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RESEARCH ARTICLE

Evaluation of Natural Compounds from Medicinal Plants for Mental Illness

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ABSTRACT

Leaves of Psidium Guajava contain flavonoids & phenolic compounds which have been implicated in antidepressant activity. Therefore the present research was aimed to evaluate the potential antidepressant activity of methanolic extract of Psidium Guajava (MEPG) leaves in mice. This extract was administered orally in a dose range of 200 and 400 mg/kg of the body weight. The antidepressant activity was evaluated using tail suspension test (TST), Locomotor activity by Actophotometer and Reserpine antagonism in mice. Results of the activity showed significant decrease in the immobility time in TST & reduction in catalepsy in locomotor activity similar to that of the imipramine (10 mg/kg) and sertraline (20mg/kg). In reserpine antagonism test, the extract showed antagonistic effect on reserpine in tested mice. The significance of difference among the various treated groups and control group were analyzed by means of one-way ANNOVA followed by Dunnett's Multiple Comparison test. In conclusion, methanolic extract of leaves of Psidium Guajava possesses potential antidepressant activity and has therapeutic potential in the treatment of CNS disorders and provides evidence at least at a preclinical level.

KEYWORDS

Psidium Guajava Linn, Antidepressant Activity, Tail Suspension Test, Locomotor Activity, Reserpine Antagonism

INTRODUCTION

Herbs are staging a comeback and herbal 'renaissance' is happening all over the globe. The herbal products today symbolize safety in contrast to the synthetics that are regarded as unsafe to human and environment. Among ancient civilizations, India has been known to be rich repository of medicinal plants, which are largely collected as raw materials for manufacture of drugs and perfumery products.[1] According to the World Health report, approximately 450 million people suffer from mental or behavioral disorders, yet only a small minority of them receives even the most basic treatment.

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This amounts to 12.3% of the global burden of disease and will rise to 15% by 2020. In the search for new therapeutic products for the treatment of neurological disorders, medicinal plant research, worldwide has progressed constantly demonstrating the pharmacological effectiveness of different plant species in a variety of animal models. Depression is an important global pubic health issue and is associated with substantial disability. It is a chronic illness that affects mood, thoughts, physical health and behavior of any individual and has been estimated to affect upto 21% of the world's population. Currently available therapy for depression treatment is often associated with several undesirable side effects, and it is effective only in a certain portion of the population. Therefore, the identification of

alternative therapeutic tools for the treatment of depression is still needed. Herbal therapies may be effective alternatives in the treatment of depression and the search for novel pharmacotherapy from medicinal plants for psychiatric illnesses, including depression, has progressed significantly in the past decade.[2]

Psidium Guajava L. known as Guava is a medicinal plant belonging to the family Myrtaceae. P. guajava is a well known traditional medicinal plant used in various indigenous systems of medicine. It is widely distributed throughout India. The leaves of P. guajava tree have long history of medicinal uses, which is still employed today. It is a native of Central America but is now widely cultivated, distributed and the fruits enrich the diets of millions of people in the tropics of the world. Guava contains broad spectrum of phytochemicals including minerals, enzymes, proteins, sesquiterpenoid alcohols and triterpenoid acids, alkaloids, glycosides, steroids, flavanoids, tannins, saponins. Guava is very rich in antioxidants and vitamins and also high in lutein, zeaxanthine and lycopene. Guavas contain carotenoids and polyphenols, the major classes of antioxidant pigments giving them relatively high potential antioxidant value among plant foods. In addition three flavonoids (quercetin, avicularin, and guaijaverin) have been isolated from the leaves. Five constituents, including one new pentacyclic triterpenoid: guajanoic acid and four known compounds beta-sitosterol, uvaol, oleanolic acid and ursolic acid, have been recently isolated from the leaves of *P. guajava* by Begum *et al*. The essential oil contains alpha pinene, caryophyllene, cineol, D-limonene, eugenol, and myrcene. The major constituents of the volatile acids include (E)-cinnamic acid and (Z)-3-hexenoic acid. P. guajava leaf is a phytotherapic used to treat gastrointestinal & respiratory disturbances, diarrhoea, cough, vertigo, menstrual disorders, piles, cholera, wounds, etc. It is used as anti-inflammatory medicine, anti-amoebic, antimalarial, antispasmodic. The leaves are used in USA as an antibiotic in the form of poultice or decoction for wounds, ulcers and tooth ache. Bronchitis, asthma attacks, cough, pulmonary diseases could be also treated with guava tea.[3]

Reactive oxygen species (ROS) have been found to play an important role in the initiation and/or progression of various diseases such as atherosclerosis, inflammatory injury, cancer and cardiovascular disease (Halliwell, 1997). Thus, recent studies have investigated the potential of plant products as antioxidants against various diseases induced by free radicals (Hou et al., 2003; Nabavi et al., 2008a). Additionally, it has been determined that the antioxidant effect of plant products is mainly attributed to phenolic compounds, such as flavonoids, phenolic acids, tannins and phenolic diterpenes (Pietta, 2000). Antioxidant capacity is widely used as a parameter to characterize nutritional health food or plants and their bioactive components. Recently, interest has considerably increased in finding naturally occurring antioxidant to replace synthetic antioxidants, which were restricted due to their side effects (Zhou et al., 2000).[4]

MATERIALS AND METHOD

Animals

Swiss albino mice (22-30 g) males were procured from registered breeders Haffkine Institute for training, research and testing, Parel. The animals were housed at 24 ± 2°C and relative humidity 55 ± 5 with 12:12 h light and dark cycle. They had free access to food and water *ad libitum*. The animals were acclimatized for a period of seven days before the study. The experimental protocol was approved by the Institutional Animals Ethics Committee (IAEC) of the college and the experiments were carried out as per CPCSEA guidelines, New Delhi (India).

Chemicals

Imipramine was gifted from Abbott Healthcare Pvt Ltd, Mumbai. Sertraline was from Amoli Organics Pvt Ltd, Mumbai. Haloperidol was gifted from RPG Life Sciences Ltd, Mumbai. Reserpine was gifted from Indo German Alkaloid, Mumbai. All other chemicals and reagents used were of analytical grade.

Method

Plant Material and Extraction

The leaves of Psidium Guajava Linn were collected from the Haffkine Institute, Parel. They were authenticated at the Blatter Herbarium, St. Xavier's College, Mumbai (Specimen Number- 4729). The leaves were carefully observed and any infected or damaged leaves were immediately discarded at this stage. The leaves were then shed dried for removing moisture from the leaves. Dried leaves were ground to a coarse powder by using a blender and stored in a screw cap bottle at room temperature. 500 gm grams of dried powder of leaves were extracted with aqueous methanol (90% v/v). The extract was concentrated under reduced pressure and residue was suspended with water. Then the extract was dissolved in 50% aqueous methanol and 2M hydrochloric acid. The solution was refluxed for 7hrs at 60 degree celcius. The reaction mixture was then concentrated under reduced pressure. The solution was successively extracted with n-hexane and chloroform to remove the hydrophobic materials. The aqueous phase was extracted with ethyl acetate. The ethyl acetate soluble fraction was concentrated under reduced pressure using rotary vaccum evaporator and dried at room temperature. Solution of Methanolic extract of Psidium Guajava (MEPG) was prepared freshly in distilled water and used for the present study.[5]

Phytochemical Analysis

The MEPG was screened for the presence of various phytochemical constituents such as flavanoids, tannins, saponin, alkaloids and phenolic compounds by employing standard screening tests.

TLC

TLC profile was developed for the MEPG. The solvent system used was Toluene (36) : Ethyl acetate (12) : Formic acid (5). The analysis was performed on precoated TLC plates of silica gel. The extract was dissolved in respective solvent and spotted over an activated plate (1 cm above from the bottom). The plates were developed at room temperature in a vertical separating chamber containing the mobile phase to the height of approximately 3/4th from the start. The chamber was previously saturated with the appropriate mobile phase (saturation time was 1 hour). After drying, visualization was done in ultraviolet light.[6]

$$Rf = \frac{\text{Distance traveled by solute}}{\text{Distance traveled by the solvent}}$$

Acute Toxicity Study

The acute toxicity study was carried out according to OECD-423 guidelines. Six swiss albino mice, males weighing in the range of 22-30 gm, were administered the test solution at a dose of 2000 mg/kg. The mice were critically observed for clinical signs, gross behavioral changes and mortality, if any, following the administration of the test formulation at different time intervals like 30min, 1h, 2h, 4h, 24h, 48h and 72h upto a period of 14 days.[7]

DPPH Free Radical Scavenging Activity

Antioxidants react with 1,1-diphenyl-2-picryl hydrazyl (DPPH) and convert it to 1,1-diphenyl-2-picryl-hydrazine and the degree of discoloration indicates the free radical scavenging activity of drug. The change in the absorbance produced at 517 nm, has been used as a measure of antioxidant activity**.** Various concentrations (2 ml) of standard and test solutions were added to a methanolic solution (1 ml) of DPPH radical (0.2mM). The mixture was shaken vigorously and left to stand in dark at room temperature for 30 min. The absorbance of the resulting solution was measured spectrophotometrically at 517 nm. The result was expressed as IC50 value which is the concentration of extract required for 50% inhibition of DPPH radicals. The percentage of DPPH radical scavenging activity was calculated according to the following equation[8]:

$$\% \text{ inhibition} = \frac{(A_0 - A_1)}{(A_0)} \times 100$$

Where

A0 is the absorbance of the control (blank)

A1 is the absorbance in the presence of the MEPG or the standard quercetin.

Evaluation of Antidepressant Activity

Tail Suspension Test

The tail suspension test has been described as a facile means of evaluating potential antidepressants. Six groups of six mice each were used. Control group received CMC (0.1%) 1hr before the test. Imipramine (10mg/kg, i.p) and sertraline (20mg/kg, p.o) was administered 30 min before the test. Test groups received MEPG (200mg/kg p.o. & 400mg/kg p.o) 1hr before the experiment. Haloperidol (50ug/kg, i.p) was administered 30min before treatment with standard and test compounds. Mice were suspended on the edge of a table at 58 cm above the floor by the adhesive tape placed approximately 2-3 cm from the tip of the tail. Immobility time was recorded during 5 min period using a stop watch. Animal was considered to be immobile when it does not show any movement of body and remain hanging passively.[9,10]

Locomotor Activity by Actophotometer

This test measures the exploration and the voluntary locomotion within an enclosed area. The objective value for the spontaneous motor activity was obtained by using a photoactometer. The mice were placed individually in a 30 × 30 cm black metal chamber with a screen floor and a light-tight lid. Six beams of red light were focused 2 cm above the floor in to photocells on the opposite side. Each beam interruption was registered as an event on the external counter. The floor of the chamber was wiped clean with a damp towel before each use. The mice were placed in the chamber one hour after the oral administration of the drug. They were allowed to acclimate for 2 min, and then the light beam breaks were counted for the next 10 min. Six groups of six mice each were used. Control group received saline (10ml/kg). MEPG (200mg/kg, p.o. & 400mg/kg, p.o) was administered to test groups 30 min prior to the haloperidol (2mg/kg, p.o) administration. Imipramine (15mg/kg, p.o) and Sertraine (20mg/kg, p.o) were administered in the similar manner. Catalepsy was determined 30 min after the haloperidol administration on the 4th, 8th and 14th day. On the 14th day after behavioural assessment, the animals will be humanely sacrificed. After scarification, whole brains were removed aseptically and 10% of brain tissue homogenate was prepared in 0.1M phosphate buffer solution (pH 7.4) which was further utilized for estimation of biochemical parameter i.e. Superoxide dismutase (SOD), Catalase (CAT) and Glutathione Peroxidase (GPx).[10,11,12]

Superoxide dismutase (Kakkar *et al*, 1979): The assay of SOD is based on the inhibition of the formation of NADH-phenazine methosulphate-nitroblue tetrazolium formazon. The colour formed at the end of the reaction can be extracted into butanol and measured at 560nm. The super oxide radicals were generated in a system with 10µM phenazine methosulphate (PMS), 78µM reduced nicotinamide adenine dinucleotide (NADH).The NADH-phenazinemethosulphate – nitro blue tetrazolium formazan inhibition reaction can be adopted for the rapid, simple, sensitive and reliable assay. The radical scavenging potential was assayed by adding 100µl of plasma. A blank was prepared with all the chemicals but without the plasma. In the PMS/NADH-NBT system; super oxide anions reduce NBT to a blue formazan compound. Acetic acid in the concentration was used to arrest the formazan formation. The addition of acetic acid instead of trichloroacetic acid to stop the reaction helps to dissolve the protein so that a subsequent centrifugation step can be avoided. The color reaction of super oxide radicals and NBT was detected on spectrophotometer at 560nm. The enzyme activity was expressed as change in optical density per milligram protein per minute.

Catalase (Patterson *et al*, 1984): The method is based on the photoelectric measurement of the color intensities of the product of the interaction between hydrogen peroxide and titanium

reagent. The decomposition of H_2O_2 was measured at 410nm.

$$Ti^{+4} + H_2O_2 + H_2O \longrightarrow H_2Ti_4 + 4H^+$$

The reaction mixture contained 0.1ml enzyme source (plasma), 1 ml phosphate buffer (pH 6.5) and 0.1 ml of H_2O_2. The enzyme reaction was stopped by the addition of 1 ml of the titanium reagent. The intensity of the yellow color of Ti-H_2O_2 complex was measured at 410 nm against blank which was prepared in similar without the plasma. The enzyme activity was expressed as µmol H_2O_2 consumed per milligram protein per minute.

Glutathione Peroxidase (Rotruck, 1973): The rate of glutathione oxidation by H2O2 as catalyzed by the GPx present in the supernatant is determined. The color that develops is read against a reagent blank at 412 nm on a spectrophotometer. The reaction mixture consisting of 0.4 ml tris HCl buffer, 0.2 ml GSH, 0.1 ml Sodium azide, 0.1 ml water, 0.1 ml H2O2 and 0.1 ml homogenate was incubated at 37°C for 15 min. 0.5 ml TCA was added and centrifuged. 0.5 ml of supernatant was taken and 2 ml Na2HPO4.2H2O & 0.5 ml Ellman's Reagent were added. Absorbance was read at 420 nm. The results were expressed as μmol/min/mg protein.

Reserpine Antagonism in Mice

Reserpine induces profound depletion of catecholamine in brain and produce depression in animals and humans. Six groups of six mice each were used. Sixty minutes after oral administration of the test compounds (200mg/kg, p.o. & 400mg/kg, p.o), control (saline 0.1ml/10gm) and standard compounds (Imipramine 25 mg/kg, p.o. & Sertraline 20mg/kg, p.o); 5 mg/kg reserpine was injected s. c. The test started 15 minutes after reserpine administration and was continued for 2hrs Ptosis was used as criteria for evaluation. The degree of ptosis was scored: eyes closed = 4, eyes ¾ closed = 3, eyes ½ closed = 2, eyes ¼ closed = 1, eyes open = 0. Sedation score was assessed using concentric circles of 5, 7 and 9 cm diameter and the distance moved by the mice placed at the centre of the circles. Score 0 - no movement, Score 1 = between 5-7 cm, Score 2 = between 7-9cm, Score 3 = moving away from 9 cm.[13,14]

RESULTS AND DISCUSSION

Preliminary Phytochemical Screening

The preliminary phytochemical screening of MEPG revealed the presence of Flavonoids, Alkaloids, Saponins, Tannins and Phenolic compounds.

Thin Layer Chromatography (TLC)

The presence of Quercetin and Morin (Flavonoids) was confirmed by TLC.

Rf value of Quercetin = 0.39

Rf value of Morin =0.23

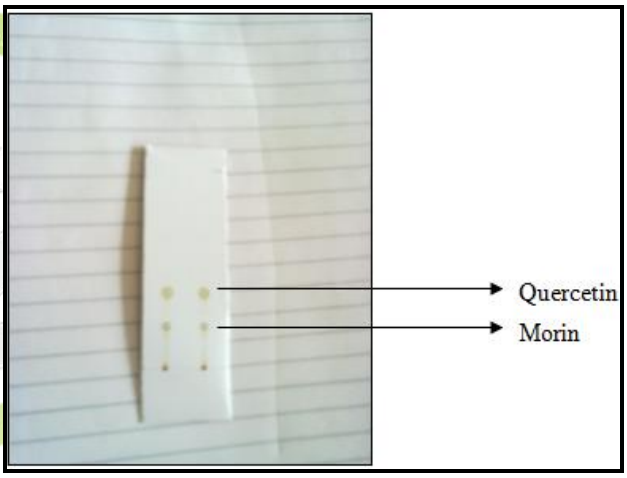

Figure 1: TLC

Acute Toxicity Test

In the acute toxicity study no deaths were observed. MEPG did not show any toxic or deleterious effects up to 2000 mg/ kg oral dose. The animals showed no symptoms associated with toxicity. CNS stimulation parameters such as hyperactivity, irritability, tremors, convulsions, straub tail were found to be negative in mice. CNS depressant parameters such as hypoactivity, narcosis, ataxia were found to be negative in mice.

DPPH Free Radical Scavenging Activity

MEPG showed promising free radical scavenging effect on DPPH radical in a

concentration dependent manner. The IC_{50} value of MEPG was found to be 39.87µg/ml. The correlation coefficient (R^2) was calculated from the graph and was found to be 0.971 for MEPG (Fig: 4.1). The extract was compared with quercetin which was used as standard antioxidant having IC_5 value 19.56 µg/ml and the correlation coefficient (R^2) was calculated from the graph and was found to be 0.968.

Table 1: DPPH Radical Scavenging Activity of MEPG

Concentration (ug/ml)	**% Inhibition of DPPH Radicals**	**IC_{50} Value**
10	33.108	
20	40.54	
40	53.04	39.87 ug/ml
60	3.176	
80	65.878	
100	78.040	

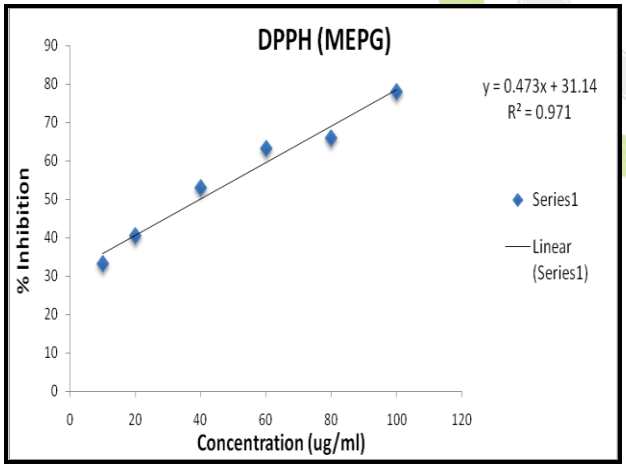

Figure 2: DPPH Radical Scavenging Activity of MEPG

Table 2: DPPH Radical Scavenging Activity of Quercetin

Concentration (ug/ml)	**% Inhibition of DPPH Radicals**	**IC_{50} Value**
10	39.452	
20	54.321	
40	63.212	19.56 ug/ml
60	72.120	
80	81.650	
100	91.050	

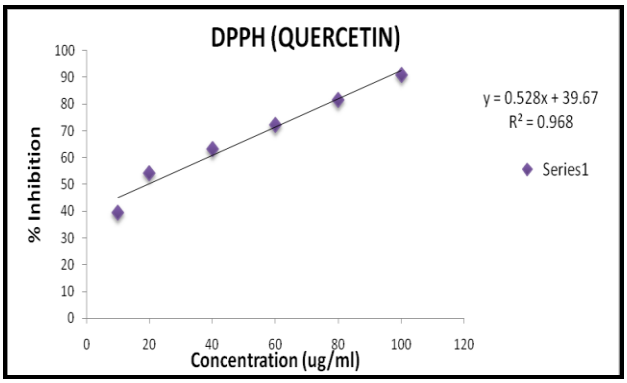

Figure 3: DPPH Radical Scavenging Activity of Quercetin

Tail Suspension Test

Haloperidol treatment showed significant increase in duration of immobility compared with vehicle control group. Treatment with MEPG (200mg/kg) showed decrease in duration of immobility, however treatment with a higher dose of MEPG (400mg/kg) showed significant decrease in duration of immobility. A dose dependent effect was observed.

Table 3: Effect of MEPG on duration of immobility in haloperidol induced immobility in mice

Groups	**Duration of Immobility**
Vehicle Control (CMC 0.1%)	0.667 ± 0.333
Disease Control (Haloperidol 50ug/kg)	20.33 ± 1.174*
Imipramine (10mg/kg)	8.667 ± 1.229**
Sertraline (20mg/kg)	11.17 ± 0.872**
MEPG (200mg/kg) (Test 1)	13.67 ± 1.022**
MEPG (400mg/kg) (Test 2)	7.167 ± 0.703**

Values are expressed as mean ± SEM for 6 animals in each group. Significance was

determined by One-way ANOVA followed by Dunnett's multiple comparison tests.

*: p<0.01 when compared with Vehicle control group

**: p<0.01 when compared with Disease control group.

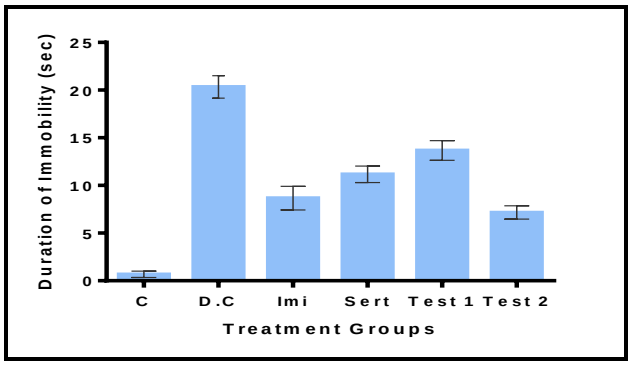

Figure 4: Effect of MEPG on duration of immobility in haloperidol induced immobility in mice

C: Vehicle Control mice, D.C: Disease Control mice, Imi: Imipramine, Sert: Sertraline, Test 1: MEPG (200mg/kg), Test 2: MEPG (400mg/kg).

Locomotor Activity by Actophotometer

Haloperidol treatment showed significant reduction in the total locomotor activity/10 min compared with Vehicle control group. Treatment with MEPG (200mg/kg) showed increase in locomotor activity on 8th & 14th day, however treatment with a higher dose of MEPG (400mg/kg) showed significant increase in locomotor activity on 8th & 14th day.

A dose dependent and time dependent effect was observed. Levels of brain tissue parameters like SOD, CAT and GPx were increased in MEPG treated groups as compared to disease control group.

Table 4: Effect of MEPG on locomotor activity in haloperidol induced catalepsy in mice

Groups	Locomotor Activity in Activity Counts/ 10 min		
	4th day	8th day	14th day
Vehicle Control (Saline 10ml/kg)	397.5 ± 3.212	407.3 ± 3.658	425.0 ± 3.055
Disease Control Group (Haloperidol 2mg/kg)	123.3 ± 1.626*	114.3 ± 2.201*	98.50 ± 3.490*
Imipramine (15mg/kg)	333.0 ± 2.733**	345.2 ± 3.371**	363.5 ± 2.473**
Sertraline (20mg/kg)	303.7 ± 4.096**	314.7 ± 3.040**	335.3 ± 3.116**
MEPG (200mg/kg)(Test 1)	253.8 ± 2.400**	266.2 ± 3.229**	286.7 ± 2.539**
MEPG (400mg/kg)(Test 2)	323.7 ± 3.201**	336.5 ± 2.604**	353.2 ± 2.600**

Values are expressed as mean ± SEM for 6 animals in each group. Significance was determined by One-way ANOVA followed by Dunnett's multiple comparison tests.

*: p<0.01 when compared with Vehicle control group.

**: p<0.01 when compared with Disease control group.

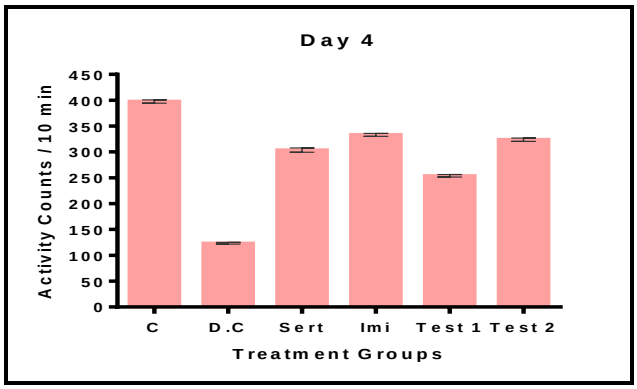

Figure 5: Effect of MEPG on locomotor activity in haloperidol induced catalepsy in mice. (Day 4)

C: Vehicle Control mice, D.C: Disease Control mice, Imi: Imipramine, Sert: Sertraline,

Test 1: MEPG (200mg/kg), Test 2: MEPG (400mg/kg).

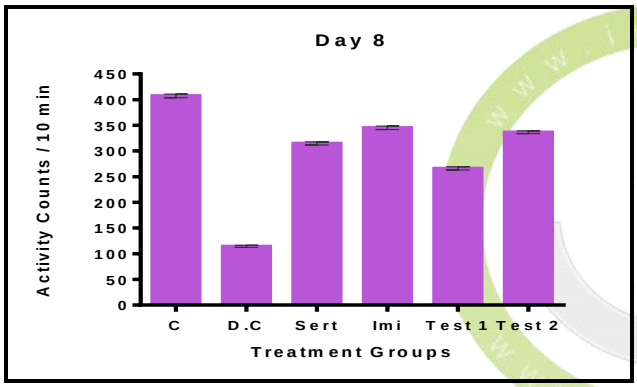

Figure 6: Effect of MEPG on locomotor activity in haloperidol induced catalepsy in mice. (Day 8)

C: Vehicle Control mice, D.C: Disease Control mice, Imi: Imipramine, Sert: Sertraline, Test 1: MEPG (200mg/kg), Test 2: MEPG (400mg/kg).

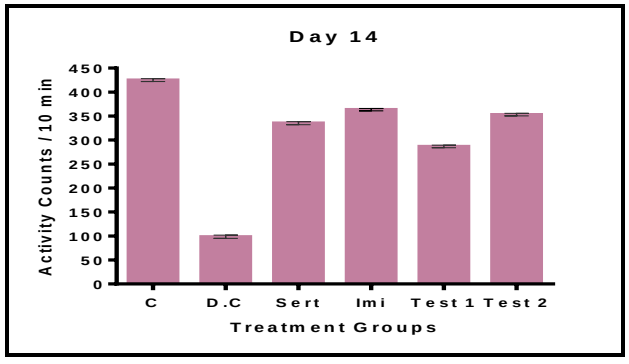

Figure 7: Effect of MEPG on locomotor activity in haloperidol induced catalepsy in mice. (Day 14)

C: Vehicle Control mice, D.C: Disease Control mice, Imi: Imipramine, Sert: Sertraline,

Test 1: MEPG (200mg/kg), Test 2: MEPG (400mg/kg).

Table 5: Effect of MEPG on biochemical brain parameters on locomotor activity in haloperidol induced catalepsy in mice

Groups	SOD U/ug of Protein	CAT U/ug of Protein	GPx ug/ug of protein
Vehicle Control (Saline 10ml/kg)	0.0389 ± 0.0037	0.0397 ± 0.004	0.3557 ± 0.0478
Disease Control Group (Haloperidol 2mg/kg)	0.0133 ± 0.0025*	0.0174 ± 0.0029*	0.1635 ± 0.0248*
Sertraline (20mg/kg)	0.0338 ± 0.0043**	0.0366 ± 0.0028**	0.3152 ± 0.0260***
MEPG (200mg/kg)	0.0261 ± 0.0018***	0.0301 ± 0.0034***	0.2885 ± 0.0192***
MEPG (400mg/kg)	0.0370 ± 0.0032**	0.0369 ± 0.0037**	0.3298 ± 0.0385**

Values are expressed as mean ± SEM for 6 animals in each group. Significance was determined by One-way ANOVA followed by Dunnett's multiple comparison tests.

*: $p<0.01$ when compared with Vehicle control group.

**: $p<0.01$ when compared with Disease control group.

***: $p<0.05$ when compared with Disease control group.

Reserpine Antagonism in Mice

Reserpine treatment showed significant increase in degree of ptosis compared with Vehicle control group. The scores were different for the Standard groups i.e. Imipramine (25mg/kg) & Sertraline (20mg/kg) and Test groups i.e. MEPG (200mg/kg) & MEPG (400mg/kg) compared to Disease control group. MEPG (200mg/kg) showed decrease in degree of ptosis, however treatment with higher dose of MEPG

(400mg/kg) showed significant decrease in degree of ptosis.

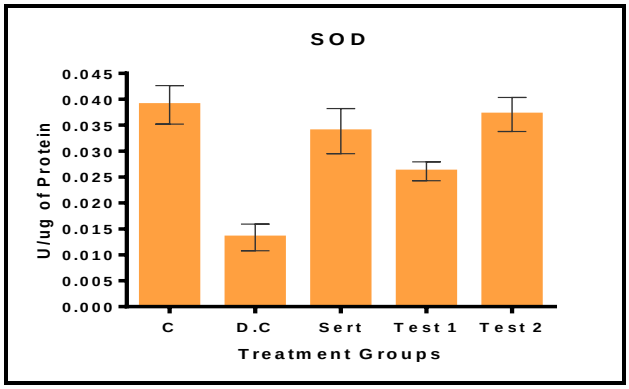

Figure 8: Effect of MEPG on SOD in haloperidol induced catalepsy in mice

C: Vehicle Control mice, D.C: Disease Control mice, Sert: Sertraline, Test 1: MEPG (200mg/kg), Test 2: MEPG (400mg/kg)

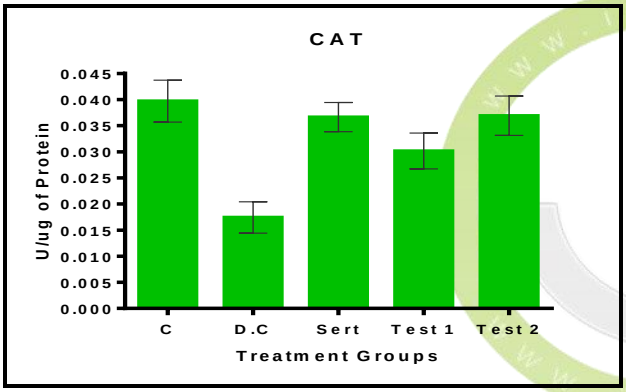

Figure 9: Effect of MEPG on CAT in haloperidol induced catalepsy in mice

C: Vehicle Control mice, D.C: Disease Control mice, Sert: Sertraline, Test 1: MEPG (200mg/kg), Test 2: MEPG (400mg/kg).

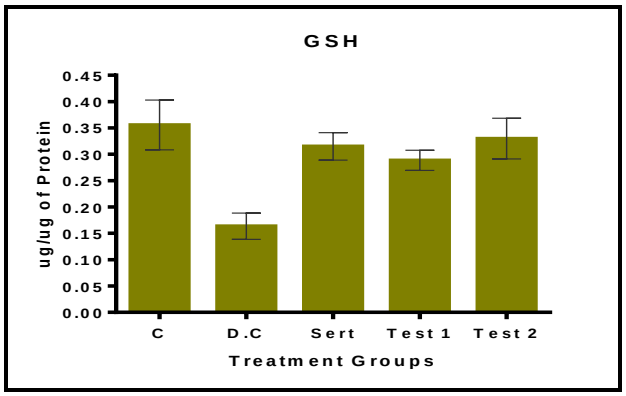

Figure 10: Effect of MEPG on GPx in haloperidol induced catalepsy in mice.

C: Vehicle Control mice, D.C: Disease Control mice, Sert: Sertraline, Test 1: MEPG (200mg/kg), Test 2: MEPG (400mg/kg).

Table 6: Effect of MEPG on Reserpine induced ptosis in mice.

Groups	Degree of Ptosis
Vehicle Control (Saline 0.1ml/10gm)	0.5317 ± 0.0703
Disease Control (Reserpine 5mg/kg)	3.207 ± 0.0493*
Imipramine (25mg/kg)	1.847 ± 0.0663**
Sertraline (20mg/kg)	2.432 ± 0.0903**
MEPG (200mg/kg) Test 1	2.538 ± 0.0773**
MEPG (400mg/kg) Test 2	1.878 ± 0.0621**

Values are expressed as mean ± SEM for 6 animals in each group. Significance was determined by One-way ANOVA followed by Dunnett's multiple comparison tests.
*: $p<0.01$ when compared with Vehicle control group.
**: $p<0.01$ when compared with Disease control group.

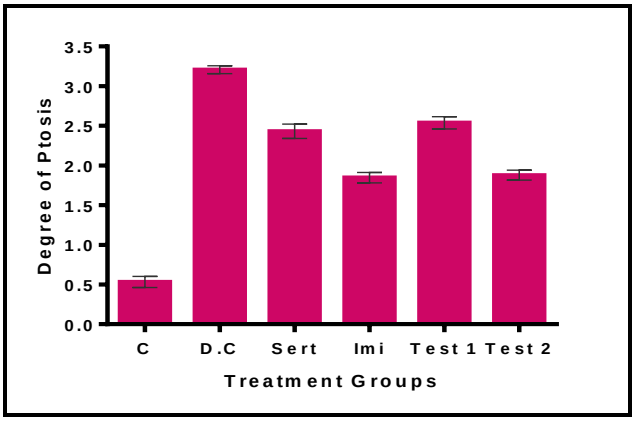

Figure 11: Effect of MEPG on degree of ptosis in mice

C: Vehicle Control mice, D.C: Disease Control mice, Imi: Imipramine, Sert: Sertraline, Test 1: MEPG (200mg/kg), Test 2: MEPG (400mg/kg).

Table 7: Effect of MEPG on Reserpine induced sedation in mice

Groups	**1st hr**	**2nd hr**	**3rd hr**	**4th hr**
Vehicle Control (Saline 0.1ml/10gm)	2.167 ± 0.3073	2.833 ± 0.1667	2.667 ± 0.2108	3.000 ± 0.0000
Disease Control (Reserpine 5mg/kg)	0.333 ± 0.2108*	0.667 ± 0.2108*	1.667 ± 0.2108*	1.833 ± 0.1667*
Imipramine (25mg/kg)	2.000 ± 0.2582**	2.167 ±0.3073**	2.833 ± 0.1667**	3.000 ± 0.0000**
Sertraline (20mg/kg)	1.667 ± 0.3333***	2.000 ± 0.2582**	2.667 ± 0.2108**	2.667 ± 0.2108**
MEPG (200mg/kg) Test 1	1.500 ± 0.3416***	1.833 ± 0.3073***	2.500 ± 0.2236***	2.500 ± 0.2236***
MEPG (400mg/kg) Test 2	1.833± 0.3073**	2.000 ± 0.2582**	2.667 ± 0.2108**	2.833 ± 0.1667**

1st hr:-

Values are expressed as mean ± SEM for 6 animals in each group. Significance was determined by One-way ANOVA followed by Dunnett's multiple comparison tests.

*: p<0.01 when compared with Vehicle control group.

**: p<0.01 when compared with Disease control group

***: p<0.05 when compared with Disease control group.

2nd hr:-

Values are expressed as mean ± SEM for 6 animals in each group. Significance was determined by One-way ANOVA followed by Dunnett's multiple comparison tests.

*: p<0.01 when compared with Vehicle control group.

**: p<0.01 when compared with Disease control group.

***: p<0.05 when compared with Disease control group.

3rd hr:-

Values are expressed as mean ± SEM for 6 animals in each group. Significance was determined by One-way ANOVA followed by Dunnett's multiple comparison tests.

*: p<0.01 when compared with Vehicle control group.

**: p<0.01 when compared with Disease control group.

***: p<0.05 when compared with Disease control group.

4th hr:-

Values are expressed as mean ± SEM for 6 animals in each group. Significance was determined by One-way ANOVA followed by Dunnett's multiple comparison tests.

*: p<0.01 when compared with Vehicle control group.

**: p<0.01 when compared with Disease control group.

***: p<0.05 when compared with Disease control group.

Reserpine treatment showed sedation effect compared with Vehicle control group. Antagonism effect on sedation was seen at 1st, 2nd, 3rd & 4th hr for the Standard groups i.e. Imipramine (25mg/kg) & Sertraline (20mg/kg) and Test groups i.e. MEPG (200mg/kg) & MEPG (400mg/kg) compared to Disease control group.

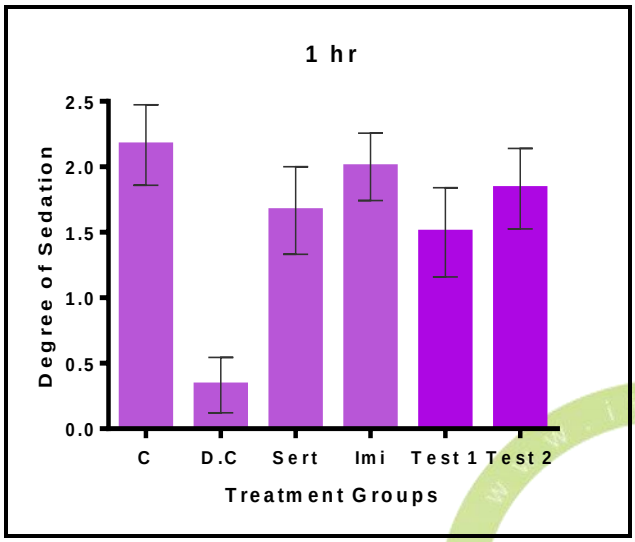

Figure 12: Effect of MEPG on Reserpine induced sedation in mice. (1st hr)

C: Vehicle Control mice, D.C: Disease Control mice, Imi: Imipramine, Sert: Sertraline, Test 1: MEPG (200mg/kg), Test 2: MEPG (400mg/kg).

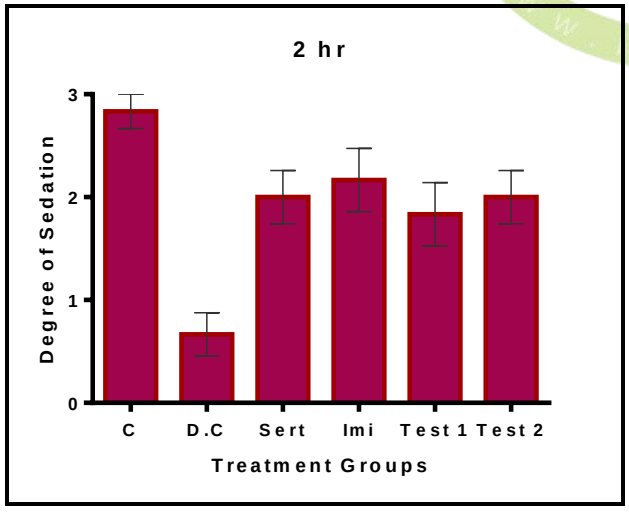

Figure 13: Effect of MEPG on Reserpine induced sedation in mice. (2nd hr)

C: Vehicle Control mice, D.C: Disease Control mice, Imi: Imipramine, Sert: Sertraline, Test 1: MEPG (200mg/kg), Test 2: MEPG (400mg/kg).

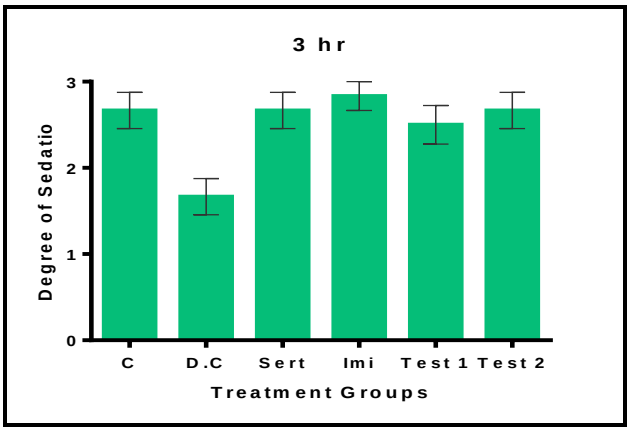

Figure 14: Effect of MEPG on Reserpine induced sedation in mice. (3rd hr)

C: Vehicle Control mice, D.C: Disease Control mice, Imi: Imipramine, Sert: Sertraline, Test 1: MEPG (200mg/kg), Test 2: MEPG (400mg/kg).

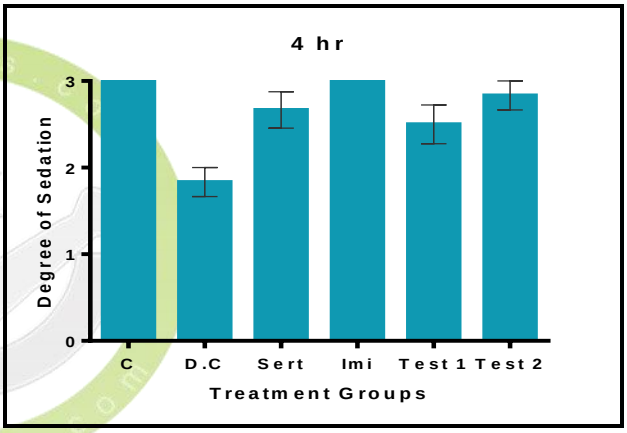

Figure 15: Effect of MEPG on Reserpine induced sedation in mice. (4th hr)

C: Vehicle Control mice, D.C: Disease Control mice, Imi: Imipramine, Sert: Sertraline, Test 1: MEPG (200mg/kg), Test 2: MEPG (400mg/kg).

DISCUSSION

Depression is a common, debilitating, life-threatening illness with a high incidence. Numerous antidepressant compounds are now available, which presumably act via different mechanisms involving the serotonergic, noradrenergic and/or dopaminergic systems. Heterogeneity of clinical response to antidepressant and mood-stabilizing drugs and susceptibility to adverse effects are major clinical problems. Therefore, new drugs are still needed for the control of depression-related disorders.[15]

The present study has shown that oral administration of the methanolic extract of Psidium Guajava was effective in producing significant antidepressant effect in the tail suspension test, locomotor activity by actophotometer and in reserpine antagonism in mice, as is evident from the reduction in the immobility time in mice, reduction in duration of catalepsy and degree of ptosis & sedation in mice respectively.

The tail suspension test has been described by Steru et al as a facile means of evaluating potential antidepressants. The immobility displayed by rodents when subjected to an unavoidable and inescapable stress has been hypothesized to reflect behavioral despair, which in turn may reflect depressive disorders in humans. Clinically effective antidepressants reduce the immobility that mice display after active and unsuccessful attempts to escape when suspended by tail.[13] The MEPG 200mg/kg and 400mg/kg significantly $p<0.01$ reduced the duration of immobility in mice as compared to Standard groups. This shows that the MEPG possesses antidepressant activity, and its specificity towards particular behavior may depend on the concentration of the extract. The monoamine theory of depression proposes that "depression is due to a deficiency in one or another of three monoamines, namely serotonin, noradrenaline and dopamine.[9] In support to monoamine hypothesis, Haloperidol pre-treated group exhibited significant increase in duration of immobility which was reduced by treatment with MEPG; suggesting involvement of dopamine in antidepressant-like activity of MEPG. Thus antidepressant effect of MEPG may be due to involvement of dopaminergic system.

Spontaneous locomotor activity is considered as an index of alertness and can be helpful to confirm the general depressive activity of any drug. The decrease in motor activity gives an indication of the level of excitability of the CNS and this decrease may be related to sedation resulting from depression of CNS.[16] In the present study, the animals who were treated for fourteen days with haloperidol showed severe cataleptic responses. MEPG 200mg/kg group showed increase in locomotor activity on 8th & 14th day, however treatment with a higher dose of MEPG 400mg/kg showed significant $p<0.01$ increase in locomotor activity on 8th & 14th day. The MEPG contains flavonoids: Quercetin and Morin. It can be speculated that antidepressant-like effect of extract might be related to the flavonoid- quercetin. Quercetin may exert beneficial actions on the central nervous system such as neuroprotective, anxiolytic and cognitive enhancing effects. It should be noted that recent studies have demonstrated the permeability of quercetin across the blood-brain barrier *in situ* and *in vivo*. Thus, this evidence provides the possibility that quercetin may exert modulatory effects on the central nervous system. Quercetin has shown MAO inhibitory capability and inhibition of MAO may alleviate symptoms of depression.[17] Therefore, the antidepressant action of MEPG may involve extract flavonoids, which inhibited uptake of monoamines or MAO activity or both in the brain.

Living tissues are endowed with innate antioxidant defense mechanisms, such as the presence of the enzymes Superoxide dismutase (SOD), Catalase (CAT) and Glutathione Peroxidase (GPx). A reduction in the activities of these enzymes is associated with the accumulation of highly reactive free radicals, leading to deleterious effects such as loss of integrity and function of cell membranes. Antioxidant enzymes such as SOD and CAT are easily inactivated by lipid peroxides or reactive oxygen species. CAT is most abundant in the liver and is responsible for the catalytic decomposition of H2O2 to oxygen and water. SOD is an extremely effective antioxidant enzyme, and is responsible for catalytic dismutation of highly reactive and potentially toxic superoxide radicals to H2O2. Glutathione Peroxidase is a substrate for glutathione related enzymes, and a regenerator for alpha-tocopherol; therefore, it plays an important role in the antioxidant defense system.[18] Some studies have also shown that Reactive Oxygen Species (ROS) play a role in the pathogenesis of

neuropsychiatric disorders (Bilici *et al*., 2001; Khanzode *et al*., 2003). It is well known that oxidation of monoamines and catecholamines can lead to ROS production in brain tissue (Fridovich, 1983). Excessive ROS production can cause oxidative damage to macromolecules including lipids, proteins, and DNA (Niebroj-Dobosz *et al*., 2004; Zhao *et al*., 2008), culminating in neuronal dysfunction and depression (Manji and Duman, 2001; Fuchs *et al*., 2004).[19] The activities of SOD, CAT and GPx in the haloperidol group were significantly $p<0.01$ decreased when compared with the vehicle control group. The results strongly suggest that the significant decrease of SOD, CAT and GPx activities observed in mice treated with haloperidol may be due to production of highly reactive oxygen metabolites (ROMs). Erythrocyte SOD levels were suggested to be a hall mark for depression. Therefore, antidepressant therapeutic intervention may be associated with a critical oxidative process along with alleviating the depressive symptoms. The brain is more vulnerable to oxidative stress because of its elevated consumption of oxygen and the consequent generation of large amounts of ROS. The restraint stress reduced the levels of SOD and CAT**.** The decrease in the activity of GPx could be due to its exhausted adoptive response to counter the effect of increased oxidative stress Interestingly, our results evidenced a parallel increase in both SOD, CAT and GPx; the most important antioxidant enzymes in response to treatment with extract to depressed animals. The increase in activity may provide an effective defense from the damaging effects of superoxide anion and hydrogen peroxide.[20] In mice treated with MEPG 200mg/kg and 400mg/kg; the activities of these antioxidant enzymes were significantly $p<0.05$ and $p<0.01$ (respectively) higher than in the haloperidol treated group.

Reserpine is a vesicular re-uptake blocker, which deplete catecholamines or lowers nor-adrenaline turnover in the brain to produce a depression like syndrome in humans and animals.[14] Depression produced by reserpine in humans is similar to naturally occurring depression; hence reserpine is used as an agent to induce depression in animal models for testing antidepressant drugs. Since antidepressant drugs are known to prevent or antagonize the effects of reserpine, prevention of reserpine induced ptosis and sedation which can be used for evaluation of antidepressants.[13] The MEPG 200mg/kg showed decrease in degree of ptosis but MEPG 400mg/kg showed significant $p<0.01$ decrease in degree of ptosis as compared to Standard groups. The MEPG 200 mg/kg showed antagonism effect on sedation at 1st hr, 2nd hr and 3rd hr; however MEPG 400mg/kg showed significant $p<0.01$ antagonism effect on sedation at 1st hr, 2nd hr, 3rd hr and 4th hr as compared to Standard groups. This suggests that the antidepressant effect of MEPG may be due to inhibition of reuptake of biogenicamines (noradrenaline, serotonin and dopamine); thus elevating the levels of biogenicamines in the brain.

CONCLUSION

It can be concluded from the results that MEPG has potential antidepressant activity. The actions of MEPG as an antidepressant may be due to either single or combination of mechanisms like inhibition of reuptake of dopamine, noradrenaline or serotonin; inhibition of Monoamine Oxidase Enzyme (MAO), presence of flavonoids & phenolic compounds in the extract or antioxidant activity of the extract. Further studies are required to be carried out to determine the actual mechanism of action and active constituents responsible for the activity of MEPG to be used as potential drug for the management of depression.

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