Diet supplements of banana fruit pulp mitigates repeated noise stress induced behavioral deficits and oxidative stress

Noreen Samad¹*, Farzana Yasmin², Saima Khaliq³, Saara Ahmad⁴, Azizuddin⁵ and Sana Mustafa⁵

¹Department of Biochemistry, Bahauddin Zakariya University, Multan, Pakistan
²Department of Food & Biomedical Engineering, NED University of Engineering and Technology, Karachi, Pakistan
³Department of Biochemistry, Federal Urdu University of Arts, Science and Technology, Karachi, Pakistan
⁴Department of Biological and Biomedical Sciences, Agha Khan University, Karachi, Pakistan
⁵Department of Chemistry, Federal Urdu University of Arts, Science and Technology, Karachi, Pakistan

Abstract: The current study was designed to determine the outcome of banana fruit pulp (BFP) on repeated noise stress exposure (NSE)-induced behavioral deficits and oxidative stress in male mice. BFP (600mg/kg b.w) was administered orally once daily for 2 weeks prior exposure to noise stress. Mice were exposed to NS for 4 h after administration of BFP for 2 weeks. Control mice were administered drinking water and similar treatment as given to test animals. At the end of the treatment behavioral changes were monitored. Animals were sacrificed following behavioral assessment and the brain and plasma samples were collected for biochemical analysis. Repeated NS-induced behavioral deficits (anxiety and depression), impaired learning and memory and produced oxidative stress. Administration of BFP inhibited NS-induced behavioral deficits (anxiolytic and antidepressant effects) and improved cognitive abilities. Brain lipid per oxidation was also decreased with concomitant increase of antioxidant enzyme activities. Repeated noise stress increased plasma corticosterone levels. A significant decrease of plasma corticosterone was observed on unstressed BFP treated animals while this decrease was comparable in stressed + BFP animals. Decreased levels of acetylcholinesterase in BPF+NS treated animals indicated increased cholinergic function which improves learning and memory. Repeated oral administration of BFP induced cognitive improving ability, anti-stress effect and potentiated antioxidant defence mechanism in both control and NS mice. Thus, it is suggested that dietary supplementation of BFP has a curative effect against NS-induced psychiatric and cognitive related disorders which merits deliberation and additional appraisal.

Keywords: Banana fruit pulp, noise stress, behavioral deficits, lipid per oxidation, antioxidant enzymes.

INTRODUCTION

It is well reported that most of the diseases are associated with unevenness between oxidant and antioxidant homeostasis (Shaw et al. 2020). Extensive studies have shown that stress not only effect the neuro-genesis and neuronal morphology it also alters neuronal plasticity (Schoenfeld et al. 2017) and deteriorating brain function with oxidative stress (Gulyaeva et al. 2019). The accurate mechanism of stress induced brain malfunctioning requires more attention. Oxidative tension is produced by unrestricted radicals associated with neurological diseases (Singh et al. 2019).

Environmental stress such as noise stress is most widespread due to rapid industrialization and urbanization. Subjection to noise greater than 100 dB is termed as a stressor (Rosenzweig and Barnes 2003). Moreover, noise exposure not only causes auditory loss but may also lead to physiological impairment (Hahad et al. 2019). However, it is notable that the brain organizes to cope up the stress condition, the physiological stress retort has mostly ended up in the liberation of hormones where they work as alarm warning signs of brain by exhibiting modulated plasticity and alterations in structure and function (McEwen 2000). Previous studies have shown that noise stress can modulate neurotransmitter secretions (Ravindran et al. 2005), alter metabolism and anatomy of neurons, minimize dendritic number, malfunction memory and cognition (Manikandan et al. 2006). Modulations in enzymatic antioxidant defense system disturb protection mechanism against reactive oxygen/nitrogen species (RNOS) which leads towards damaging exposed sites like unsaturated fatty acyl chains in the cell membrane, proteins and DNA (Maes et al. 2001). With increasing efforts in indigenous herbal plants for their therapeutic efficiency, several attempts are now being exercised in characterizing herbal plants as a potential modern medicine source.

Banana is a condimental fruit. All parts of its plant used as foodstuff and as medicine (Pierera et al. 2015). It is used for the treatment of kidney stones, ulcers, skin diseases, gout, etc. (Pellai and Aashan 1955). Banana plant contains various antioxidant compounds, biogenic amines which are pertinent to the pathophysiology of psychiatric illness (Edenta et al. 2014).

*Corresponding author: e-mail: noreen.samad@bzu.edu.pk
Diet supplements of banana fruit pulp mitigates repeated noise stress induced behavioral deficits and oxidative stress

The proof of antioxidant activity of banana (Musa sapientum) in former experiments encourage us to use banana fruit pulp (BFP) for mitigation of repeated noise stress induced anxiety, depression, impaired cognition and increased oxidative tension, which to the unsurpassed of our information has not been done in any earlier study. Hence, the current work was steered to appraise the outcome of administration/supplementation of BFP on repeated noise stress exposure (NSE)-induced behavioral deficits and oxidative stress in male mice.

MATERIALS AND METHODS

Animals
Adult Albino male mice (22-26 g) were obtained from University of Lahore, Lahore-Pakistan. The rodents were placed alone to evade societal activity under 12-hrs dark and light timings and at fixed temperature (21±2°C) with independent intake of diet and water. All experimental practices were performed in a well-adjusted environment to avert the external biasness of direction and period. The study was performed after getting consent from Bioethical Committee of Department.

Chemicals and reagent
All chemicals and reagents were bought from Sigma Chemicals Co. (St. Louis, USA).

Plant collection and identification
Banana fruit were collected from the nearby areas of Multan, Punjab, Pakistan and identified by the taxonomist as Musa sapientum. BFP was freshly prepared in deionized water and administered to mice by gavage procedure.

Estimation of dose related effect of BFP on activity in the novel and familiar environment
Dosage (0.0, 75.0, 150.0, 300.0, 600.0 mg/kg/day) results of BFP on activity in open field and home cage to determine a BFP dose that attenuates the repeated noise stress responses. BFP was given through oral tract for 4 weeks (5 animals in each batch). All activities were monitored for 5 min after 15 days’ delivery of BFP.

Estimation of the effect of designated BFP dose (600 mg/kg/day) on repeated noise stress induced behavioral deficits, cognitive impairment and oxidative stress.
24 Mice were arbitrarily alienated into four groups (n=6); (i) control+stressed; (ii) control+unstressed; (iii) BFP+stressed; (iv) BFP+unstressed. Animals were treated with 600 mg/kg BFP. The mice were given treatment according to respective batch and 20 minutes’ post treatment animals were subjected to 4-h NS. The stressed batch was subjected to NSE in a separate room for 4-h. Behavioral activities@ [ Forced swimming test (FST), light dark box activity (LDA), elevated plus maze (EPM) and Morris water maze (MWM) test] of all groups were examined on day 16th after NSE. After behavioral activities rats were slaughtered, and their brains were separated. Brain was removed and kept frozen at −60°C for biochemical analysis.

Noise stress experience (NSE)
Noise of generator was recorded and intensified by speakers to train mice to noise stress in a separate room. Speakers were accustomed 32 cm on apex of the animal cages. The noise strength was fixed at 100 dB (3KHz) and adjusted by an echo level measuring device DS102 (Range: 70–120 dB, Accuracy: ±1.4 dB (30Hz), made in Taiwan) (Chabuck et al. 2013).

Behavioral assessment
Light dark activity and elevated plus maze test was done to check anxiety profile as mentioned before (Samad and Haleem 2018). Duration of time spent in a light box and open arm were checked to evaluate anxiety-like behavior deficit. Forced swim test which is a proven tool to check the depressive symptoms was used (Samad and Haleem 2018) in which, immobility time duration was documented to check the depression like signs. Morris water maze test which is known to assess memory was executed as before (Samad and Haleem 2018). Time to grasp the stage was recorded for acquisition, short term memory and long-term memory for 120 sec.

Biochemical analysis
Entire brain was eroded with saline solution and weighed. A ten percent w/v tissue homogenate was made with 0.1M phosphate buffer (pH 7.4) and centrifuged at 10,000g for 10 min at 4°C. The supernatant was then utilized to estimate and quantify the oxidative stress marker malondialdehyde (MDA) (Chow and Tappel 1972) and enzymatic antioxidant potential including super oxide dismutase (SOD) (Naskar et al. 2011), catalase (CAT) (Pari and Latha, 2004) and glutathione peroxidase (Gpx) (Flohé and Gunzler 1984). The method described by Ellman et al (1961) was performed to check the activity of AChE in the brain tissues of animal

STATISTICAL ANALYSIS

One way and Two-way ANOVA followed by tukey’s test was done to analyze results. P less than 0.05 were taken as significant. SPSS version 20.0 was used for statistical analysis.

RESULTS

Exploratory activity of BFP fed animal in an open field and home cage is shown in fig. 1. Statistics of square crossed in an open field was done by 1-way Anova [F(5,24)=80.55, p=0.024] showed substantial effect of BFP. Tukey’s test showed that with the increase of drug dose square crossed were also increased.
Statistics of cage crossings in home cage was done by 1-way Anova \( F(5,24)=50.55, p=0.001 \) showed substantial role of BFP. Tukey’s test showed that with the increase of drug dose square crossed were also increased.

![Graph showing role of various doses of BFP exploratory activity](image)

**Fig. 1:** Role of various doses of BFP exploratory activity. Values are means ± SD (n = 5) 15 days after the administration of drug. Substantial changes by Tukey’s test *p<0.05 from control animals following 1-way Anova.

Effect of administration of BFP on LDA and EPM in unstressed and repeated stressed mice is shown in fig. 2. Statistic for time spent in light box examined by ANOVA (2-way, df 1,20) exhibited that significant effects of NSE \( F= 9.28p < 0.01 \), BFP \( F= 104.28p <0.01 \), and interaction of NSE x BFP \( F(1,20) = 10.85p < 0.01 \). Tukey’s test for multiple comparisons showed that 4-hrsNSE significantly decreased time spent in light box in comparative mice. Time spent in light box eventually enhanced in BFP treated non-NSE and NSE mice than control.

![Graph showing time spent in light box and open arm for the control and BFP treated unstressed and stressed groups](image)

**Fig. 2:** Time spent in light box and open arm for the control and BFP treated unstressed and stressed groups following single 4-h NSE. Values are mean ± SD (n = 6). Data was analyzed by Tukey’s test following 2-way Anova. Statistical difference is represented as** *p<0.01 versus respective control and ++p<0.01 versus unstressed groups.

Effect of administration of BFP following repeated4-hrsNSE on immobility time in FST is shown in fig. 3. Effects of NSE \( F= 43.11p < 0.01 \), BFP \( F = 343.902 p < 0.01 \) and NSE x BPE interaction \( F= 61.67 p < 0.01 \) in FST were significant following ANOVA (2-way, df 1,20).

Tukey’s test showed that BFP significantly decreased immobility time in unstressed and repeated stressed animals. Immobility time in FST was greater in control stressed than unstressed mice.

![Graph showing effect of administration of BFP on immobility time in FST](image)

**Fig. 3:** Effect of administration of BFP following repeated4-hrsNSE on immobility time in FST is shown in fig. 3. Effects of NSE \( F= 43.11p < 0.01 \), BFP \( F = 343.902 p < 0.01 \) and NSE x BPE interaction \( F= 61.67 p < 0.01 \) in FST were significant following ANOVA (2-way, df 1,20).

**Fig. 4:** Effect of administration of BFP on MWM test to assess function of memory is shown in fig. 4. Data of training time analyzed by ANOVA (2-way, df 1,20) showed significant effects of BFP \( F= 19.33 p < 0.01 \) and NSE \( F = 13.64 p < 0.01 \). While interaction between BFP x NSE \( F=0.93p > 0.05 \) were not significant. ANOVA for STM revealed substantial effects of BFP \( F= 20.48 p < 0.01 \), while effect of NSE \( F= 1.49 p > 0.05 \), and interaction \( F= 2.73p >0.05 \) were not significant. ANOVA for LTM revealed significant effect of BFP \( F= 152.80p <0.01 \). Effects of NSE \( F= 3.11p > 0.05 \) and interaction \( F= 0.02 p > 0.05 \) were not significant. Tukey’s test revealed that during acquisition time to attain the platform is reduced in BFP+unstressed and control+stressed mice. Whereas analysis of STM and LTM reveal that BFP substantially
Diet supplements of banana fruit pulp mitigates repeated noise stress induced behavioral deficits and oxidative stress

Effect of administration of BFP following repeated NSE on MDA levels is shown in fig. 5. Statistics by ANOVA (2-way, df 1,20) exhibited a substantial effect of NSE [F=29.28 p < 0.01] and BFP [F=144.77 p < 0.01] and NSE x BFP [F=12.17 p < 0.01]. Tukey’s test showed that NSE elevated MDA levels in control stressed animals. Whereas, subjection of BFP reduced oxidative stress in unstressed and stressed than control mice.

Effect of administration of BFP on antioxidant enzymes following repeated NSE is shown in fig. 6. Data on the activity of SOD evaluated by ANOVA (2-way, df 1,20) exhibits substantial property of BFP [F=179.25 p < 0.01], and NSE [F=48.38 p<0.01]. While the interaction between NSE x BFP [F=0.95 p>0.05] was not substantial. Tukey’s test showed BFP elevated the activity of CAT in unstressed and stressed than control animals.

Data on the activity of CAT is evaluated by ANOVA (2-way, df 1,20) exhibited substantial effects of BFP [F=64.71 p < 0.01]. Whereas NSE [F=0.07 p=0.78] and NSE x BFP [F=4.33 p=0.05] exhibited non-substantial effects. Tukey’s test revealed that BFP enhanced the activity of GPx in stressed and unstressed than control. Effect of BFP on activity of brain AChE activity following NSE is shown in fig. 7. ANOVA (2-way, df 1,20) showed significant effect of BFP [F = 18.35 p < 0.01] and NSE [F= 4.34 p<0.01]. Interaction between BFP xNSE [F= 3.76 p>0.05] insignificant. Tukey’s test exhibited BFP significantly reduced the activity of AChE in unstressed than control. The levels of AChE activity were also reduced in control stressed than unstressed animals.
DISCUSSION

Experience to stress condition is associated with functional alteration and anatomical impairment of neurons (Chen et al. 2017). The stress response is involved in behavioral alteration as well as changes in the mechanism of antioxidant enzymes (Wang et al. 2019). The cells in the body are regularly exposed to oxidants from both internal and external environment but the body has a strong mechanism of antioxidants (Tabrez and Ahmed 2009; 2010). The LPO is caused when free radical production becomes increased and the antioxidant defense machinery becomes reduced. The LPO may cause oxidation of poly unsaturated fatty acids that are rich in brain and cause damage of brain and tissue and disease (Yadav et al. 2018). Exposure to noise stress contributes to the oxidants production and thus produces oxidative stress in the brain (Samad et al. 2020; Wankhar et al. 2017). The current research work evaluated the protective role of BFP on repeated NSE (daily for 14 days) induced anxiety and depression like symptoms, imbalance between oxidative damage and antioxidant enzyme status and impaired cognitive ability in male mice. It was observed that NSE induced decreased time spent in light compartment of light dark activity box and open arm of elevated plus maze as well as increased immobility time in forced swim test that were recognized by behavioral test for % anxiety and * depression like symptoms. These symptoms were attenuated by co-subjection of BFP, suggesting the antidepressant and anxiolytic role of BFP. It was also evident that NSE-induced memory impairment in Morris water maze test was also improved by administration of BFP, indicating memory enhancing role of BFP. It is also indicated by present study that NSE induced deficits of behavioral activity and cognition could be due to the generation of oxidants resulting in damage of nerve cells and decreased neuronal/synaptic plasticity as evident before (Fetoni et al. 2019). Synaptic plasticity has a vital role in psychiatric disorders and memory function (Andre et al. 2018). It was observed that following administration of BFP anxiolytic, antidepressant and improved cognitive ability was observed in stressed and unstressed mice. (Singh et al. 2016).
respective control and ++p < 0.01 versus unstressed groups.

**Fig. 7:** Effect of BFP administration following single 4-h NSE on brain acetylcholinesterase activity. Values are mean ± SD (n = 6). Data was analyzed by Tukey’s test following 2-way Anova. Statistical difference is represented as **p<0.01 versus respective control and +p < 0.05 versus unstressed groups.

Increased levels of free radicals/oxidants were found in subjects suffering from anxiety, depression, dementia, and Alzheimer’s disease and cause molecular and cellular changes in the brain and play a vital role in expansion and succession of neurological diseases (Olloquequi et al. 2018). LPO is a biomarker of oxidative harm to the cells (Liu et al. 1996). In present study repeated NSE caused oxidative damage that is evidenced by enhanced LPO in brain of stressed than unstressed mice. Hence, anxiety, depression and impaired cognitive ability observed in stressed mice in the present study might be due to excessive production of free oxidants which peroxidized membranous poly unsaturated fatty acid of cell, deteriorate the structure of proteins and DNA (Das and Khanna 1997). In the present study, administration of BFP reduced MDA levels in stressed as well as unstressed mice which suggest that increased LPO could be prevented by inhibiting free radical/oxidants production. The present findings demonstrated significant decrease in the activity of antioxidant enzymes SOD while comparable reduction in CAT and GPx after NSE in mice. A failure of antioxidant defense mechanism caused oxidative obliteration by changing the balance between oxidant and antioxidant factor and producing free radicals (Fontella et al. 2005). Antioxidants may scavenge oxidant/ free radical and may afterward mitigate oxidative stress. Hence, it is suggested that reduced action of antioxidant enzyme seen in the current study is due to excessive generation of free radicals in stressed mice. In the present study the reduced activity of antioxidant enzymes was mitigated by the administration/supplementation of BFP in stressed and unstressed mice than control. Hence, it can be suggested that decrease in oxidative stress by decreasing MDA levels and increasing in antioxidant enzyme activity could be due to inhibition of free radical production by bioactive compounds present in BFP. It appears that supplementation of BFP could be the possible therapy due to antioxidants potential for the mitigation of NSE induced alterations in antioxidant defence system.

Many researches have reported the connection between AChE and memory function, but still there is a need of more research because exact pattern for this association is ambiguous. Impaired memory function following neonatal metal ion exposure (Perez et al. 2010) and restraint stress (Samad and Saleem 2018) reduced action of AChE. The result on AChE activity in the current study is novel and suggesting that BFP may be involved in availability of acetylcholine to improve the memory function. BFP and NSE have shown to enhance the memory function, which is also seen in the current study. It is therefore suggested that BFP may possess inhibitory functions analogous to that of AChE inhibitors which may lead to an enhance acetylcholine levels available for receptor binding at synapse (Samad and Saleem 2018) and this may possibly be the basis of enhanced cognitive ability in stressed mice.

**CONCLUSION**

In conclusion, NSE caused oxidative stress , reduction in antioxidant enzymatic potentials, behavioral alteration (anxiety and depression) and increased AChE activity leading to impairment in memory functions, which were mitigated by administration of BFP. Our results may have implications for novel curative strategies to treat/prevent neurological diseases with antioxidant potential of BFP. Prior administration/supplementation of BFP possibly will help to avert the development of diseases such as behavioral deficits, anxiety, depression, dementia, Alzheimer’s disease, and improve cognitive processes.

**REFERENCES**


Samad N and Saleem A (2018). Administration of Allium cepa L. bulb attenuates stress-produced anxiety and
Diet supplements of banana fruit pulp mitigates repeated noise stress induced behavioral deficits and oxidative stress


