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Methanol extracts of *Basella alba* leaves alleviate stress in rats

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ABSTRACT

Objective: Stress is becoming an unavoidable threat in recent times, there has been increasing interest by researchers in the use of naturally occurring biologically active compounds with medicinal value to cure body ailments. The present work was carried out to investigate the effect of methanol extract of *Basella alba* leaves on stress in Wistar rats (Rattus norvegicus).

Methods: A total of 35 male rats were used in this study. They were grouped into seven groups of five rats each. Group 1 (normal control) was received 10 mL/kg normal saline. Group 2 contained restraint stress rats only. Group 3 contained forced swim stress rats only. Group 4 and 5 were treated with 60 mg/kg of *B. alba* extract (BAE) thereafter subjected to restraint and forced swim stresses respectively. Group 6 and 7 were treated with 120 mg/kg of BAE thereafter subjected to restraint and forced swim stresses respectively. Stress procedures were carried out at the end of first and third weeks.

Results: In the stressed rats, there were significant increases ($P < 0.05$) in fasting blood glucose and white blood cell count while there were significant decreases in superoxide dismutase activity and glutathione concentration when compared to group 1. There were significant decreases ($P < 0.05$) in blood glucose and white blood cell count and significant increases in superoxide dismutase and glutathione concentrations in BAE treated rats when compared to group 2 and 3. Some of the significant differences were either dose or duration dependent.

Conclusion: In conclusion, results from this research suggest that BAE alleviates hyperglycaemia, chronic activation of immune system and generation of free radicals due to stress in Wistar rats.

1. Introduction

Stress is a biological response to aversive conditions that tend to threaten or perturb the homoeostasis of the organism (Bhattacharya & Ghosal, 2000). It has become an increasingly popular and widely applied term in our everyday languages (Kholoud et al., 2015). A person's response towards stress is hinged on whether an event is appraised as a challenge or a threat (Lazarus & Folkman, 1984). Challenging stimulus can lead to desirable outcomes such as motivation and improved task performance whereas threatening ones or distress can result in anxiety, depression, social dysfunction and even suicidal intention. Stress and its related disorders are responsible for over 70% of the global illnesses. Studies on the pathophysiological aspects of stress has been a subject of research for long and Cannon (1929) first suggested that any aversive emotional stimulus can cause physical damage to the body and can produce disease states like anxiety and depression, hypertension, immunosuppression, endocrine disorders, diabetes mellitus and peptic ulcer. During stress there is an increased generation of reactive oxygen species (ROS), associated with the oxidative stress (Finkel & Holbrook, 2000). The negative effects caused by the oxidative stress are known to decrease after supplementation with certain dietary antioxidants (Halliwell, 2007). Increased level of blood glucose has been reported in the immobilization stressed rats due to the decreased secretion of insulin level (Zardooz, Zahedi Asl, Gharib Naser & Hedayati, 2006). Moreover, it has been demonstrated that stress induces adverse effects on haematological parameters in rats. (Kholoud et al., 2015). Many drugs have been reported to be widely used by people to combat stress. amongst

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these drugs are: amphetamine, benzodiazepines, caffeine and anabolic steroids. However, the incidence of toxicity, non-specific resistance and dependence on these drugs were observed to limit their therapeutic importance in the regulation of stressful events (Hoffman, 2001).

Medicinal plants play an important role in the traditional systems of medicine and have been used to treat body ailments since antiquity. In recent years, there has been increasing interest by researchers in the use of naturally occurring biologically active compounds with medicinal value (Anandarajagopal, Sudhahar, Ajaykumar & Muthukumaran, 2011). This is due to the fact that they are cheap and accessible to most of the population in the world. Therefore, there has been a rise in the use of herbal remedies in several parts of the world with many of the herbal remedies being incorporated into orthodox medical practice. Many medicinal plants have been reported in the literature to have antistress properties and are utilised for such purpose owing to the fact that they are safer and cheaper than orthodox drug. One of these plants is *Basella alba* L. It is a perennial vine which grows rapidly and extremely heat tolerant (Grubben & Denton, 2004). It belongs to the Basellaceae family (Rathee, Ahuja, Rathee, Thanki & Rathee, 2010). It is popularly known as Malabar spinach, Indian spinach, Ceylon spinach, vine spinach, climbing spinach, Chinese spinach (Bamidele et al., 2010) and cyclone spinach (Nirmala, Saroja & Gayathri, 2011). It is a wild leafy vegetable, which is rare in its natural habitat, but in recent times, it is an important leafy vegetable grown for its nutritive value throughout the temperate regions as an annual and the tropics as a perennial crop (Bamidele et al., 2010). It was reported that the leaves of *B. alba* are traditionally used in Ayurveda system of medicine to bring sound refreshing sleep when it is applied on the head about half an hour before bathing (Panda, 2004). Also, studies have shown that *B. alba* plant has anti-inflammatory (Kachchhava, 2006), antioxidant (Nirmala et al., 2011), anti-diabetic (Bamidele, Arokoyo, Akinnuga & Oluwarole, 2014) and central nervous system (CNS) depressant activities (Anandarajagopal et al., 2011). In the light of its traditional importance mentioned above and the information about its anti-diabetic, antioxidant and CNS depressant activities, this present work is therefore conducted to investigate the effect of methanol extract of *B. alba* leaves on stress in male Wistar rats.

2. Materials and methods

2.1. Animals

A total of 35 male albino rats, weighing between 150−200 g were purchased from Olu Research Farm in Ibadan, Oyo State and housed in Bowen University Animal House in cages where they were maintained under standard environmental and nutritional condition, room temperature and 12 h light/dark cycle was maintained. The animals were allowed to acclimatize for two weeks not a week with access to standard pellet diet and water given *ad libitum*. The rats were grouped into seven groups, including the control group. Their cages were made of wood and metal wire mesh to allow for good ventilation and were cleaned from time to time. All procedures involving the use of animals in this study follow the guiding principles for research involving animals as recommended by the declaration of Helsinki and the Guiding principles in the care and use of animals (World Medical Association, 2002).

2.2. Plants materials

Basella alba L. plants were obtained from different humid locations in Ibadan, Oyo state, Nigeria. The leaves were washed and air dried under shade preventing direct sunlight. The plant materials were identified and authenticated in the Department of Botany, University of Ibadan with voucher number (UIH-22,391). *B. alba* leaves were washed in tap water and dried at room temperature for 8 weeks after which they were ground into fine powder. The methanol extract was prepared by soaking 431 g of the plant powder in 2.65 litres of 70% methanol for 72 h. The mixture was sieved using a cheese cloth after which it was filtered with filter paper. The filtrate was then concentrated by evaporation using a rotary evaporator to obtain a solid mass. The resulting mass of the paste-like extract was 26.5 g with a percentage yield of 6.15%. The solid extract was then re-dissolved in normal saline and stored in capped bottles in a refrigerator at 4 °C until required.

2.3. Extract administration

The rats were weighed prior to the administration of the methanol extract of *B. alba* to determine the amount to be administered by using a weighing scale. *B. alba* leaf extract was administered via oral route with the aid of oro-pharyngeal cannula at low dose of 60 mg/kg and high dose of 120 mg/kg (Anandarajagopal et al., 2011; Saibal, Sanyib, Anand & Somnath, 2014). During administration, the rats were handled carefully to restrict movement and prevent trauma to them. Extract was administered in two divided doses per day for one week and this was conducted twice.

2.4. Experimental design

The male Wistar rats were grouped into seven different groups containing five rats per group. Each group was kept in different cages. The grouping was done as follows: Group 1 (Normal control): Neither stress was induced in the animals in this group nor treated with *B. alba* leaf extract. Group 2 (Stress control-one hour restraint stress model): Stress was induced by retraining the animals for 1 h without treating them with *B. alba* leaf extract. Group 3 (Stress control - forced swim stress model): Stress was induced by forcing the animals to swim without treating them with *B. alba* leaf extract. Group 4 contained one hour restraint stress animals treated with low dose (60 mg/kg) of *B. alba* leaf extract. Group 5 contained forced swim stress animals treated with low dose (60 mg/kg) of *B. alba* leaf extract. Group 6 contained one hour restraint stress animals treated with high dose of (120 mg/kg) *B. alba* leaf extract. Group 7 contained forced swim stress animals treated with high dose of (120 mg/kg) *B. alba* leaf extract.

2.5. Ethical approval

The study was approved to be conducted by College of Health Sciences Ethical Committee, Bowen University, Iwo, Osun State, Nigeria.

2.6. Stress procedure

2.6.1. Restraint stress test

The stress was induced in the rats after drug administration for 7 d individually by placing them for one hour each day for 7 d inside plastic cylindrical restrainers (21 cm in lenght × 6 cm in diameter) with ventilated sliding doors at the ambient temperature (Gudipudi, Puranik, Alla, Ajjarapu & Kistammagari, 2015). This test was conducted twice.

2.6.2. Forced swim test

The method described by Gudipudi et al. (2015) was used. The rats were individually subjected to fresh water swimming stress for 7 d after drug administration for 7d. After the test, the rats were towel dried and placed back in their cages.

2.7. Behavioural test

2.7.1. Forced swim test behavioural response assay

The assay was assessed in the first minute and separated into three categories: floating: This is defined as the rat remaining still, other than slight twitching or small involuntary movements required to remain upright and afloat. Swimming: relatively calm paddling, including all four legs, or just the two hind limbs. Struggling: vigorous swimming, thrashing, and/or climbing in an attempt to escape.

2.7.2. Open field test

The test was conducted to assay locomotor activity levels and anxiety in the stressed rats and also willingness to explore (Anandarajagopal et al., 2011).

2.7.3. Body grooming

The level of aggressiveness and food intake were also studied.

2.8. Collection of blood sample

Collection of blood sample was done at the end of the experiment through the cardiac puncture method and chloroform was used to anaesthetize the animals. Sufficient blood was drawn via the needle and syringe and was immediately transferred to an EDTA bottle. After separation of the whole blood, the plasma was put in plain bottles. The animals were euthanised by exsanguination after the experiment.

2.9. Analysis of blood samples

2.9.1. Blood glucose analysis

Blood glucose level was measured using a glucometer. Baseline fasting blood glucose (FBG) was recorded after the two weeks of acclimatization in all rats. After one week of administration and one week of stress induction, FBG was determined in the rats in groups 1−7 and recorded. To obtain the FBG level, the rats were fasted overnight (about 12−18 h). During overnight fasting, food and water were removed from their cages. The second phase of administration and stress induction occurred for another one week each after which FBG was determined again. Blood samples were obtained by pricking the tail of the rats with a sterile lancet and allowing one or two drops of blood to come in contact with the tip of the glucometer strip already inserted. The results were then obtained from the glucometer. The result of blood glucose measurement by glucometer correlates excellently well with the result obtained from standard laboratory methods (Ajala, Oladipo, Fasanmade & Adewole, 2003).

2.9.2. Haematological parameters analysis

The blood samples were analysed to determine the haematological parameters in the Lab Master Laboratories, Ibadan, Oyo State, Nigeria using Mindray BC-2800 auto haematology analyser, an automated analyser and leucocytes differential counter. BC-2800 directly measures the white blood cell (WBC), red blood cell (RBC), haemoglobin, platelet and haematocrit. The automated haematology analyser reading correlated well with readings by the standard manual methods (Samuel et al., 2010).

2.9.3. Antioxidant biomarker assay

Glutathione (GSH) was assayed according to the method of Ellman (1959). The sample (0.2 mL) was added to 1.8 mL of distilled water, then 3 mL of the precipitating solution was added and allowed to stand for 5 min. (The precipitating solution was made up of 1.67 g glacial metaphosphoric acid, 0.2 disodium EDTA (ethylenediaminetetraacetic acid) and 30 g sodium chloride in 100 mL of distilled water). After standing for 5 min the solution was filtered and 2 mL of the filtrate was added to 8.0 mL of phosphate (0.3 mol/L disodium orthophosphate solution in distilled water). Then 5 mL of the resulting solution was added to 1 mL of DTNB (5.5 dithiobis-2-nitrobenzoic acid) reagent. The DTNB reagent was prepared by adding 40 mg of DTNB in 100 mL of 1% sodium citrate. It was measured at 412 nm against blank. Activities were expressed as U/L.

Superoxide dismutase (SOD) concentration was determined using the method described by Ravi, Seema, Sripoorna, Jeeva and Renu (2015). A fortress diagnostic kit was used to determine the concentration of SOD in blood. The role of this enzyme is to accelerate the dismutation of toxic superoxide radicals produced during oxidative energy processes to hydrogen peroxide and molecular oxygen. Fortress method employs xanthine and xanthine oxidase (XOD) to generate superoxide radicals that react with 2-(4-iodophenyl *O*-3-(4-nitrophenol)−5-phenyltetrazolium chloride (I.N.T) to forma red formazan dye. The SOD activity was then measured by the degree of inhibition of this reaction.

2.9.4. Statistical analysis

The results were expressed as mean ± SEM; Number of observation (n) was five. Significant difference between control groups and treated groups were analysed by one way analysis of variance (ANOVA) followed by Newman-Keuls Multiple Comparison (Post HOC) Test using Graphpad prism (6.0). P values < 0.05 was considered significant.

3. Results

3.1. Results of stress on fasting blood glucose

The results of the experiment were tabulated as mean ± SEM as shown in Table 1. Stress procedures in groups 2, 4, 6 (restraint stress groups) and groups 3, 5, 7 (forced swim stress groups) did not commence until the end of first week and values were taken at the end of the second and fourth week.

3.1.1. Effect of stress on fasting blood glucose in rats

The FBG levels of rats that were subjected to stress models (groups 2 and 3) were taken twice throughout the experiment. At the end of second week, the fasting blood glucose levels in group 2 (106 ± 4.55 mg/dL) and group 3 (114.8 ± 6.12 mg/dL) were significantly increased compared to the normal control (54.4 ± 1.86 mg/dL) confirming the induction of stress.

At the end of 4th week, there was also a significant increase of FBG in group 2 (136.8 ± 5.29 mg/dL) and group 3 (120.8 ± 4.51 mg/dL) when compared to group 1 (normal control) (62.6 ± 1.21 mg/dL).

3.1.2. Effects of B. alba on fasting blood glucose in stressed rats

The FBG of group 4 (85.2 ± 1.49 mg/dL) and group 5 (72.8 ± 1.36 mg/dL) rats given 60 mg/kg of *B. alba* extract at

Table 1
Fasting blood glucose in different experimental groups of forced swim stress and restraint stress rats (mean ± SEM, $n = 5$).

Groups	Week 2	Week 4
1 (normal control)	54.4 ± 1.86	62.6 ± 1.21
2 (restraint control)	106.2 ± 4.55*	136.8 ± 5.29*
3 (forced swim test control)	114.8 ± 6.12*	120.8 ± 4.51*
4 (restraint test + *B. alba* 60 mg/kg)	85.2 ± 1.49*Δ	85.8 ± 4.90*Δ
5 (forced swim test +*B. alba* 60 mg/kg)	72.8 ± 1.36*Δ	67.6 ± 1.36Δ
6 (restraint test + *B. alba* 120 mg/kg)	80 ± 3.37*Δ	91.4 ± 2.46*Δ
7 (forced swim test +*B. alba* 120 mg/kg)	82 ± 2.04*Δ	101.6 ± 1.72*Δ

* $P < 0.05$ *vs* Group 1 (normal control).
Δ $P < 0.05$ *vs* appropriate stress control.

the end of the second week were significantly higher than normal control (54.4 ± 1.86 mg/dL) but significantly lower than stress control (groups 2) (106.2 ± 4.55 mg/dL) and group 3 (114.86 ± 6.12 mg/dL).

At the end of 4th week, there was significant increase in FBG level in group 4 (85.8 ± 4.90 mg/dL) when compared to the normal control (62.6 ± 1.21 mg/dL). Group 4 (85.8 ± 4.90 mg/dL) and group 5 (67.6 ± 1.36 mg/dL) rats showed significant decrease when compared to the stress control group 2 (136.8 ± 5.29 mg/dL) and group 3 (120.8 ± 4.51 mg/dL).

For group 6 (80 ± 3.37 mg/dL) and 7 (82 ± 2.04 mg/dL) rats given 120 mg/kg of *B. alba*, at the end of 2nd week, there were significant increases in blood glucose level when compared to group 1 (54.4 ± 1.86 mg/dL) but were significant decreased when compared to the rats in the stress control group 2 (106.2 ± 4.55 mg/dL) and group 3 (114.8 ± 6.12 mg/dL).

At the end of the fourth week, the FBG levels in group 6 (91.4 ± 2.46 mg/dL) and 7 (101.6 ± 1.72 mg/dL) were significantly increased when compared to group 1 (62.6 ± 1.21 mg/dL) and significantly decreased when compared to the stress control (group 2) (136.8 ± 5.29 mg/dL) and group 3 (120.8 ± 4.51 mg/dL).

3.2. Result of stress on haematological parameters in rats

3.2.1. Effect of restraint stress model on haematological parameters in rats

Table 2 showed the effect of restraint stress model on haematological parameters in rats.

There were no statistical differences in the RBC, PCV (packed cell volume) and HbC (haemoglobin concentration) between the restraint stress group (Group 2) and the normal control group (Group 1) both at the second week and fourth week. Group 2 rats ($11.80 \pm 0.36 \times 10^9$ L) at the end of the second week showed a significant increase in WBC count when compared with the normal control group ($7.80 \pm 0.79 \times 10^9$ L). Group 2 rats (1052.40×10^9 L) also showed a significant increase in platelet count at the end of the second week when compared to normal control group ($639.60 \pm 33.07 \times 10^9$ L).

3.2.2. Effect of B. alba on haematological parameters in restraint stress model rats

There were significant increases in RBC, PCV and HbC in group 4 when compared to group 1 at the end of the second week as shown in Table 2. Similarly, the increases in RBC and HbC were statistically significant when compared to group 2 at the end of the second week. Comparison of group 6 to group 1 showed no statistical difference in RBC, PCV and HbC at the end of week 2 and 4 except for PCV that was statistically significant at the end of week 4.

There was a dose dependent significant increase in WBC count in group 4 (restraint test + *B. alba* 60 mg/kg) when compared to group 1 (normal control) from $7.80 \pm 0.79 \times 10^9$ L (group 1) to $15.60 \pm 0.84 \times 10^9$ L (group 4) at the end of 2nd week.

Also, there was a significant increase in WBC in group 4 rats when compared to group 1 from ($11.38 \pm 0.79 \times 10^9$ L) to group 4 ($15.60 \pm 0.84 \times 10^9$ L) at the end of the week 4.

There was a significant decrease in WBC count in group 6 (restraint test +*B. alba* 120 mg/kg) when compared to group 2 rats (restraint test control) from $11.80 \pm 0.36 \times 10^9$ L (group 2) to $7.98 \pm 0.57 \times 10^9$ L (group 6).

Animals treated with 60 mg/kg of *B. alba* showed statistical decrease in platelet count in group 4 when compared to group 2 at the end of week 2 only. There was significant decrease in platelet count in animals treated with 120 mg/kg of *B. alba* after induction of stress both at the end of week 2 and 4 as shown in Table 2.

3.2.3. Effects of forced swim stress model on haematological parameters in rats

The haematological parameters of rats in forced swim stress group (groups 3) were analysed at the end of 2nd and 4th week after inducing stress. Results from Table 3 showed no significant difference in RBC, PCV and HbC when group 3 was compared to group 1 (normal control) both at the end of second week and fourth week. Rats in forced swim control (group 3) ($10.64 \pm 0.12 \times 10^9$ L) showed a significant increase in WBC count at the end of the second week when compared to the normal control ($7.80 \pm 0.79 \times 10^9$ L) and during the fourth week there was also a significant increase ($P < 0.05$) in WBC count in group 3 ($18.82 \pm 1.32 \times 10^9$ L) when compared to normal control group ($11.38 \pm 0.79 \times 10^9$ L).

Rats in group 3 ($800.8 \pm 29.58 \times 109$ L) showed a significant increase in platelet count at the end of week 2 when compared to the normal control ($639.6 \pm 33.07 \times 109$ L) but at the end of week 4 there was a significant decrease in platelet count from $730.4 \pm 10.25 \times 109$L (normal control) to ($579.0 \pm 28.69 \times 109$ L) in group 3.

3.2.4. Effect of B. alba on haematological parameters in forced swim stress model rats

At the end of the second and fourth week of experiment, no statistical differences were found in RBC, PCV and HbC in forced swim stress model animals treated with 60 mg/kg *B. alba* (group 5) when compared to normal control (group 1). Comparison of group 5 to group 3 showed increases in RBC, PCV and HbC which were not statistically significant. When the forced swim stress model animals were treated with 120 mg/kg of *B. alba*, only PCV showed statistical increase ($P < 0.05$) at the end of fourth week of experiment amongst RBC, PCV and HbC when compared to both group 1 and 3 as shown in Table 3.

At the end of week two, there was a significant decrease in WBC count in group 5 rats (forced swim control + *B. alba*

Table 2
Haematological parameters in different experimental groups of restraint stress rats (mean ± SEM, $n = 5$).

Blood parameters	Week	Group 1	Group 2	Group 4	Group 6
RBC ($\times 10^9$L)	2	6.94 ± 0.41	7.16 ± 0.46	8.14 ± 0.23*Δ	7.38 ± 0.19
	4	8.14 ± 0.21	7.62 ± 0.33	7.58 ± 0.20	7.66 ± 0.22
PCV (%)	2	42.6 ± 2.38	47.2 ± 2.71	52.2 ± 1.69*	47.2 ± 1.49
	4	47.2 ± 1.49	47.6 ± 2.25	45.8 ± 1.01	42 ± 2.02*
HbC (g/100 mL)	2	12.6 ± 0.42	12.74 ± 0.39	14.24 ± 0.13*Δ	13.12 ± 0.45
	4	14.02 ± 0.32	13.48 ± 0.47	14.08 ± 0.29	13.18 ± 0.43
WBC ($\times 10^9$L)	2	7.8 ± 0.79	11.80 ± 0.36*	10.06 ± 0.20*	7.98 ± 0.57Δ
	4	11.38 ± 0.79	13.34 ± 0.49	15.60 ± 0.84*	10.80 ± 0.75
PLATELET ($\times 10^9$L)	2	639.6 ± 33.07	1052.4 ± 47.82*	624.6 ± 22.32Δ	483.4 ± 12.57*Δ
	4	730.4 ± 10.25	711.2 ± 24.11	690.6 ± 45.38	619.6 ± 8.59*Δ

* $P < 0.05$ *vs* Group 1 (normal control).
Δ $P < 0.05$ *vs* appropriate stress control.

Table 3
Haematological parameters in different experimental group of forced swim stress rats (Mean ± SEM, $n = 5$).

Blood parameters	Week	Group 1	Group 3	Group 5	Group 7
RBC × 10^9L	2	6.94 ± 0.41	6.42 ± 0.27	6.88 ± 0.23	7.10 ± 0.41
	4	8.14 ± 0.21	7.8 ± 0.17	7.98 ± 0.23	6.84 ± 0.24*Δ
PCV (%)	2	42.6 ± 2.38	43 ± 1.52	45.2 ± 1.02	46.6 ± 1.63
	4	47.2 ± 1.49	44.2 ± 0.37	47.8 ± 0.86	45.6 ± 3.14
HbC (g/100 mL)	2	12.6 ± 0.42	11.84 ± 0.28	12.62 ± 0.25	12.06 ± 0.25
	4	14.02 ± 0.32	13.7 ± 0.24	14.54 ± 0.26	14.56 ± 1.53
WBC (× 10^9L)	2	7.8 ± 0.79	10.64 ± 0.12*	8.36 ± 0.24α	9.24 ± 1.19
	4	11.38 ± 0.79	18.82 ± 1.32*	17.68 ± 1.49*	17.16 ± 1.54*
PLT (× 10^9L)	2	639.6 ± 33.07	800.8 ± 29.58*	694.6 ± 50.10Δ	397 ± 20.12*Δ
	4	730.4 ± 10.25	579 ± 28.69*	676.4 ± 39.35Δ	605.6 ± 13.63*

* $P < 0.05$ *vs* Group 1 (normal control).
Δ $P < 0.05$ *vs* appropriate stress control.

60 mg/kg) when compared to group 3 rats (forced swim control) from 10.64 ± 0.12 × 10^9 L (group 3) to 8.36 ± 0.24 × 10^9 L (group 5).

There was a significant increase ($P < 0.05$) in WBC in group 5 (17.68 ± 1.49 × 10^9 L) when compared to group 1(11.38 ± 0.79 × 10^9 L) at the end of fourth week.

At the end of 2nd and 4th week, group 7 showed a decrease in WBC when compared to group 3 but not statistically significant. Comparison of group 7 to group 1 at the end of fourth week showed an increase in WBC which was significant at $P < 0.05$.

There were significant decreases in platelet count in group 5 (694.6 ± 50.10 × 10^9 L) and 7 (397.0 ± 20.12 × 10^9 L) when compared to group 3 (800.8 ± 29.58 × 10^9 L) at the end of 2nd week.

At the end of week 4, there was a significant increase in platelet count in group 5 (676.40 ± 39.35 × 10^9 L) when compared to group 3 (579.0 ± 28.69 × 10^9 L) and also there was a significant decrease in platelet count in group 7 (605.60 ± 13.63 × 10^9 L) when compared to group 1 (730.40 ± 10.25 × 10^9 L) as depicted in Table 3.

3.3. Results on antioxidant biomarkers

The results on antioxidant biomarkers were analysed at the end of the second week and fourth, the results were shown in Figs. 1–4.

3.3.1. Effect of restraint stress model on antioxidant biomarkers

At the end of the second week, group 2 (0.12 ± 0.007 U/L) showed a significant decrease in SOD when compared to group 1 (0.18 ± 0.007 U/L) as seen in Fig. 1. At the end of the fourth week, group 2 (0.24 ± 0.009 U/L) showed significant decrease in SOD concentration when compared to group 1 (0.33 ± 0.007 U/L).

At the end of the second week, there was no significant difference in GSH in group 2 (0.01 ± 0.002 U/L) when compared to group 1 (0.02 ± 0.004 U/L) as seen in Fig. 3. At the end of the fourth week, there was a significant difference ($P < 0.05$) in GSH in group 2 (0.03 ± 0.004 U/L) when compared to group 1 (0.02 ± 0.002 U/L).

3.3.2. Effect of B. alba on antioxidant biomarkers in restraint stress model rats

In restraint stress, at the end of the second week (Fig. 1), group 4 (0.23 ± 0.008 U/L) showed significant increase in SOD concentration when compared to group 1 (0.18 ± 0.007 U/L) and group 2 (0.12 ± 0.007 U/L). Group 6 (0.27 ± 0.007 U/L) also showed significant increase in SOD concentration when compared to group 1 (0.18 ± 0.007 U/L) and group 2 (0.12 ± 0.007 U/L).

At the end of the fourth week, group 4 (0.37 ± 0.007 U/L) there was significant increase in SOD concentration when compared to group 1 (0.33 ± 0.007 U/L) and group 2 (0.24 ± 0.009 U/L). Group 6 (0.38 ± 0.007 U/L) showed significant increase in SOD concentration when compared to group 1 (0.33 ± 0.007 U/L) and group 2 (0.24 ± 0.009 U/L). Group 4 (0.03 ± 0.001 U/L) and group 6 (0.04 ± 0.004 U/L) showed significant increase in GSH concentration when compared to group 2 (0.01 ± 0.002 U/L) at the end of second week. Group 4 (0.05 ± 0.002 U/L) group 6 (0.09 ± 0.004 U/L) showed significant increase in glutathione concentration when compared to group 2 (0.02 ± 0.002 U/L) at the end of fourth week (Fig. 3).

3.3.3. Effect of forced swim stress model on antioxidant enzymes

Fig. 2 showed that at the end of the second week, there was a significant decrease in SOD concentration between group 3 (0.10 ± 0.000 U/L) and group 1 (0.18 ± 0.007 U/L). Group 3 (0.26 ± 0.005 U/L) showed a significant decrease when compared to group 1 (0.33 ± 0.007 U/L) at the end of fourth week. There was no significant increase in GSH concentration in group 3 (0.02 ± 0.002 U/L) when compared to group 1 (0.02 ± 0.004 U/L) at the end of second week (Fig. 4). At the end of fourth week,

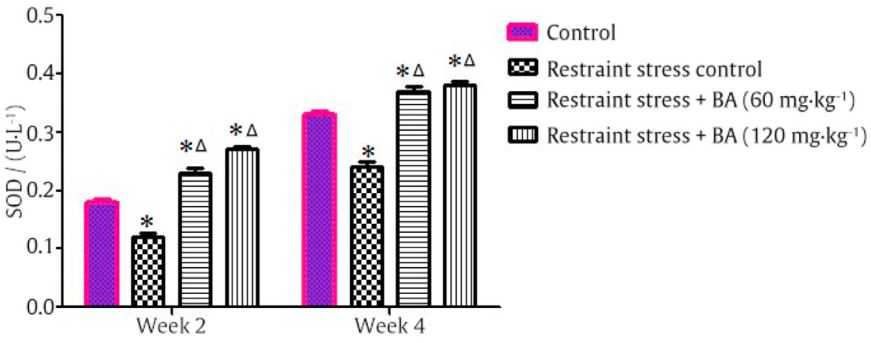

Fig. 1. Superoxide dismutase enzyme concentration in different groups of restraint stress model in rats.
*$P < 0.05$ *vs* normal control group; Δ$P < 0.05$ *vs* appropriate stress control group.

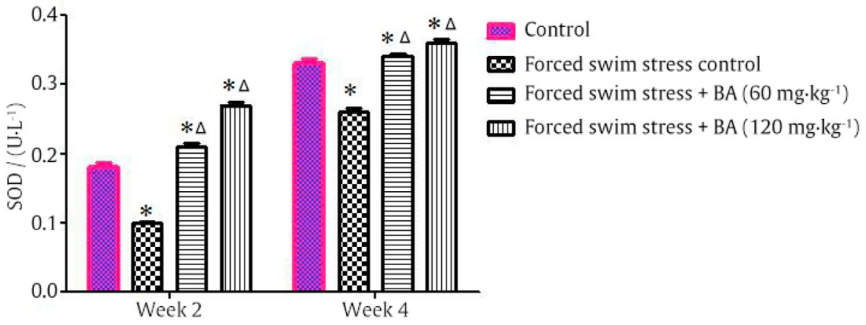

Fig. 2. Superoxide dismutase enzyme concentration in the different groups of forced swim stress model in rats. $^{*}P < 0.05$ *vs* normal control group; $^{\Delta}P < 0.05$ *vs* appropriate stress control group.

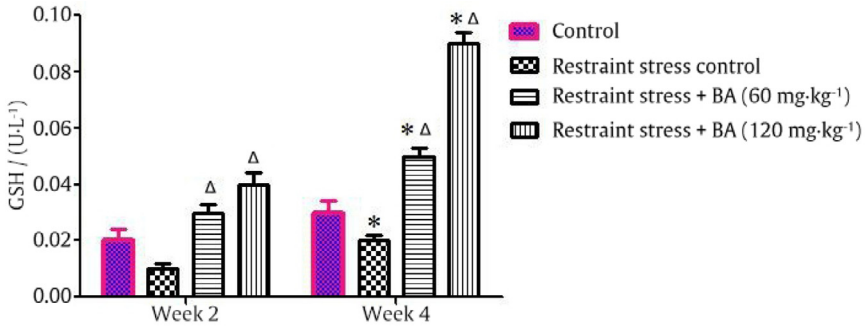

Fig. 3. Glutathione concentrations in the different groups of restraint stress model in rats. $^{*}P < 0.05$ *vs* normal control group; $^{\Delta}P < 0.05$ *vs* appropriate stress control group.

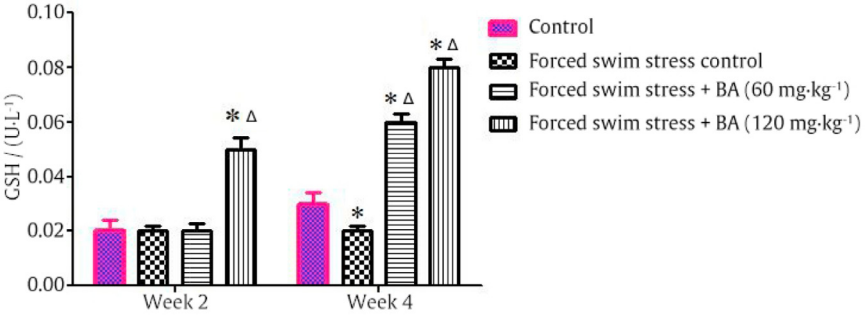

Fig. 4. Glutathione concentration in the different groups of forced swim stress model in rats. $^{*}P < 0.05$ *vs* normal control group; $^{\Delta}P < 0.05$ *vs* appropriate stress control group.

there was a significant decrease ($P < 0.05$) between group 3 (0.02 ± 0.002 U/L) and group 1 (0.03 ± 0.004 U/L).

3.3.4. *Effect of* B. alba *on antioxidant enzymes in force swim stress model rats*

In Fig. 2, group 5 (0.21 ± 0.005 U/L) showed significant increase in SOD concentration when compared to group 1 (0.18 ± 0.007 U/L) and group 3 (0.10 ± 0.000 U/L) respectively at the end of the second week. Group 7 (0.27 ± 0.005 U/L) showed significant increase in SOD concentration when compared to both group 1 (0.18 ± 0.007 U/L) and group 3 (0.10 ± 0.000 U/L) at the end of second week. There was no significant difference in SOD concentration between group 5 (0.34 ± 0.004 U/L) and group 1 (0.33 ± 0.007 U/L) while comparison of group 5 (0.34 ± 0.004 U/L) with group 3 (0.26 ± 0.005 U/L) showed a significant increase at the end of fourth week. Group 7 (0.36 ± 0.005 U/L) showed a significant increase in SOD concentration when compared to both group 1 (0.33 ± 0.007 U/L) and group 3 (0.26 ± 0.005 U/L) at the end of fourth week. There was no statistical difference in GSH concentration when group 5 (0.02 ± 0.003 U/L) was compared to both group 1 (0.02 ± 0.004 U/L) and group 3 (0.02 ± 0.002 U/L) respectively at the end of second week (Fig. 4). Group 7 (0.05 ± 0.004 U/L) showed a significant increase in GSH concentration ($P < 0.05$) when compared to group 1 (0.02 ± 0.004 U/L) and group 3 (0.02 ± 0.002 U/L) respectively at the end of second week. At the end of fourth week, there were significant increases in groups 5 and 7 when compared to group 1. Group 7 only showed significant increase when compared to group 3.

3.4. Results on behavioural test

3.4.1. Open field test

There was a significant increase in the number of faecal pallets in group 2 (2.4 ± 0.75) when compared to group 1 (1.0 ± 0.0) and there was also significant decrease in faecal pallets in group 4 (1.0 ± 0.0) when compared to group 2 (2.4 ± 0.75) as shown in Table 4. There was a significant increase in line crossing in group 6 (68.8 ± 5.19) when compared to group 1 (34.6 ± 1.03) and also when compared to group 2 (28.6 ± 6.52). Group 7 (2.0 ± 0.45)

showed a significant increase in duration in quadrant when compared with group 1 (1.0 ± 0.0).

3.4.2. Forced swim test assessment

Group 3 rats were observed to spend more time floating and less time struggling when compared with rats in groups 5 and 7. Group 5 and 7 rats were observed to spend more time struggling and swimming when compared to rats in group 3.

3.4.3. Body grooming

At the end of the fourth week, rats that passed through stress protocols were observed to have their body hairs and whiskers in a scattered and standing position as shown in Fig. 5A. The rats in the normal control group showed good grooming (Fig. 5B).

3.4.4. Appetite and aggression

Rats that underwent stress procedures were observed to have lesser food intake and became more aggressive towards any form of contact whereas those of control group had normal food intake and were less aggressive.

4. Discussion

The effects of methanol extract of *B. alba* on stress were examined in this study. We investigated its effects on blood glucose, haematological parameters, antioxidant enzymes (superoxide dismutase and glutathione) and behavioural tests.

Results from this study showed an increase in fasting blood glucose in the stressed groups. The increase in fasting blood glucose seems to be connected to an increase in adrenalin and corticosterone release from adrenal gland which occur during stress. This shows that stress has a vital role to play in the elevation of blood glucose and could initiate the onset of type 2 diabetes (Surwit et al., 2002). When the stressed animals were treated with *B. alba* extract, there was a significant decrease in fasting blood glucose level. The decrease might be due to the anti-hyperglycaemic effect of *B. alba* (Bamidele et al., 2014) which is likely connected to the plant antioxidant activity through its total phenolic content (Olajide & Azeez, 2011). Plants that contain the active components such as glycosides, saponins and flavonoids have antioxidant activity and are said to possess anti-hyperglyaemic effect.

The increase in white blood cell (WBC) counts in the present work is in contrast to the work done by Kholoud et al. (2015) in animal exposed to immobilized stress in which WBC was decreased. Increase in WBC count that occurred during the induced stress might be due to the body's response to stress that sends signals to the brain which are processed and led to the release of the stress hormone, cortisol, to put the body on alert to release immune boosting molecules such as white blood cells and also, during chronic stress beta adrenergic binding leads to an increase in white blood cells, showing that stress may play a role in elevation of WBC count in correlation with the reports of Nishitani and Sakakibara (2014). *B. alba* extract decreased the stress-induced increase in WBC count and this may be associated with reduction in blood glucose due to the presence of antioxidant in the plant, as an increase in WBC count is linked with a worsen insulin action thus inhibiting breakdown of glucose and thus may play a role in pathogenesis of type 2 diabetes (Vozarova et al., 2002). Results showed that long term intake of *B. alba* was more effective than short term consumption.

There was no significant change in RBC count and packed cell volume in response to stress. This is consistent with the observation of Ebunlomo et al. (2012). An increase in platelet count in stress control group was observed when compared to normal control group which is in consonance with the report by Levine et al. (1985), these could be due to alpha-adrenergic receptors located in platelet membranes which are activated by catecholamine secreted cells during stress (Newman, Lewis, Williams, Bishopric & Lefkowitz, 1978) whereas *B. alba* reduced the elevated platelet count and this could be due to the presence of lupeol in the plant which has antiplatelet activity (Sankaranarayanan et al., 2010).

There was a significant increase in haemoglobin concentration in *B. alba* treated rats when compared to normal control and stress control showing that *B. alba* may increase haemoglobin concentration as reported by Bamidele et al. (2010). This significant increase may be related to its chemical composition which includes proteins, fats, vitamin A, vitamin C, vitamin E, vitamin K, vitamin B9, riboflavin, niacin, thiamine, and minerals such as calcium, magne-

Table 4
Results of open field test in different experimental groups (Mean ± SEM, $n = 5$).

Groups	Rearing	Line crossing	Quadrant entry	Duration in quadrant	Defecation
1	10.0 ± 1.87	34.6 ± 1.03	143 ± 0.24	1.0 ± 0.0	1.0 ± 0.00
2	7.8 ± 2.35	28.6 ± 6.52	1.0 ± 0.00	1.0 ± 0.0	2.4 ± 0.75*
3	7.0 ± 1.30	28.6 ± 7.17	1.20 ± 0.20	1.6 ± 0.4	1.0 ± 0.00
4	7.0 ± 2.30	26.2 ± 5.41	1.2 ± 0.20	1.2 ± 0.2	1.0 ± 0.00Δ
5	9.0 ± 3.83	36.2 ± 4.96	1.2 ± 0.20	1.2 ± 0.2	1.2 ± 0.20
6	12 ± 2.43	68.8 ± 5.19*Δ	1.4 ± 0.25	1.6 ± 0.60	1.8 ± 0.49
7	5.4 ± 1.96	35.40 ± 7.16	1.0 ± 0.00	2.0 ± 0.45*	1.0 ± 0.00

* $P < 0.05$ *vs* Group 1 (normal control).
Δ $P < 0.05$ *vs* appropriate stress control.

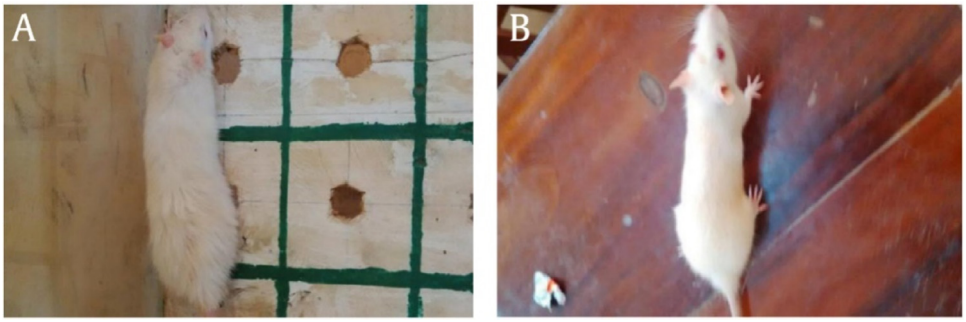

Fig. 5. A stressed rat with poor body grooming (A) and normal control rat with neat body grooming (B).

sium and iron (Duke & Ayensu, 1985). These minerals and some of the vitamins are hematinics necessary for the formation of blood cells (Alada, Akande & Ajayi, 2004).

Results from this study shows that prolonged stress decreased SOD enzymes. This might be as a result of the induced long term stress causing an insufficient amount of antioxidant enzymes to counter the free radicals and increase in enzyme activity after treatment with *B. alba* could be due to the total phenol content of the plant which is responsible for its antioxidant activity (Olajide & Azeez, 2011) and also due to the presence of β sitosterol and lupeol in the plant, this leads to scavenging and trapping of free radicals released during stress (Brewer, 2011). The increase in the antioxidant enzyme concentration could be beneficial in helping to destroy the free radicals production due to the stress. However, *B. alba* has a higher antioxidant activity at high doses than low doses in correlation with the reports by Nantia, Manfo, Beboy and Moundipa (2013).

Results from the behavioural studies showed that *B. alba* seemed to increase exploratory and anxiety behaviours in depressed state in stressed rats which correlate well with the CNS depressant activity reported by Anandarajagopal et al. (2011).

5. Conclusion

The results of this study have shown that *B. alba* is effective in mitigating stress through its hypoglycaemic effect, antioxidant potential and its role in the reversal of chronic activation of the immune system. Hence, consumption of *B. alba* as part of daily diet by humans should be strongly advised and encouraged across diverse populations as stress is an unavoidable event.

Declaration of Competing Interest

We declare that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

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