration of the volatile oil of *Z. officinale* and Aripiprazole to male albino *Wistar* rats following the exposure to repeated restraint stress showed significant increase in locomotor/exploratory activity on an open field arena (Figure 11). It is therefore, suggested that the volatile oil of *Z. officinale* and Aripiprazole has the ability to reverse or normalize the locomotor suppressant behavior in experimental animals and hence may help to cope with immobility associated with depression in humans. The results are in accordance with previous studies, indicates the possible increase in brain serotonin levels.

Stress-related anxiety is also one of the components of major depression in humans. EPM is a rat model of anxiety that is used for the screening of compounds with anxiolytic potential and used as a general research tool in neurobiology of anxiety and depression (Batool et al., 2011). In the present study, increase in the proportion of numbers of entries and time spent in open arms are taken as best index of reduced anxiety levels or reduction in stress-induced immobility (Figures 8 and 9). It has been shown that depressive disorders have been associated with disturbances in brain serotonin and there is a great relationship between serotonin and depression as serotonergic system play a major role in the action of antidepressants (Batool et al., 2011). The level of serotonin determines the involvement of CNS in the control of locomotion and emotions. The present results also supports the above mentioned studies, indicates antidepressant activity of the volatile oil of *Z.officinale* and Aripiprazole.

The learned helplessness paradigm is one model based on the observation that animals exposed to uncontrollable stress (electric shocks) in one situation, subsequently fail to escape shock in a different situation when escape is possible. Thus ‘‘helpless’’ rats treated pharmacologically with imipramine, volatile oil of *Z. officinale* and Aripiprazole produced not only a significant average improvement of failures, but also, in more than half of the animals a reversal from ‘‘helpless’’ to ‘‘non-helpless’’ has been produced (Figure 12). The present results are in general agreement with previous studies, indicating that induction of behavioral depression/learned helplessness may be regulated, at least in part, by sero-

tonergic input into the hippocampal CA3 subfield (Mitchell et al., 2005).

In general the docking studies of Gingerol, Shogoal and Aripiprazole against the $5HT_{1A}$ protein had shown high binding affinity against the protein. The *in vivo* study such as FST shows a decrease in immobility time after treatment of volatile oil of *Z.officinale* and Aripiprazole that may be due to the inhibition of monoamine uptake. The OFT behavioral model shows an increase in exploratory and locomotor activity indicates a possible increase in serotonin metabolism. An increase in the proportion of numbers of entries and time spent in open arms in EPM and a decrease in escape failures in learned helplessness test after treatment of volatile oil of *Z.officinale* and Aripiprazole indicates that depression is regulated by serotonergic transmission. The *in vivo* studies of volatile oil of *Zingiber officinale* and Aripiprazole exhibited significant antidepressant activity which may be mediated through inhibition of monoamine uptake or by increasing serotonin metabolism.

However further studies are required in the field of non-invasive neuroimaging to explore neural pathways and neurochemical mechanisms underlying depression, and to find the influence of genetic expressions on the function of these pathways and the emotional behaviors they mediate.

Conclusion

The docking studies of the Gingerol, Shogoal and Aripiprazole against the $5HT_{1A}$ protein had shown high binding affinity. The ligands were observed as the best candidates, which may be considered as potential ligands for treatment of depression.

The *in vivo* studies of volatile oil of *Z.officinale* and Aripiprazole exhibited significant antidepressant activity which may be mediated through inhibition of monoamine uptake or by increasing serotonin metabolism.

These findings implicate volatile oil of *Z.officinale* and Aripiprazole as candidates for treatment of depression.

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