ORIGINAL ARTICLE

Effects of *Adhatoda vasica* leaf extract in depression co-morbid with alloxan-induced diabetes in mice

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Context: Increased neuronal oxidative stress as a consequence of diabetes may result in neuropsychological complications such as depression. Depression co-morbid with diabetes further hampers the quality life years in diabetic patients. **Aim:** Thus, the present study was aimed at investigating the effects of *Adhatoda vasica* leaf extract (EAV), as a natural remedy, in alloxan-induced diabetes and co-morbid depression in mice. **Materials and Methods:** Experimentally, mice were rendered diabetic with a single dose of alloxan of 200 mg/kg, intraperitoneally (i.p.). After 3 weeks of having chronic diabetic state, mice were given EAV (100-400 mg/kg, orally)/ vehicle/standard control (escitalopram, ESC; 10 mg/kg, orally) for 7 days. After dosing, anti-diabetic effect was detected by the fasted blood glucose levels and anti-depressant effect was evaluated by behavioural despair tests, followed by monoamine oxidase (MAO) activity and oxidative stress analysis. **Results and Discussion:** EAV treatment effectively reduced the elevated blood glucose levels and reversed co-morbid depressive behaviour. Furthermore, EAV inhibited diabetes induced increased oxidative stress and MAO activity in the brain. Thus, EAV demonstrated the potential protective action against oxidative stress and revealed monoamine modulatory activity in the brain, which may contribute to its anti-depressant effect. **Conclusion:** This work demonstrates the efficacious effect of EAV in reversing the depression co-morbid with alloxan-induced diabetes in mice.

Key words: *Adhatoda vasica*, co-morbid depression, diabetes, forced swim test, monoamine oxidase activity, oxidative stress

INTRODUCTION

Depression is one of the severe neuropsychological complications associated with diabetes. The prevalence rate of depression in diabetic individual is nearly twice as that of the normal population.[1] Depression co-morbid with diabetes correlates with poor health management, reduced quality life years and higher risk of complications.[2] There exists a complex pathophysiological correlation though; the altered neuronal monoaminegic function due to increased metabolic oxidative stress as a consequence of diabetes may raise depressive episodes.[3] Diabetes progressively increases cellular oxidative load and results in accumulation of reactive oxygen species (ROS) in the brain.[4] The highly toxic ROS in neuronal tissue cause cellular damage and cell death, which is believed to be one of the major aetiologies of depression co-morbid with diabetes.[5]

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The pathological mechanism of diabetes associated depression can be studied by screening the neuro-behavioural alterations in diabetes animal models. Alloxan-induced diabetes model is one of well known and well utilised models. Alloxan develops diabetes through specific inhibition of glucokinase enzyme and selective necrosis of the insulin secreting beta cells in the pancreas, which result in insulin-dependent (type-1) diabetic condition.[6] Earlier studies have demonstrated the consequences of depression in alloxan-induced diabetes such as decrease in duration of immobility in forced swim test (FST) and prolonged desperate behaviour and decreased exploratory activity in open field test.[7,8] Reports have shown that alloxan-induced diabetes results in increased brain monoamine oxidase (MAO) activity.[9,10] Furthermore, alloxan-induced diabetes is known to increase oxidative stress in the brain resulting in altered neuronal activity.[11,12]

In recent years, there is a phenomenal rise in the interest in herbs to explore their active constituents, therapeutic activity and pharmacological actions. *Adhatoda vasica* or *Justicia Adhatoda*, one of the Ayurvedic plants, is widely known for its medicinal activity for over two thousand years. It is commonly known as Malabar nut tree (or Adosa in Hindi), belong to the *Acanthaceae* family. It is a small evergreen, sub-herbaceous shrub,

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which grows commonly in open plains, especially in the lower Himalayas.[13] Earlier studies have shown the anti-oxidant effect of *Adhatoda vasica*.[14,15] The leaf extract of *Adhatoda vasica* contains quinazoline class of alkaloids namely, vasicine and vasicinone, which are reported to have anti-oxidant activity.[16] The other active principle includes essential oil rich in vitamin C and b-carotene, which are also shown to have an anti-oxidant effect in depression as well as in cognitive condition.[17-20] In addition, the leaf extract has also been reported to have anti-diabetic action in rodents.[21] However, the effects of *Adhatoda vasica* leaf extract for the treatment of depression co-morbid with diabetes, still remains an area of interest.

Thus, the present investigation was aimed at studying the anti-depressant and anti-diabetic effects of the Indian herb *Adhatoda vasica* leaf extract (EAV) in alloxan-induced diabetes in mice and to assess if the anti-oxidant activity of EAV is involved in the protective effect against depression co-morbid with alloxan-induced diabetes.

MATERIALS AND METHODS

Animals

In the present study, 42 Swiss albino mice (Mus musculus) of either sex, with body weight of 24 ± 2 g, were used. Mice were obtained from the Hisar Agricultural University, Hisar, India. Mice were maintained in standard laboratory conditions with alternating light and dark cycle of 12 h each, temperature 23 ± 2° C and humidity conditions 62 ± 5% relative humidity (RH) in the housing for at least 1 week before the commencement of the experiments. Mice had free access to food (standard pellet chow feed) except 18–24 h before the blood glucose monitoring test and were given filtered water *ad libitum*. Mice were used only once for each experiment.

Ethical Approval

The experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC, Protocol No. IAEC/RES/04/01, dated 22 April 2009) of Birla Institute of Technology and Science, India (417/01/a/CPCSEA). All the experiments were conducted according to the guidelines of the Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA) and proper care and handling of animals were assured throughout the experimental work.

Drugs and Chemicals

Escitalopram (ESC) was given by Ranbaxy Research Laboratory, India as a gift sample. Alloxan was purchased from Sigma Aldrich, USA. The ethanolic extract (EAV) was freshly prepared in 0.25% w/v sodium carboxymethyl cellulose salt (sod CMC), while ESC was freshly prepared in distilled water. ESC and EAV were given orally at 9.00 to 10.00 hours for 7 days in a constant volume of 10 ml/kg.

Plant Material

Sample Collection

The fresh leaves of the plant *Adhatoda vasica* [Figure 1] were collected in the month of January from the botanical garden, Birla Institute of Technology and Science (BITS-Pilani), India and authenticated by pharmacognosy expert, Department of Pharmacy, BITS-Pilani, India. The leaves were dried in shade for 25 days and then crushed to powder using an electronic blender. The powdered sample was subjected to successive extraction.

Preparation of Ethanolic Extract of Adhatoda Vasica Leaves

A powdered sample of 100 g was packed in soxhlet apparatus and extracted with 95% ethyl alcohol until clear and colourless solvent was appeared in the siphon tube.[22] The plant extract was then collected and filtered through Whatman No. 1 filter paper. The extract was air-dried and stored at 8°C. The extract was analysed by thin layer chromatography (TLC) and compared with control. The percentage yield of ethanolic extract was calculated as percentage of mass of the recovered product/mass of the raw material and was found to be 10.7% (w/w).

Acute Toxicity Studies

Swiss albino mice of either sex (20-25 g weight) were used for acute oral toxicity study. Mice were fasted 3 h prior to the experiment. The study was carried out as per OECD guidelines 425 and animals were observed for mortality and behavioural changes for 48 h (short-term toxicity).[23]

Induction of Diabetes and Assessment

Alloxan was freshly prepared in acetate buffer (0.15 M, pH 4.5) and was given to overnight fasted mice at a single dose of 200 mg/kg, i.p. Induction of hyperglycaemia was

Figure 1: *Adhatoda vasica* leaves

assessed by a portable glucometer (Akkiscan, Nepro, India) 72 h after alloxan dosing. After 3 weeks, mice with fasted blood glucose levels above 200 mg/dl were considered diabetic and animals with lower levels were discarded.

Treatment

Animals were randomly divided into six groups and each group consisted of six mice and following treatments were given for 7 days to their respective groups as follows:

Group I - Normal control group received 0.25% w/v sod CMC
Group II - Diabetic control group received 0.25% w/v sod CMC
Group III - Diabetic mice received EAV (100 mg/kg)
Group IV - Diabetic mice received EAV (200 mg/kg)
Group V - Diabetic mice received EAV (400 mg/kg)
Group VI - Diabetic mice received ESC (10 mg/kg).

Behavioural Assays

Forced swim test

During the test, mice were individually placed in a plexiglass cylinder (height: 30 cm, diameter: 22.5 cm) filled with water up to a height of 15 cm, at 23-25°C. In this test, after an initial vigorous activity (2 min), mice acquired an immobile posture, which was characterised by motionless floating in the water, making only those movements necessary to keep the head above the water. The duration of immobility (s) was recorded during the past 4 min of the 6 min test. Mice were subjected to 15 min training session under similar conditions, 24 h before the test.[24]

Tail Suspension Test

In tail suspension test (TST), mice were individually suspended by the tail to a horizontal bar (distance from floor was 50 cm) using scotch tape (distance from tip of tail was approximately 1 cm). Typically, mice exhibited several escapes-oriented behaviour interspersed with temporally increasing bouts of immobility. The duration of immobility(s) during the 6 min test session was recorded.[25]

Locomotor Activity

The locomotor activity can be easily studied with the help of actophotometer. Each mouse was placed individually in actophotometer and the spontaneous locomotor score of all mice were recorded for the last 8 min of 10 min period.[26]

Biochemical Assays

MAO-A and MAO-B activity

The MAO-A and MAO-B activity was estimated in whole brain content as described elsewhere.[27] Mice were sacrificed and brains were collected immediately on ice bath and washed with sodium phosphate buffer (0.1 M, pH 7.4). The collected whole brain samples were homogenised in 10 volumes of sodium phosphate buffer (0.1 M, pH 7.4) and centrifuged (Remi, cooling compufuge, CPR-24, India) at 12,000 rpm for 20 min. Pellets were discarded. Supernatant was pipette out and analysed for MAO-A and MAO-B enzymatic activity. For estimating MAO-A activity, 2.75 ml Tris buffer (0.1 M, pH 7.4) and 100 µl of 4 mM 5-hydroxytryptamine were mixed in quartz cuvettes, which were then placed in double beam spectrophotometer (UV-1800, Shimadzu, Japan). This was followed by the addition of 150 µl solution of brain homogenate to initiate the enzymatic reaction and the change in absorbance was recorded at a wavelength of 280 nm for 5 min against the blank. For estimating MAO-B activity, 2.75 ml Tris buffer (0.1 M, pH 7.4) and 100 µl of 0.1 M benzylamine were mixed in quartz cuvettes, which were then placed in double beam spectrophotometer. This was followed by the addition of 150 µl solution of brain homogenate to initiate the enzymatic reaction and the change in absorbance was recorded at a wavelength of 250 nm for 5 min against the blank.

Estimation of Lipid Peroxidation

Malondialdehyde (MDA) content, a measure of lipid peroxidation, was measured in the whole brain content in the form of thiobarbituric acid reactive substance (TBARS) as per the reported method.[28] Briefly, 0.5 ml of brain homogenate and 0.5 ml of Tris–HCl were incubated at 37°C for 2 h. After incubation, 1 ml of 10% trichloroacetic acid was added and centrifuged at 1000 rpm for 10 min. To 1 ml of supernatant, 1 ml of 0.67% thiobarbituric acid was added and the tubes were kept in boiling water for 10 min. After cooling, 1 ml double distilled water was added and absorbance was measured at 532 nm. TBARS were quantified using an extinction coefficient of 1.56×10^5/M/cm. Protein (mg/g of tissue) was estimated by commercial available kit. The brain MDA content was expressed as nmols of MDA per mg of protein.

Nitrite Estimation

Nitrite levels were estimated in the whole brain content using the Greiss reagent, which served as an indicator of nitric oxide production.[29] A measure of 500 µl of Greiss reagent (1:1 solution of 1% sulphanilamide in 5% phosphoric acid and 0.1% napthaylamine diamine dihydrochloric acid in water) was added to 100 µl of brain homogenate and absorbance was measured at 546 nm. Nitrite levels were calculated using a standard curve for sodium nitrite. Nitrite levels were expressed as percentage of control (unit activity).

Estimation of Catalase

Catalase activity was assessed in the whole brain content by the standard method.[30] Briefly, the assay mixture consisted of 1.95 ml phosphate buffer (0.05 M, pH 7.0), 1.0 ml hydrogen peroxide (0.019 M) and 0.05 ml brain homogenate (10%) in a final volume of 3.0 ml. Changes in

absorbance were recorded at 240 nm. Catalase activity was calculated in terms of k/min and expressed as mean ± SEM.

Statistical Analysis

The intergroup variation was measured by one way analysis of variance (ANOVA) followed by *post hoc* Dunnet's test. For the analysis of within group variation as in case of fasted blood glucose monitoring and body weight variation during the period of study was measured by repeated measures ANOVA followed by *post hoc* Bonferroni's test. The values are expressed as mean ± SEM.

RESULTS

Acute Toxicity

The acute toxicity study revealed the non-toxic nature of the extract even at a higher dose of 2000 mg/kg body weight of mice for oral route of administration. For the present study, three doses of 100 mg/kg p.o. (low), 200 mg/kg p.o. (medium) and 400 mg/kg, p.o. (high) were selected, which were then assessed for effects on locomotor activity of mice.

Effect of EAV on Blood Glucose Levels and Body Weights

In the present study, behavioural analysis followed by biochemical assays was used to investigate the effect of EAV in alloxan-induced diabetes in mice. For assessment of diabetes, fasted blood glucose levels were measured. After 3 weeks of alloxan injection, blood glucose levels were highly elevated in alloxan-treated mice as compared with the untreated control mice [Table 1]. Sub-chronic EAV treatment significantly and dose dependently regulated diabetes induced elevated blood glucose levels ($P < 0.01$). The body weights of mice were measured weekly and were compared between day 1 and 7of EAV/vehicle dosing. It was found that diabetic mice (Group II) showed a significant decrease in body weights compared with non-diabetic mice (Group I), $P < 0.01$. Further, unlike diabetic group (Group II), the EAV treatment group did not show decrease in body weights during the successive dosing period.

Effect of EAV on FST, TST and Locomotor Activity

After 3 weeks, mice were subjected to behavioural tests such as FST and TST for assessment of depression. Alloxan-treated diabetic mice (Group II) exhibited significantly increased duration of immobility(s) both in FST and TST assays as compared with non-diabetic mice (Group I). EAV and ESC treatment (Group III, IV, V and Group VI, respectively) significantly reversed the increased duration of immobility in alloxan-treated diabetic mice, ($P < 0.01$), [Figure 2a and b]. Spontaneous locomotor scores were measured using actophotometer. Alloxan dosing in mice showed insignificant changes in spontaneous locomotor scores. Also EAV and ESC treatment showed no significant changes in spontaneous locomotor scores in mice ($P < 0.05$), [Figure 3].

Effect of EAV on MAO Activity

Following behavioural studies, biochemical assays were performed to explore the possible mechanism underlying the effects of EAV. Consequently, MAO activity was measured in the brain and it was found that alloxan-induced diabetic mice (Group II) showed significantly elevated MAO-A and MAO-B activity in the whole brain content as compared with non-diabetic

Table 1: Effects of EAV on body weight and fasted blood glucose level

Group	Body weight (g)		Fasting glucose level (mg/dl)	
	Start of dosing	End of dosing	Start of dosing	End of dosing
I	25.00±0.36	26.83±0.60	94.00±3.45	92.83±4.12
II	25.16±0.31	22.33±0.76$^{\$\$}$	349.00±28.97**	352.33±25.94**
III	26.33±1.35	27.16±1.50	365.50±29.14**	248.83±54.68$^{\$\$}$
IV	25.83±0.65	26.16±1.01	316.17±26.04**	201.33±18.91$^{\#\#\$\$}$
V	25.33±0.98	26.33±1.36	329.00±26.24**	159±25.05$^{\#\#\$}$
VI	23.16±0.40	24.00±0.63	334.50±21.02**	321.67±18.24

Start of dosing indicates day 1 of EAV treatment and end of dosing indicates day 7 of EAV treatment. Data are reported as mean±SEM of six mice/group. *P<0.05, **P<0.01: Statistically significant as compared with Group I. $^{\#}P$<0.05, $^{\#\#}P$<0.01: statistically significant as compared with Group II. $^{\$}P$<0.001, $^{\$\$}P$<0.01: statistically significant as compared with start of the dosing. Group I (normal mice received vechile), II (diabetic mice received vechile), III (diabetic mice received EAV, 100 mg/kg), IV (diabetic mice received EAV, 200 mg/kg), V (diabetic mice received EAV, 400 mg/kg), VI (diabetic mice received ESC, 10 mg/kg), EAV – Adhatoda vasica leaf extract

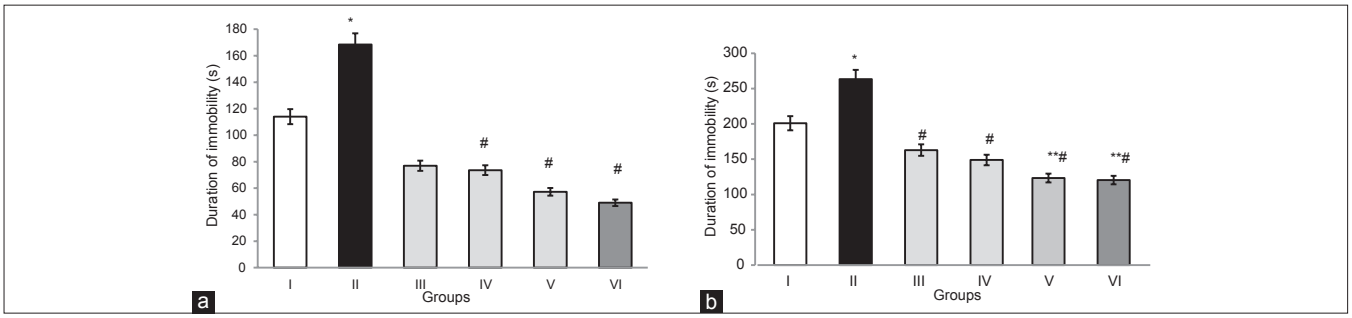

Figure 2: Effect of EAV (100, 200, 400 mg/kg) and ESC (10 mg/kg) on duration of immobility(s) during FST (a) and TST (b). Results are expressed as mean ± SEM (n = 6 mice/group). The columns represent the duration of immobility(s). Error bars represent SEM. *P < 0.05, **P < 0.01 compared with Group I, # P < 0.01 compared with Group II. Group I (normal mice received vechile), II (diabetic mice received vechile), III (diabetic mice received EAV, 100 mg/kg), IV (diabetic mice received EAV, 200 mg/kg), V (diabetic mice received EAV, 400 mg/kg), VI (diabetic mice received ESC, 10 mg/kg)

mice (Group I) ($P < 0.05$). EAV dosing (Group III, IV and V) reversed diabetes induced increased MAO-A and MAO-B enzyme activities, significantly and dose dependently ($P < 0.01$), [Figure 4a and b].

Effect of EAV on Oxidative Stress

Oxidative stress was assessed using oxidant and anti-oxidant markers such as lipid peroxidation, nitrite levels and catalase activity profile in the whole brain content of mice. Lipid peroxidation was assessed using MDA as a marker of lipid peroxidation. Alloxan-induced diabetic mice (Group II) exhibited significantly increased MDA levels in the brain as compared with non-diabetic mice (Group I), [Table 2]. EAV treatment (Group III, IV and V) showed significant control of diabetes mediated alteration in the brain lipid oxidative function ($P < 0.05$). Further, the effect was comparable to that produced by ESC (Group VI), $P < 0.05$. Similarly, nitrite levels were measured and found to be significantly increased in alloxan-induced diabetic mouse brain (Group II). Treatment of EAV (Group III, IV and V) significantly inhibited diabetes-induced elevated nitrite levels ($P < 0.01$). Further the effect was dose dependent and comparable to the effect of ESC treatment (Group VI), $P < 0.01$.

The anti-oxidant profile was measured using catalase enzymatic function. There was a significant reduction of catalase activity in the whole brain content of the alloxan-induced diabetic mice (Group II) as compared with non-diabetic group (Group I), $P < 0.01$. EAV sub-chronic treatment at 400 mg/kg (Group V) reversed diabetes-induced stress condition with significant elevation of catalase activity in the brain ($P < 0.01$). However, lower doses of EAV (100 and 200 mg/kg; Group III and IV, respectively), showed some increased catalase activity, but the increase in the activity was not up to the statistically significant levels.

DISCUSSION

Depression is prevalent in diabetic patients as a neurological complication. The pathophysiology of depression co-morbid with diabetes shows complicated neurochemical phenomenon, which includes altered brain monoamine (namely serotonin, dopamine and norepinephrine) functions, elevated MAO activity

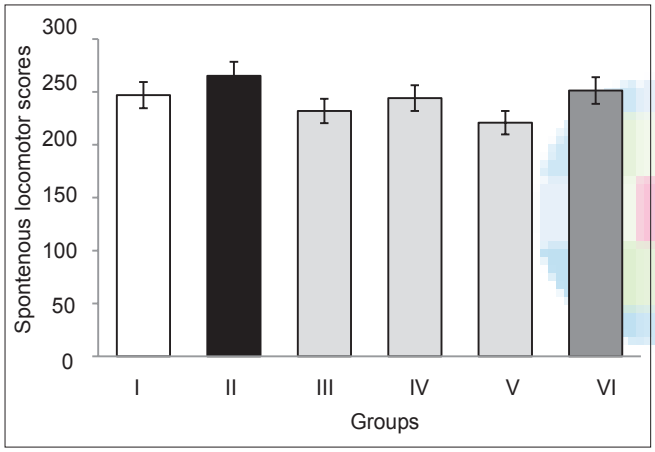

Figure 3: Effect of EAV (100, 200, 400 mg/kg) and ESC (10 mg/kg) on locomotor activity. Results are expressed as mean ± SEM (n = 6 mice/group). The columns represent the mean spontaneous locomotor scores. Error bars represent SEM (n = 6/group). Group I (normal mice received vechile), II (diabetic mice received vechile), III (diabetic mice received EAV, 100 mg/kg), IV (diabetic mice received EAV, 200 mg/kg), V (diabetic mice received EAV, 400 mg/kg), VI (diabetic mice received ESC, 10 mg/kg)

Table 2: Effects of EAV on oxidative stress

Groups	Lipid peroxidation (MDA nmols/mg protein)	Nitrite (unit activity)	Catalase (k/min)
I	0.448±0.095	33.812±2.192	0.728±0.099
II	0.960±0.086*	77.589±6.432**	0.171±0.055**
III	0.560±0.141	49.067±5.331##	0.332±0.065
IV	0.365±0.094##	41.774±2.099##	0.427±0.053
V	0.517±0.121#	32.411±6.929##	0.690±0.094##
VI	0.462±0.110#	38.930±7.743##	0.798±0.062##

Oxidative stress parameters namely, lipid peroxidation, nitrite activity and catalase were measured in whole brain samples. Data are reported as mean±SEM of 6 mice/group. *P<0.05, **P<0.01: Statistically significant as compared with Group I. #P<0.05, ##P<0.01: Statistically significant as compared with Group II. Group I (normal mice received vechile), II (diabetic mice received vechile), III (diabetic mice received EAV, 100 mg/kg), IV (diabetic mice received EAV, 200 mg/kg), V (diabetic mice received EAV, 400 mg/kg), VI (diabetic mice received ESC, 10 mg/kg), MDA – Malondialdehyde, EAV – Adhatoda vasica leaf extract

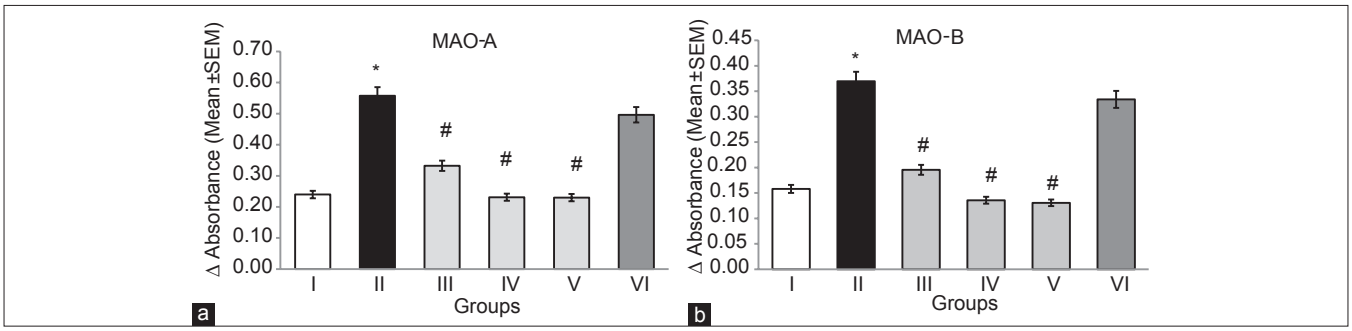

Figure 4: Effect of EAV (100, 200, 400 mg/kg) and ESC (10 mg/kg) on MAO-A (a) and MAO-B (b). The columns represent change in absorbance from the baseline and are expressed as mean ± SEM (n = 6 mice/group). Error bars represent SEM. *P < 0.05 compared with Group I, # P < 0.01 compared with Group II. Group I (normal mice received vechile), II (diabetic mice received vechile), III (diabetic mice received EAV, 100 mg/kg), IV (diabetic mice received EAV, 200 mg/kg), V (diabetic mice received EAV, 400 mg/kg), VI (diabetic mice received ESC, 10 mg/kg)

and increased oxidative stress.[31,32] Thus, the present study was designed to assess the neuro-behavioural consequences using behavioural despair tests followed by biochemical analysis with estimation of MAO enzymatic function and oxidative stress in alloxan-induced diabetes and to evaluate if EAV sub-chronic treatment can attenuate depression co-morbid with diabetes in mice.

The present study revealed that alloxan treatment resulted in diabetes and subsequent depression in mice. EAV significantly produced anti-diabetic and anti-depressant effects in alloxan-induced diabetes and co-morbid depression. Alloxan treatment produced hyperglycaemia and decreased body weights in mice, the common signs of the diabetic condition. EAV showed significant effect on body weights as diabetic mice received EAV treatment showed insignificant decrease in body weights at the end of the dosing period unlike diabetic mice with vehicle treatment. Further, EAV exhibited significant control in elevated blood glucose levels, a key marker of diabetes, which revealed the regulatory effects of EAV on glucose metabolism. The results are in line with the previous reports that suggest the blood glucose lowering effects of the leaf extract.[21,33,34] Previous studies have shown that *Adhatoda vasica* potentiates insulin release from pancreas and enhances mobilisation of glucose to the peripheral tissues.[21] In addition, it has also demonstrated that it has anti-oxidant and radical scavenging activity that may be associated with anti-diabetic activity of EAV.[33] *Adhatoda vasica* has also been reported to inhibit α-glucosidase, the key enzyme that catalyses the final step of carbohydrates digestion and hence can retard liberation of glucose from dietary carbohydrate complex.[35] Overall, it may be suggested that EAV has anti-diabetic activity that may be associated with its complex glucose regulatory effects. Further, the study focused on effects of EAV on diabetes-induced depressive behaviour in mice. Alloxan-induced diabetic mice were assessed for the depressive behaviour regularly in a period of 7 days. After 3 weeks of chronic diabetic state mice exhibited increased behavioural despair effects in FST and TST, increased brain MAO-A and MAO-B enzyme activity and elevated neuronal oxidative stress condition. EAV reversed depressive behaviour evoked by chronic persistence of alloxan-induced diabetic state.

The behavioural despair tests such as FST and TST are most widely accepted models for screening of anti-depressant activity of the compounds in rodents.[24,25] The duration of immobility reflects a state of hopelessness, which is a cardinal symptom of depression in humans.[36] Alloxan-induced diabetes in mice exhibited increased duration of immobility in FST and TST, which is consistent with the results obtained in previous studies.[7] It revealed the development of depressive episodes as a consequence of diabetes in mice.[36,37] EAV effectively reversed diabetes-induced behavioural despair effects in FST and TST without altering the generalised motor performance and the effect was similar to that of the anti-depressant drug, ESC.

Interestingly, earlier reports have linked the brain monoamine alterations to the despair effects in FST and TST models.[38] Thus, one possible mechanism underlying the effectiveness of EAV in this study may involve monoaminergic modulation. Further, it is unlikely that hyperglycaemia could result in despair behaviour and subsequent reduction in blood glucose levels by EAV administration could produce anti-depressant effect, as previous findings have shown that increase in blood glucose levels due to repeated administration of glucose does not elicit despair behaviour in non-diabetic mice.[37] Thus, chronic diabetic state might possibly result in complex physiological alterations that could account for depressive behaviour in mice.

Behavioural assays revealed that the consequences of depression in alloxan-induced diabetes could be related to decreased brain monoaminergic levels, which may be due to increased breakdown, decreased release or reuptake of these molecules. Consequently, MAO enzyme activity as a marker of the monoamine-lowering process was estimated. MAO is an oxidative enzyme, which arrests the activity of monoamines by oxidising them.[39] Alloxan-induced mice exhibited elevated MAO-A and MAO-B activity compared with non-diabetic mice, which corroborates the results obtained by previous studies.[9] EAV effectively attenuated diabetes-induced MAO-A and MAO-B activity. It revealed an indirect elevation of monoamine levels by specific inhibition of their metabolism, which may contribute to the anti-depressant effect of EAV.[38]

The specific necrosis of insulin secreting beta cells in alloxan-treated mice results in insulin deficiency and elevated blood glucose levels.[6] Earlier studies have reported that the excessive glucose levels in neuronal tissue results in overproduction of ROS. The glucose oxidation causes excessive production of electrons, which are taken up by oxygen in the electron transport chain to form ROS.[4] Due to decrease in endogenous anti-oxidant levels and increase in oxidative load, excessive ROS lead to detrimental effects on neuronal tissues.[40] In the present study, alloxan-induced diabetes produced significant elevations of oxidative stress markers in the brain, which is in line with the results obtained in previous studies.[11,12]

Peroxidation of lipids forms lipid radical species that damage cellular macromolecules.[41] Alloxan-induced diabetes significantly elevated MDA levels in the whole brain content, which is a biomarker for lipid peroxidation.

EAV treatment reversed alloxan-induced diabetes stimulated excessive lipid peroxidation in the brain, which showed the protective effect of EAV against oxidative load increased in neuronal tissue.

Nitric oxide is one of the important signalling molecules in neurotransmission. However, the excessively accumulated nitric oxide by nitrosative stress can react with ROS to form a toxic reactive nitrogen species, which is a potent inducer of neurocellular death.[42] Alloxan-induced diabetic mice showed highly increased nitrite levels in the brain, which was curtailed by treatment with EAV. It demonstrated a significant effect of EAV against nitrosative stress in the brain.

Catalase is a key anti-oxidant enzyme in biological system. Similar to the previous studies, a significant decrease in catalase activity was found in alloxan-induced diabetic mice.[12] In contrast, EAV treatment showed a significant elevation of catalase activity in the brain, which revealed the modulatory effect of EAV in anti-oxidant function in biosystem. Altogether, these data suggested the protective effect of EAV against oxidative stress in alloxan-induced diabetes, plausibly preventing the damage of monoaminergic neurons by ROS that could contribute to the consequences of depression co-morbid with diabetes.[11,38]

Conclusively, the present study demonstrated the anti-depressant and anti-diabetic effects of EAV in depression co-morbid with alloxan-induced diabetes in mice. EAV exerted a significant anti-depressant effect on the depressive behaviour exhibited by alloxan-induced diabetic mice in behavioural despair tests (FST and TST). Moreover, EAV inhibited diabetes induced elevated MAO activity and produced a potential protective effect against oxidative stress in the brain, which may contribute to its anti-depressant effect. The results also indicated that monoaminergic modulation may possibly involve in the anti-depressant action of EAV.

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