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BY Sathish Kumar
Assistant Professor and HOD Department of Biotechnology JSS college of Arts, Commerce and Science Ooty road, Mysore, India

Eram Fathima
Postgraduate Department of Biotechnology, JSS College, Ooty road, Mysore, India

Correspondence
BY Sathish Kumar
Assistant Professor and HOD Department of Biotechnology JSS college of Arts, Commerce and Science Ooty road, Mysore, India

Mirabilis jalapa: Phytochemical screening and anti-stress activity of methanolic leaf extract

BY Sathish Kumar and Eram Fathima

Abstract
Mirabilis jalapa (M. jalapa) is widely used as a traditional medicine in the treatment of various ailments. In the present study we aimed to evaluate the phytochemical constituents and anti-stress activity of the methanolic extract of aerial parts *of M. jalapa*. The extract was screened for preliminary phytochemical tests. Phytochemical analysis revealed the presence of alkaloids, flavonoids, phenols, tannins, and saponins. This is one of the first studies reporting the anti-stress property of *M. jalapa* using Drosophila melanogaster where different groups were assayed for stress related marker enzymes like SOD and CAT. The activity in stress induced flies has increased compared to that of control flies. After incorporation of the plant extract there was reduction in level of these defensive enzymes there by increased the ability to scavenge ROS reducing the free radical concentration in the stress induced flies. Thus, *M. jalapa* may have anti-stress property.

Keywords: *Mirabilis jalapa* L; Methanolic extract; Phytochemicals; anti-stress property; Drosophila melanogaster.

1. Introduction
Mirabilis jalapa Linn. *(M. jalapa*), well-known as Beauty of night, Four O' clock, Sweet Marvel of Peru in English, belongs to the family Nyctaginaceae. [1] Four O'clock are leafy, shrub like, multibranched perennials that produce flowers in summer, and are tall herbaceous climbing plant with opposite leaves, large showy flowers, coriaceous obovoid fruits and prominent tuberous roots which are planted as ornamental plant all over the world [2]. This species is cultivated for the brilliant color and pleasing odor of its flowers which are used in food coloring. The leaves can be cooked and eaten as well, but only as an emergency food. *M. jalapa* got the popular name "Four O' clock" because the flower opens in late afternoon. They remain open until morning and on cloudy days; they may not close at all. Its flowers come in pink, red, yellow, white and some bi-colors and have a slight vanilla scent.
M. jalapa is used extensively as a medicinal plant in almost all folklore remedies around the world for treating various diseases. However, studies have evidenced its antibacterial, antiviral and antioxidant activities [3-5].Chemical analysis of various parts of *M. jalapa* revealed the presence of alkaloids, flavonoids, phenols, steroids, triterpenes, glycosides, tannins, saponins and lignins. The complete study of these compounds from TLC visualized alanine, arabinose, campesterol, daucosterol and dopamine, d-glucan, hexacon-1-ol, indicaxanthin, isobetanin, 6-methoxyboeravinone, C-methylabronisoflavones, miraxanthins, n-dotriacontane, n nonacosane, npentacosane, n-triacontane. Flowers generally contain anthocyanins and flavonoids. Many active compounds were extracted from different organs of *M. jalapa*, along with anti-fungal phenolic compounds, ribosome-in activating protein (RIP) which is connected with anti-viral activity, anti-microbial peptides and rotenoids that are potent inhibitors of HIV-1 reverse transcriptase [6]. The alcoholic extract of M. jalapa is a possible source of active compounds against pathogenic enteric organisms [7]. From the Methanolic extract of the whole plant of *M. jalapa*, about twenty different chemical constituents have been identified from the by Gas chromatogram Mass spectrometry (GC-MS) analysis. The presence of various bioactive compounds validates the use of whole plant for various ailments by traditional practitioners [8].
M. jalapa is a beneficial medicinal plant and has a great importance in the field of ethnobotany. Leaves of *M. jalapa* has Purgative and emetic properties [9]. Decoction of leaves is used against genitourinary system disorders and poultice of subterranean parts is used to treat injuries [10]. The native people of Mexico use *M. jalapa* for treatment of many gastrointestinal disorders, including dysentery, diarrhea, muscle pain and abdominal colic. The extract of *M. jalapa* shows an inhibitory effect on digestive gut and smooth muscle contractility [11] whereas decoction is used for Constipation [12].

The leaves are crushed, mixed with salt and use in Sprain and bruise [13]. Leaves of *M. jalapa* are applied on external wounds until recovery [14] and also used for treating amenorrhea and dysmenorrhea in women [15]. Leaves juice is mixed with water and can be used for treatment of jaundice [16] and also is taken orally in Hepatitis [17]. Paste of leaves has soothing property [18] and is useful in Skin eruption. Extract of roots has Hypolipidemic and hypoglycemic activity [19] and also are used to arouse aphrodisiac activity [20]. Stem with leaves are utilized for depigmentation [21]. Juice of leaves is used as eye drop to soothe eye inflammation. Boiled Leaves are consumed to reduce body pains. Tuber is administered in small quantities to cure piles [22].

The present study aims to evaluate the methanolic extracts of the aerial parts of the plant for its preliminary phytochemical studies such as alkaloids, saponins, flavonoids, tanins, proteins and carbohydrates, and also to determine its anti-stress property by Oxidative stress analysis using fruit fly Drosophila melanogaster model.

Materials and Methods

Collection of Plant Samples

The fresh leaves of *M. jalapa* were collected from local areas of Mysore, Karnataka state, India in mid- February 2014. Fresh leaves were identified and authenticated prior to phytochemical analysis.

Preparation of the extract

The extraction of the *M. jalapa* aerial parts was carried out according to the known standard procedures [23]. The plant material (leaves) was dried in shade and powdered in a mechanical grinder. In this process, the whole or coarsely powdered crude leaves were placed in a conical flask with the solvent (methanol and water) and allowed to stand at room temperature for a period of at least 3 days with frequent agitation until the soluble matter has dissolved. The mixture then was strained, the marc (the damp solid material) was pressed, and the combined liquids were clarified by filtration or decantation after standing. The extract was dried in a vacuum desiccator to obtain constant weight. The methanolic extract of the aerial parts yielded a dark brown residue (2.5%). The extracts were then kept in sterile bottles, under refrigerated conditions, until further use. The dry weight of the plant extracts was obtained by the solvent evaporation and used to determine the concentration in mg/ml.

Phytochemical Screening

The methanolic extract of powdered leaves of *M. jalapa* was subjected to phytochemical tests and to identify the constituents such as alkaloids, saponins, flavonoids, tannins and carbohydrates.

Qualitative Analysis

Test for Alkaloides: This was performed according to the method of Magadula and Tewtrakul [24]. In this test, about 1ml of the plant extract was dissolved in 5 ml of 27% HCl and warmed on steam bath. The filtrate (1ml) was mixed with drops of Dragendorff's reagent. Reddish orange precipitation was considered as indicative of the presence of alkaloids.

Test for saponins (frothing test): This was carried out according to the method of Horbone [25]. 3ml of plant extract was taken in test tube and shaken vigorously. It was then allowed to stand for about a minute and observation was made for the formation of stable froths.

Test for flavonoids: To 1ml of the plant extract in a test tube, 1ml of 5% lead acetate was added and allowed to stand. Observation was made for any precipitation.

Test for tannins: To 2ml of the plant extract, three drops of 10% $FeCl_3$ was added and observation was made for the appearance of blackish-blue or blackish green coloration.

Estimation of total flavonoids by aluminum chloride method:

The aluminum chloride colorimetric method was modified from the procedure described by Woisky and Salatino [26]. Quercetin was used to make the calibration curve. 10 mg of quercetin was dissolved in 80% ethanol and then diluted to 25, 50 and 100 µg/ml. The diluted standard solutions (0.5 mL) were separately mixed with 1.5 mL of 95% ethanol, 0.1 mL of 10% aluminum chloride, 0.1 mL of 1M potassium acetate and 2.8 mL of distilled water. After incubation at room temperature for 30 minutes, the absorbance of the reaction mixture was measured at 415 nm with a Shimadzu UV-160A spectrophotometer (Kyoto, Japan). The amount of 10% aluminum chloride was substituted by the same amount of distilled water in blank.

Estimation Of total phenolics by FolinCiocalteu method:

The total phenolics in the extract were evaluated using Folin-Ciocalteu method as described by Kujala *et al*. [27]. 5 ml of Folin-Ciocalteu (Sigma-Aldrich) and 4 ml Sodium carbonate (7% w/v) was added to each sample solution (1.0 ml) and the standard (gallic acid) and shaken. The solution was allowed to stand for 30 minutes in the dark at room temperature, after which absorbance was measured at 765 nm using a spectrophotometer. The amount of total phenolics was expressed as gallic acid equivalent (GAE) in milligram per gram dry plant extract using the expression;

$C = c \times V/m$

Estimation of total carbohydrates by Phenol-Sulphuric acid method:

The total sugar concentration of the extract was estimated by Dubois method [28]. Glucose working standard (1mg/ml) was pipetted out into different test tubes in 0-0.1ml range, 0.2ml fraction was taken and made up to 1ml by adding water, 5ml of 96% H_2S0_4 was added to all the tubes and incubate at for 5 minutes. 1ml of Phenol was added to the tubes and incubated at 30˚ C for 20 minutes. Absorbance was read at 490 nm.

Estimation of Total Protein by Lowry's method:

Protein was estimated by Lowry's method [29] using bovine serum albumin as standard working solution (75µg/ml). The working solution from 0-1.0ml was pipetted out and the volume was made up to 1.0 ml by adding distilled water. 0.2ml of the fraction was taken up from these different dilutions and made up to 1ml by adding distilled water. Then 3ml of Lowry's reagent was added, incubate all the tubes for 10 minutes at room temperature. 0.5ml of FC reagent was then added to tubes and incubated at room temperature for 20 minutes, which gave blue-voilet complex read at 660nm.

Evaluation of Anti-Stress Activity

Drosophila melanogaster Stock and Media: *Drosophila melanogaster* (wild type) was obtained from the Drosophila stock Centre, Department of Zoology, University of Mysore. The flies were maintained at 25 ± 1°C and 60–70% relative humidity. Flies were reared on 'Rava-Agar Medium'. The diet

was composed of Rava-10g, Jaggery-100g, propionic acid-7.5ml, Agar-agar-10g and distilled water-1000ml as described previously [30].

Oxidative Stress Analysis: The 4 groups of Drosophila melanogaster were cultured in the laboratory. In the first group only control flies were cultured. In the second group MTX induced flies, in the third group MTX along with plant extract (0.5g) treated flies and in the fourth group only plant extracts (0.5g) treated flies were cultured. Later the stress induction and reduction parameters were found by the evaluating antioxidant defense enzymes like Catalase (CAT) and Super oxide dismutase (SOD) in every group of flies.

Enzyme collection: All four groups of flies were taken in different Eppendorf tubes (4 flies in each tube). They were homogenized in a 200µl of 250mM phosphate buffer (pH 7.8); 50 mM (pH 7) & 0.4M (pH 7) for SOD & CAT assay respectively, and are centrifuged at 8000 rpm for 20 minutes in a cooling microfuge. 100µl of this supernatant serves as enzyme source for SOD & CAT enzymatic assays.

Superoxide dismutase (SOD): SOD enzyme (EC.1.15.1.1) was assayed using a method described by Beauchamp and Fridovich [31] with slight modifications. For this assay Mix 3 ml of cocktail solution containing Phosphate buffer (0.8 ml), Methionine (1 ml), riboflavin (0.5 ml), EDTA (0.1 ml), NBT (0.5 ml), and the volume is made up to3 ml by adding distilled water. A blank was set without the enzyme and NBT to calibrate the spectrophotometer having buffer (1.0 ml), Methionine (1 ml), riboflavin (0.7 ml), and EDTA (0.3 ml). Another control was prepared having NBT but no enzyme and is taken as a reference. The protein estimation was carried out by Lowry's method [29] and the specific activity of SOD was calculated.

Catalase enzyme assay (CAT): CAT enzyme (EC.1.11.1.6) was assayed using a method reported earlier [32]. 0.1ml of crude Enzyme extract is mixed with 2.9ml of 30% of hydrogen peroxide. Decrease in the absorbance at A240nm indicates the action of CAT on H_2O_2. Protein estimation is done by Lowry's method [29]. Specific activity is expressed in units/mg of protein.

Statistical Analysis: The results are shown as mean±SEM (standard error of mean) of experiments performed in triplicates.

Results

Preliminary Phytochemical screening

Table 1: Preliminary Phytochemical screening of plant extract of *M. jalapa*.

Phytochemical components	Plant extract
Alkaloids	+
Saponins	+
Flavonoids	+
Tannins	+
Phenols	+

"+" =Present; "-" = absent

The phytochemical active compounds of plant extract were qualitatively analyzed and the results are presented in Table 1. The data indicate that the methanolic extract of aerial part showed the presence of phytochemical active compounds such as alkaloids, flavonoids, phenols, tannins and saponins. The methanolic extract of *M. jalapa* found to contain a noticeable amount of total flavonoids which plays a major role in controlling antioxidants. The plant exhibited significant antioxidant properties and could serve as a free radical inhibitor/scavenger. The amount of protein present in the methanol extract was found to be 3.7µg/ml and also 46% of total carbohydrate content.

Effect from extract of *M. jalapa* to SOD activity

Culturing of flies in culture media along with different concentrations of MTX (a stress inducer) increased the activity of SOD (stress marker enzyme). The activity is further enhanced as the concentration of MTX increased from 5ppm to 10ppm in the flies in concentration dependent manner when compared to that of control flies [Fig 1 & Table 2]. Considerable decrease in the activity of SOD was observed in the third group of flies by the introduction of 0.5g of plant sample [Fig 2 & Table 2].

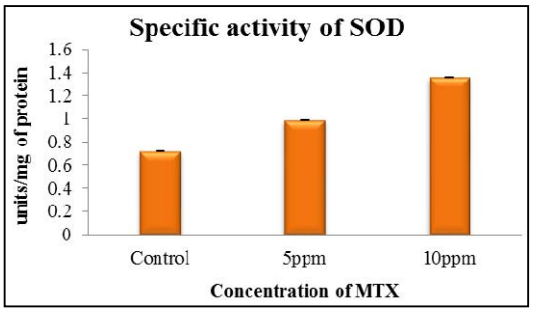

Fig 1: Activity of SOD increased gradually in the flies (second group) reared on media containing increased Concentration of MTX.

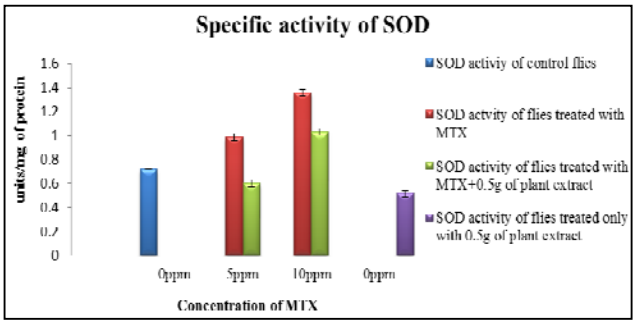

Fig 2: Activity of SOD increased gradually in flies reared on media containing increasing concentration of MTX (second group) and reduction of activity was observed in the presence of 0.5g of plant extract (third group).

Table 2: Comparison table of CAT and SOD activity of stress induced flies (second group) and MTX+0.5 gm of plant extract at different concentrations (third group).

	Concentration of MTX	5ppm	10ppm
CAT activity in units/mg of protein	MTX alone	70	81
	MTX + Plant extract	38	65
SOD activity in units/mg of protein	MTX alone	0.99	1.36
	MTX + Plant extract	0.6	1.03

Effect from extract of *M. jalapa* to CAT activity

The CAT enzyme activity also got increased similar to the SOD in second group of flies. And its activity was further enhanced in increased concentration of MTX from 5ppm to 10ppm in the culture media in a concentration dependent manner compared to that of control flies [Fig 3 & Table 2]. There was a considerable decrease in the activity of CAT

when 0.5 g of plant extract was added along with MTX of different concentration in third group of flies [Fig 4 & Table 2].

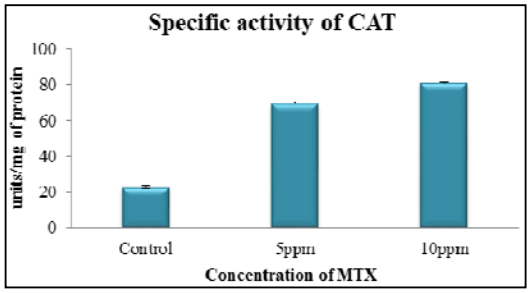

Fig 3: Activity of CAT increases gradually in flies (second group) reared on media containing increasing concentration of MTX.

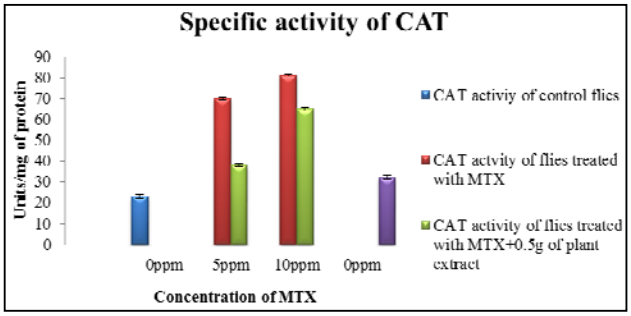

Fig 4: Activity of CAT increases gradually in flies reared on media containing increasing concentration of MTX (second group) and reduction of activity was observed in the presence of 0.5g of plant sample (third group).

Variation of enzyme activity in normal flies treated with plant sample alone

Comparative study was performed by growing the fruit flies in the media containing only 0.5gm of plant extract with that of control. Difference in the enzyme activity was observed in the fourth group of flies reared on the media containing only 0.5 gm of the plant sample [Table 3].

Table 3: Variation of enzyme activity in normal *D. Melanogaster* and the flies treated with plant exract.

	CAT activity in units/mg of protein	SOD activity in units/mg of protein
Control flies (1st group)	23	0.72
Plant extract treated flies (4th group)	32	0.52

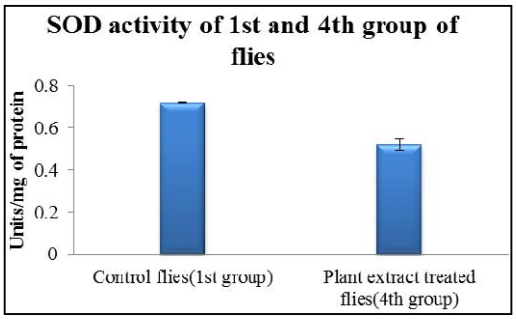

Fig 5: SOD activity in fruit flies of control (first group) and plant extract (fourth group) comparative study.

Reduction in the SOD activity was seen in the fourth group flies over the control flies [Fig 5], and the activity of CAT was also found to be decreased in the same flies grown on medium containing only 0.5g of plant extract to that of control flies [Fig 6 and Table 3]

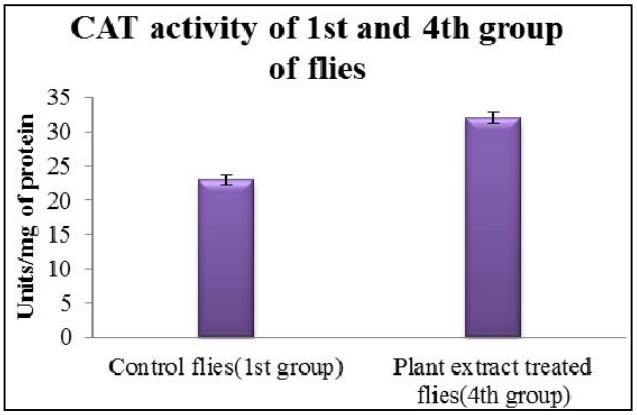

Fig 6: CAT activity in fruit flies of control (first group) and plant sample (fourth group) comparative study.

From the above result, it was confirmed that SOD & CAT activity was increased when MTX is added and the activity was reduced when treated with plant extract. Hence *M. jalapa* has the ability to reduce the stress induced by MTX.

Discussion

The preliminary phytochemical investigation of methanol extract of *M. jalapa* indicated the presence of alkaloids, flavonoids, phenols, tannins and saponins. Most of the polar compounds such as phenolic and flavonoid substances are potent inhibitors of reactive oxygen species attack [33]. Phenolics and flavonoids also show cytotoxicity in Hoechst 33258 fluorescence assay by inhibiting cellular DNA in a concentration-dependent manner [34]. The biological properties, comprising cytotoxic and antioxidant properties, of flavonoids are considered in an evaluation of the medicinal and nutritional values of these compounds [35]. From literature review and phytochemical analysis it was found that the plant, *M. jalapa*, contains phenolic as well as flavonoid type compounds.

Stress is defined as a physical/ psychological stimulus that can produce mental tension or physiological reactions that may lead to illness [36]. Origin of stress may vary but its effect is harmful. It is a condition which can disturb the normal physiological and psychological functioning of an individual. It is a well-known fact that stress of any nature causes a non-specific state in the organism i.e. "stress syndrome" which is characterized by adrenal hypertrophy, depletion of adrenal ascorbic acid and cortisol and a reduction in the size of lymphoid tissue [37, 38].

An imbalance between antioxidant defense and ROS results in oxidative stress, leading to cellular damage. Oxidative stress is a condition characterized by higher levels of intracellular ROS. Either are, or break down to form, free radicals. ROS include superoxide anion (O_2-), singlet oxygen (O_2), hydroxyl radical (OH-), and hydrogen peroxide (H_2O_2), that are capable of reacting with, and damaging not only DNA, even proteins, and lipids as well [39]. The results of the present study revealed increase in the activity of the stress related marker enzymes in stress induced flies. Under normal conditions, ROS are cleared from the cell by the actions of SOD and CAT. Low level of intracellular ROS have been identified as second messengers in signaling pathways and implicated in

transcriptional regulation to stimulate cell growth, but higher doses of ROS result in growth arrest and cell death [40]. Oxidative damage to proteins plays a crucial role in ageing because oxidized proteins lose catalytic function and are preferentially hydrolysed. It is hypothesized that oxidative damage to specific proteins constitutes one of the mechanisms linking oxidative stress damage and age-associated losses in physiological functions [41].

Cells have a variety of defense mechanisms to ameliorate the harmful effects of ROS. Superoxide dismutase (SOD) catalyses the conversion of two superoxide anions into a molecule of hydrogen peroxide (H_2O_2) and oxygen (O_2) [42, 43]. In the peroxisomes of eukaryotic cells, the enzyme Catalase converts H_2O_2 to water and oxygen, and thus completes the detoxification initiated by SOD. In addition to this Cells can able to protect themselves against ROS damages using Guaiacol peroxidase, Ascorbate peroxidase.

In the present work, the activity of SOD and CAT increases significantly in a concentration dependent manner after inducing stress by MTX [Fig 1, 3 & Table 2]. One possible reason is that the stress inducing agent MTX an anticancerous drug which acts by inhibiting the metabolism of folic acid where it is needed for the de novo synthesis of nucleosides may cause drug induced oxidative stress and much ROS is produced [44].

Similarly Increased activity levels in Arginase, GPx, CAT and SOD enzyme levels was observed on prolonged ammonia exposure in kidney tissues of fish due to oxidative stress [45]. Oxidative stress has been implicated as the main causal factor in aging and antioxidant defense is therefore considered critically important in longevity assurance [39]. In order to antagonize the elevated level of ROS, the level of the defensive enzymes such as CAT and SOD also got increased under stressful condition to protect the organism from drug induced oxidative stress [Fig1 & 3]. Similar result of increase in the antioxidant enzyme levels in the medium fed with same concentration of MTX was found by Fathima *et al*., [46].

MTX has a greater toxic effect on rapidly dividing cells (such as malignant and myeloid cells, and gastrointestinal and oral mucosa), which replicate their DNA repeatedly, and thus inhibits the growth and proliferation of these noncancerous cells, as well as producing several side effects. Facing a scarcity of dTMP, rapidly dividing cancerous cells undergo cell death via thymine less death [47]. Thus MTX causes drug induced oxidative stress. Oxidative stress is the steady state level of oxidative damage in a cell, tissues or organ caused by the ROS. Oxidative modification of nucleic acids by ROS is of remarkable biological importance, as it results in the transformation of nonmalignant cells into malignant ones [48]. Oxidative stress has been linked to various diseases, including cancer, atherosclerosis, ischemic injury, inflammation, and neurodegenerative diseases, such as Alzheimer's disease and Parkinson's disease [39]. There are a number of non-enzymatic small molecule antioxidants plays a role in detoxification. Glutathione may be the most important intra-cellular defense against the deleterious effects of ROS. Other small molecule antioxidants such as Ascorbic acid (vitamin C), Tocopherol (vit E), Uric acid, and β-carotene also play important roles as cellular antioxidants [39]. Similarly, polyphenol antioxidants contribute in avoiding ROS damages by scavenging [49].

In recent herbal drug scenario, plant derived antioxidants are attaining importance because of their potential health benefits, no toxicity and side effects over synthetic antioxidants like butyl hydroxy anisole and butyl hydroxy toluene (BHA and BHT, respectively) [50].

As per our results, the plant extract (*M. jalapa*) used in this study is found to reduce the stress induced by MTX and was confirmed by comparing the activity of antioxidant enzymes in second group of flies with that of third group flies containing both MTX as well as 0.5g of plant extract [Fig 2, 4 and Table 2].

Similarly Acorus calamus [46], Convolvulus pluricaulis [51], Glycyrrhiza glabra [52], and Rauwolfia serpentine [53] has reduced the level of antioxidant enzymes in stress induced fruit flies treated with respective plant sample in different concentrations.

In fourth group the flies were reared in the media containing only 0.5 gm of plant sample where the specific activity of the SOD is less to that of control [Fig 5]. The hypothetical reason behind this is may be the plant molecules are reducing the level of ROS much lesser to that of normal level which intern reduced the antioxidant enzyme levels. Whereas the fruit flies reared in the medium containing Convolvulus pluricaulis [51] and Glycyrrhiza glabra [52], the activity of SOD in control and fourth group of flies were almost similar to that of control.

In the current study, specific activity of CAT has increased slightly in fourth group of flies over the control flies [Fig 6 & Table 3] which may indicate the antioxidant and free radical scavenging property of *M. jalapa*. Slight increase in the level of defensive enzymes within the normal range aid in better scavenging of ROS and also it shows the healthiness of fruit flies. But the specific activity of CAT has decreased in flies reared on medium containing Glycyrrhiza glabra [52] and there is a slight increase in CAT activity in the flies with Convolvulus pluricaulis [51] to that of control flies.

Especially when it comes to decreasing the effects of stress on the body, herbs are a perfect solution to reduce stress related build ups of toxins, to calm the overactive mind, to strengthen the heart and breathing systems, to help break down adrenaline, all of which are under attack by ongoing stress [54].

The stress induction in the Drosophila was confirmed by the increase activity of cellular defensive enzymes like SOD and CAT. As per the results plant extract was found to reduce the stress induced by MTX in fruit flies. This may open up a new avenue of research in identifying the plants which possess anti-stress property and exploiting its action by using D. melanogaster as model organism.

Conclusion

The plant sample used as an anti-stress agent here may be used to combat stress related disorders. The anti- stress property (Reduction of elevated ROS) was confirmed by employing the SOD and CAT activity assay, compared to the stressor induced group. The stressor group treated with plant extract revealed the decreased level of ROS thereby reducing antioxidant enzyme activity. Thus, *M. jalapa* tends to balance between ROS and a variety of enzyme system that can deactivate ROS, thereby it aids in improving and maintaining the health of D. melanogaster even under stressful conditions. This experiment have a profound implication for the broad scope of applications of anti-stress molecules to humans before which fruit flies can be used as models to study its power of action.

From the present work it can be concluded that *Mirabilis jalapa* Linn, traditionally widely used medicinal plant shows a wide range of biological activities. However, here remains an immense scope for further exploration of this plant and needs the attention of scientists to exploit the full potential activities of this plant.

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References

1. Kayser O, Quax WJ Eds., Medicinal plant biotechnology: from basic research to industrial applications. Weinheim, Wiley VCH, 2007.
2. Daniel M. Medicinal plants chemistry and properties.
3. Kataoba J, Habuqa N, Furuno M, Miyano M, Takanami Y, Koiwai A. DNA sequence of Mirabilis antiviral protein (MAP), a ribosome inactivating protein with an antiviral property from *M. Jalapa* and its expression in Escherichia coli. J. Biol.Chem. 1991; 266:8426-8430.
4. kusamba C, Byamana K, Mbuyi WM. Antibacterial activity of Mirabilis jalapa seed powder. J Ethnopharmocol. 1991; 35:197-199.
5. Dimayuga E, Virgem M, Ochoa N. Antimicrobial activity of medicinal plants of Baja Californi Sur/Mexico. Pharmaceutical Biology. 1998; 35:33-43.
6. Kaladhar D, Nandikolla SK. Antimicrobial studies, Biochemical and image analysis in Mirabilis lalapa Linn. Int J Farm &Tech. 2010; 2(3):683-93.
7. Eneji SM, Inuwa HM, Ibrahim S, Ibrahim AB, Abdulfattah A. *In vitro* assessment of bioactive components of Mirabilis jalapa ethanolic extract on clinical isolates of Salmonella typhi and Bacillus cereus. Afr J Biotechnol. 2011; 10(7):16006-11.
8. Mahalingam R, Bhirathidasan R, Ambikapathy V, Panneerselvam A. GC-MS Determination of Bioactive compounds of Mirabilis jalapa. Asian J Plant Sci Res. 2012; 2(3):224-7.
9. Goleniowski ME, Bongiovanni GA, Palacio L, Nunez CO, Cantero JJ. Medicinal plants from the Sierra de Comechingones, Argentina. J Ethnopharmacol. 2006; 107(3):324-41.
10. Weckerle CS, Ineichena R, Huber FK, Yang Y. Mao's heritage. Medicinal plant knowledge among the Bai in Shaxi, China, at a crossroads between distinct local and common widespread practice. J Ethnopharmacol. 2009; 123:213-28.
11. Aoki K, Cortes AR, Ramırez MC, Gomez-Hern M, Francisco J. L-M. Pharmacological study of antispasmodic activity of Mirabilis Jalapa Linn flowers. J Ethnopharmacol. 2008; 116:96-101.
12. Lee S, Xiao C, Pei S. Ethnobotanical survey of medicinal plants at periodic markets of Honghe Prefecture in Yunnan Province, SW China. J Ethnopharmacol. 2008; 117:362-77.
13. Boulogne I, Lionel GR, Harry OL, Marie F, Gladys LM. Tramil. Ethnopharmacological survey in Les Saintes (Guadeloupe, French West Indies): A comparative studies. J Ethnopharmacol. 2011; 133:1039-50.
14. Polat R, Fatih S. An ethnobotanical survey of medicinal plants in Edremit Gulf. J Ethnopharmacol. 2012; 139:626-41.
15. Srithi K, Chosie T, Prasit W, Henrik B. Medicinal plants used in Hmong women's health care in Northern Thailand. J Ethnopharmacol. 2012; 139:119-35.
16. Sharma J, Sumeet G, RG Gour, RM Painuli. The treatment of jaundice with medicinal plants in indigenous communities of Sub Himalayan of Uttarakhand India. J Ethnopharmacol. 2012; 143:262-91.
17. Muhammad A, Adeel M, Rifat NM, Zabta KS. Indigenous knowledge of medicinal plants from Guranwala district Pakistan. J Ethnopharmacol. 2013; 148:714-23.
18. Muhammad A, Adeel M, Rifat NM. Indigenous knowledge of medicinal plants from Leepa valley, Azad Jamu and Kashmir, Pakistan. J Ethnopharmacol. 2012; 143:338-46.
19. Sarkar P, Mahmud AK, Mohanty JP. Anti-diabetic activity of ethanolic Extract of Mirabilis Jalapa roots. Int J Pharm Technol. 2011; 3:1470-9.
20. Singh R, Sarabjeet S, Jeyabalan G, Ashraf A. An Overview on Traditional Medicinal Plants as Aphrodisiac Agent. J Pharmacogn Phytochem. 2012; 4:43-56.
21. Kamagaju L, Bizuru E, Minani V, Renato Morandini, Stevigny C, Ghanem G, Duez P. An ethnobotanical survey of medicinal plants used in Rwanda for voluntary depigmentation. J Ethnopharmacol. 2013; 150:708-17.
22. Bhatia H, Manhasb RK, Rani M. Traditional knowledge of poisonous plants of Udhampur district of Jammu and Kashmir, India. J Ethnopharmacol. 2014; 152:207-16.
23. Gilda GL, Suzana GL, Wagner V. Quick preparative separation of natural naphthopyranones with antioxidant activity by high speed counter current chromstography. Z. Naturforsch57c, 2002, 1051-1055.
24. Magadula JJ, Tewtrakul S. Anti-HIV-1 protease activities of crude extracts of some Garcinia species growing in Tanzania. Afr. J. Biotechnol. 2010; 9(12):1848-1852.
25. Harbourne JB. Phytochemical Methods: A Guide to Modern Technique of plants Analysis. Chapman and Hall, London, 1983.
26. Woisky RG, Saltino A. Analysis of propolis: some parameters and procedures for chemical quality control. J. of Apicultural research. 1998; 37(2):99-105.
27. Kujala TS, Loonen JM, Klika KD, Pihlaja K. Phenolics and betacyanins in red beetroot (Beta vulgaris) root: Distribution and effect of cold storage on the content of total phenolics and three individual compunds. J Agric. Food Chem. 2000; 48:5388-5342.
28. Dubois M, Gilles KA, Hamilton JK, Rober PA, Smith F. colorimeric method for determination of sugars and related substances. Analytical Chemistry. 1956; 28:350-356.
29. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 1951; 193:265.
30. Ashburner M, Thompson JN. The laboratory culture of Drosophila in Ashburner M, Wright TRF. The genetics and Biology of Drosophila 2A. Academic Press, 1978, 1-81.
31. Beauchamp CO, Fridovich I. Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. Analytical Biochemistry. 1971; 44:276-287.
32. Beers RF, Sizer IW. A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. J. Biol. Chem. 1952; 195:133-140.
33. Owen RW, Haubner R, Mier W, Giacosa A, Hull WE, Spiegelhalder B, *et al*. Isolation, structure elucidation and antioxidant potential of the major phenolic and flavonoid compounds in brined olive drupes. Food Chem. Toxicol. 2003; 41(5):703-717.
34. Chang YC, Tai KW, Huang FM, Huang MF. Cytotoxic and Nongenotoxic Effects of Phenolic Compounds in Human Pulp Cell Cultures. J endodontics. 2000; 26(8):440-443.
35. Harborne JB, Williams CA. Advances in flavonoids research since, Phytochem. 1992-2000; 55(6):481-504.

36. Stedman: American Psychological Association (APA): stress. (n.d.). The American Heritage® Stedman's Medical Dictionary. Available: from Dictionary.com website: http://dictionary.reference.com/browse/stress. Accessed Mar 11, 2013.
37. Singh N, Nath R, Mishra N, Kohli RP. An Experimental Evaluation of Anti-stress Effects of Geriforte (An Ayurvedic Drug). Quarterly J. of Crude Drug Res. 1978; 3:125-132.
38. Selye H. Experimental evidence supporting the conception of Adaptation Energy, American J. of Physiology. 1938; 123:758-765.
39. Jan Vijg. Aging of the genome: The dual role of DNA in life and death. Oxford university press, New York. 2007; 1:138-190.
40. Chelikani P, Fita I, Loewen PC. Diversity of structures and properties among catalases. Cellular Mol. Life Science. 2004; 61:192-208.
41. Rajindar S Sohal. Role of oxidative stress and protein oxidation in the aging process Free radical Bio. and Med. 2002; 33:37-44.
42. McCord JM, Fridovich I. The Reduction of Cytochrome C by Milk Xanthinse Oxidase. Journal of Bio. Chem. 1968; 243:5733-5760.
43. Anitha T, Usha R. Effect of salinity stress on physiological, biochemical and antioxidant defense systems of high yeilding cultivars of Soyabean. International Journal of Pharma and Bio Sciences. 2012; 3(4):851-864.
44. Nigar Vardi, Hakan Parlakpinar, Aslı Cetin, Ali Erdogan, Cetin Ozturk. Protective Effect of Carotene on Methotrexate–Induced Oxidative Liver Damage. Toxicologic Pathology. 2010; 38:592-597.
45. Hari P, Neeraja P. Ambient Ammonia Stress on Certain Detoxifying Enzymes in Kidney Tissues of Fish, *Cyprinus Carpio*. International Journal of Pharma and Bio Sciences. 2012; 3(4):213-217.
46. Fathima A, Patil V, Kumar S. Suppression of elevated reactive oxygen species by acorus calamus (vacha) a sweet flag in drosophila melanogaster under stress full conditions. IJPSR. 2014; 5(4):1431-1439.
47. Johnston A, Gudjonsson JE, Sigmundsdottir H, Ludviksson BR, Valdimarsson H. The anti-inflammatory action of methotrexate is not mediated by lymphocyte apoptosis, but by the suppression of activation and adhesion molecules. Clinical Immunology. 2005; 114:154-163.
48. N. Sivakumar S, Niranjali Devaraj S. Enzymatic and Non-Enzymatic Anti-Oxidant Status of Breast Cancer Patients In Tamilnadu. International Journal of Pharma and Bio Science. 2011; 2(4):46-53.
49. Sarma AD, Mallick AR, Ghosh AK. Free Radicals and Their Role in Different Clinical Conditions: An Overview. International Journal of Pharma Sciences and Res. 2010; 1(3):185-192.
50. Kumar V, Kumar U, Mishra M, Prakash V. *In Vitro* Antioxidants status in selected Indian medicinal plants. International Journal of Pharma and Bio Sciences. 2012; 3(4):511-520.
51. Kumar A, Kumar S. A study of anti-stress property of *Convolvulus pluricaulis* (Shankhapushpi) on stress induced *Drosophila melanogaster*. Drosophila Information Service. 2010; 93:30-35.
52. Sowmya M, Kumar S: Anti-stress property of *Glycyrrhiza glabra* (Athimadhura) on stress induced *Drosophila melanogaster*. Journal of Stress Physiology & Biochemistry. 2010; 6:18-27.
53. Deepthi BK, Kumar S. Anti-stress property of *Rauwolfia serpentine* (Sarpagandha) on stress induced *Drosophila melanogaster*. Drosophila Information Service. 2011; 94:34-40.
54. Wang T, Xu H, Oberwinkler J, Gu Y, Hardie R, Montell C. Light activation, adaptation, and cell survival Functions of the Na+/Ca2+ exchanger CalX. Neuron. 2005; 45:367-378.