

# Nanoparticulate BDNF as a potential antidepressant via neuroendocrine mechanisms in experimental model of depression.



**SRDC** Sarawak Research and Development Council

**Scope:** Bioindustry and Biomedical Sciences

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**Introduction.** Depression is the most common mental problem worldwide (WHO, 2024), and its fast spread is threatening. The number of incident cases of depression worldwide increased from 172 million in 1990 to 258 million in 2017, comprising an increase of 49.86% [1]. So far the effective treatment of depression remains to be an unresolved problem, as at least 30% of people suffer from its treatment-resistant form, which is a tremendous individual and societal burden [2]. In the pathophysiology of depression, the hypothalamic-hypophyseal-adrenal axis (HHAA) is recognized as a major neurobiological link between its inducing factors, such as dysfunctional neurogenesis and neurotransmission, and immunological and endocrine factors [3]. Experimental research works showed that in modeled stress, activation of HHAA results in the development of depression [4], but it remains unknown how depression itself affects HHAA. Nanoparticulate BDNF was shown to be effective in the experimental treatment of neurodegenerative diseases, brain trauma, and stroke, while data regarding nano-BDNF treatment of depression are missing.

The objective of the study is to assess the effect of nano-BDNF on modelled depression in mice and the underlying and investigate the underlying mechanism of its action on the HHAA.

**Methods:** To evaluate the efficiency of Poloxamer 188 and Polysorbate 80 as surfactants for BDNF loading in 100nm PLGA nanoparticles, an enzyme-linked immunoassay (ELISA) test was performed using different concentrations of BDNF. The absorbance of each concentration was measured at 450 nm using a microplate reader. The obtained data were analyzed using Microsoft Excel and one-way ANOVA in IBM SPSS Statistics 26; mean, standard deviation, mean error by Tukey's post-hocs test. Shapiro-Wilk test was used for normality of distributions.

Thirty-six C57BL/6 mice weighing 20-25 g were involved in the study. The reserpine model of depression was applied to all the animals. Thereafter, mice were divided into 3 groups, with 12 per group. Group I (negative control) included mice receiving i.v. injections of normal saline as a treatment. Group II (positive control) involved animals receiving treatment with a standard antidepressant (fluoxetine). Group III (experimental) was treated with nanoparticulate BDNF with surfactant for seven days.

Behavioral tests were performed at the end of the experiment to assess the severity of depression. They included an open field test (Panlab Harvard with USB digital camera and SMART 3.0 video tracking software, Barcelona, Spain) with the distance walked, rearing, urination and grooming frequencies evaluated (Fig.1); and a forced swimming test (Fig.2). The corticosterone concentration in blood was measured by ELISA assay using a microplate reader (SpectraMax iD3, USA). Thereafter, the animals were sacrificed by decapitation. Adrenal glands and thymus were sampled for microscopic examination (Fig.3). The histological slides (Fig.4) were stained with hematoxylin-eosin and viewed under the microscope Zeiss Primostar 3 (Jena, Germany). Image analysis was performed using Image Pro+ 7.0 software (Media Cybernetics, USA). All results were statistically processed using SPSS 27.0.1 software; one-way ANOVA dispersion analysis was applied with Tukey's test used; for the image analysis of histological slides, Student's t-test was applied with  $p < 0.05$ , indicating the significance of differences.

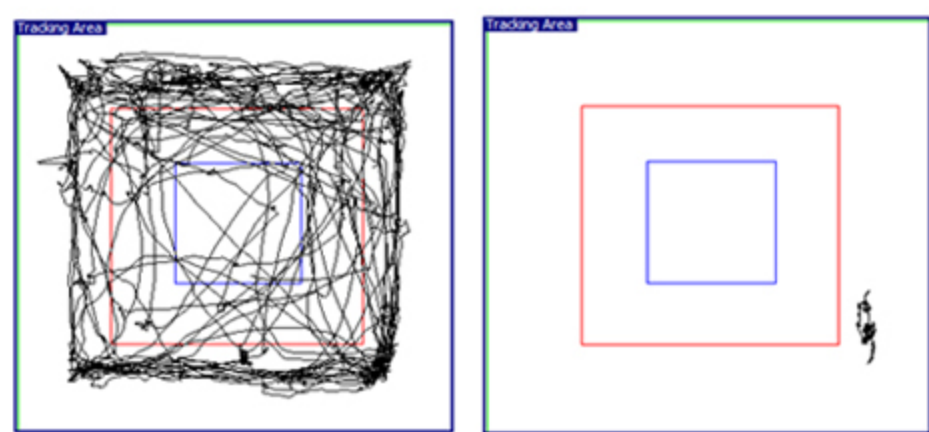


Fig.1. Open Field Test, Group III and Group I animals

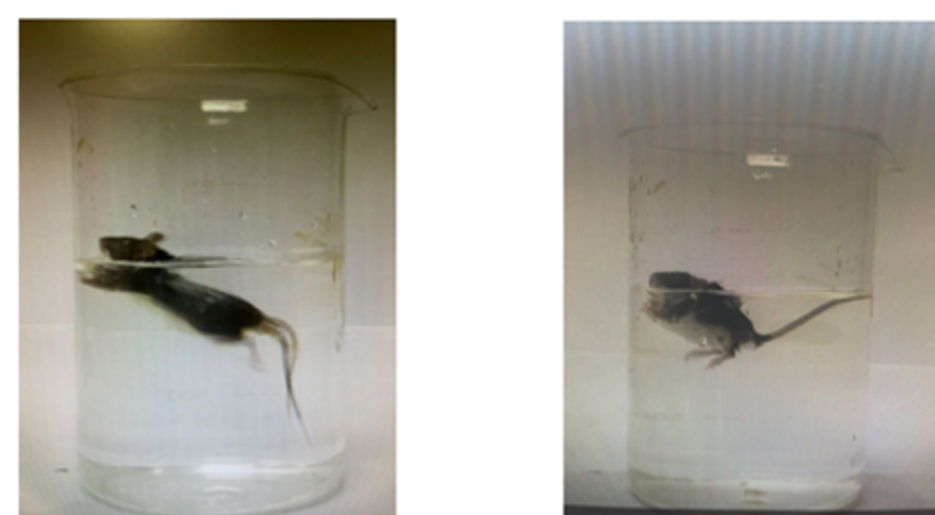


Fig.2. Forced swimming test for the experimental and control mice.

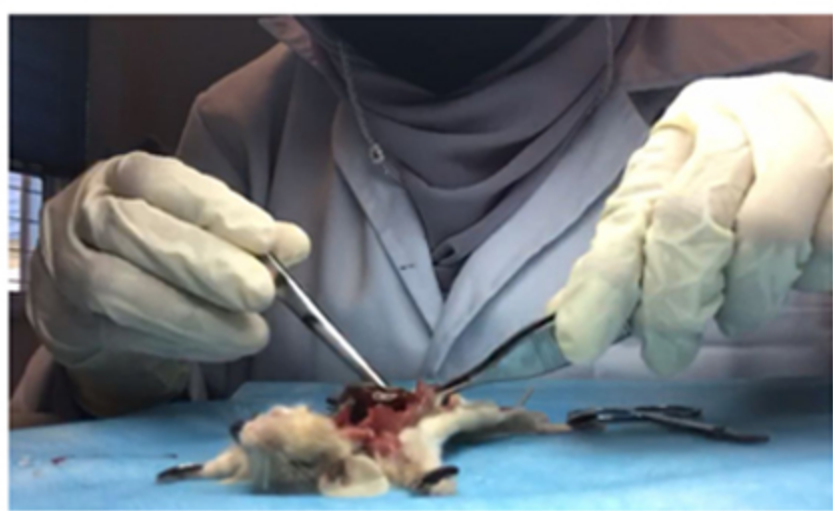


Fig.3. Autopsy and tissue sampling of the experimental animal



Fig.4. Sectioning of the histological slides for the image analysis

**Legend to graphs**  
 □ - Group I    ■ - Group II    ■ - Group III  
 \* -  $p < 0.05$  compared with Group I  
 \*\* -  $p < 0.01$  compared with Group I  
 # -  $p < 0.05$  compared with Group II

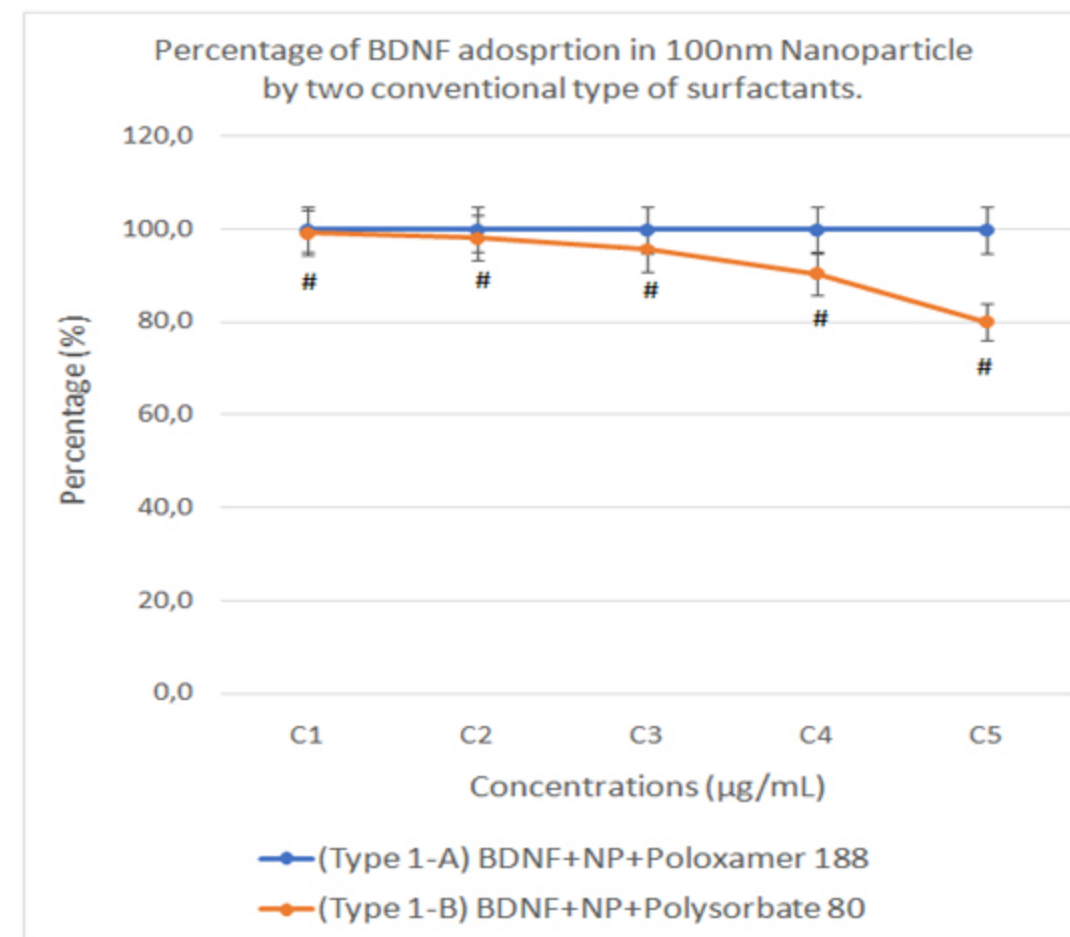


Fig.5. The results of the sorption test.

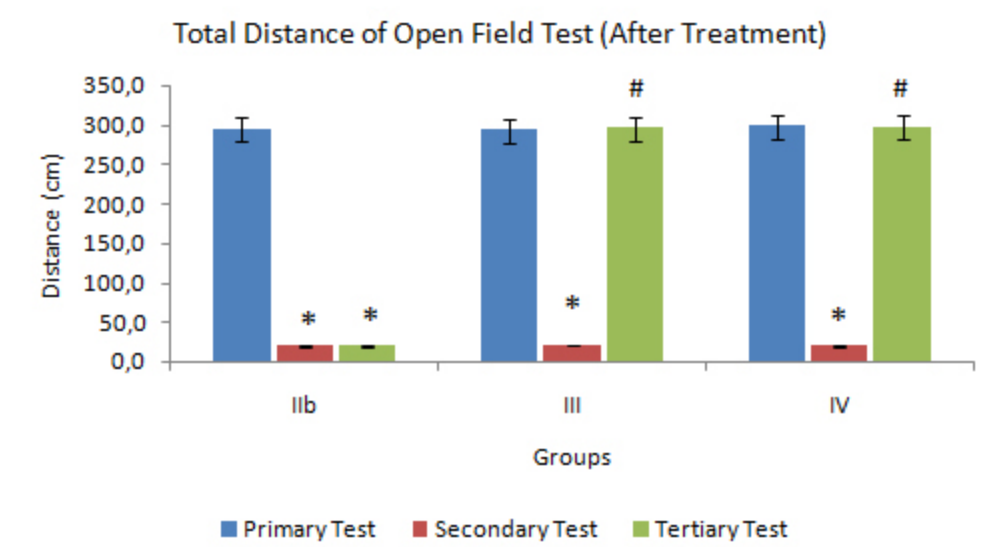
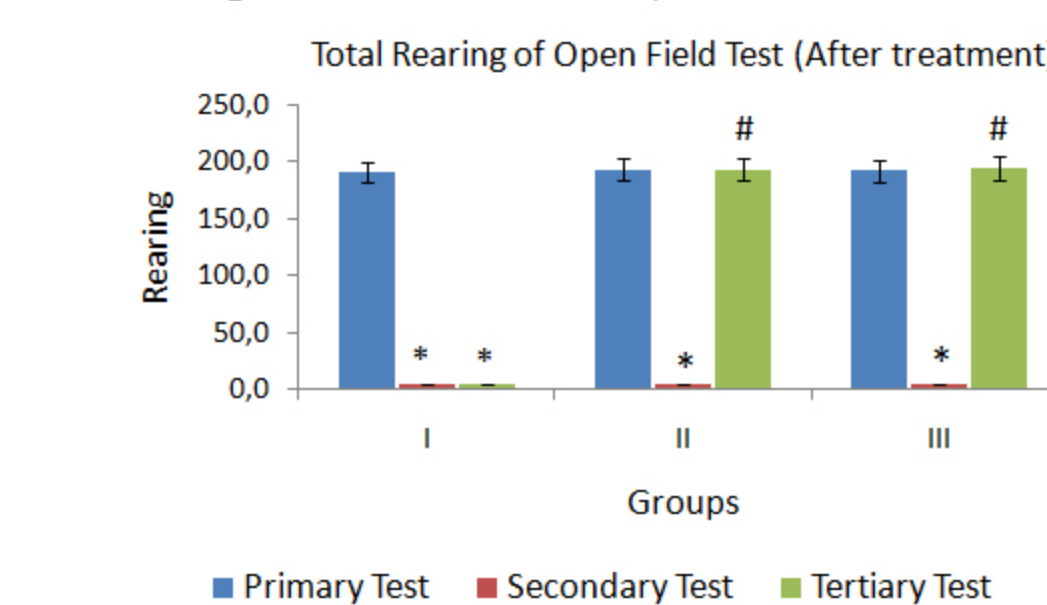


Fig.7. Rearing frequency in the negative and positive control, and the experimental (for depression modeling) animals (M±S); # -  $p < 0.01$  compared to the primary test, \* -  $p < 0.01$  compared to the Group IIb in the tertiary test

Fig.6. Distance walked by the negative and positive control, and experimental

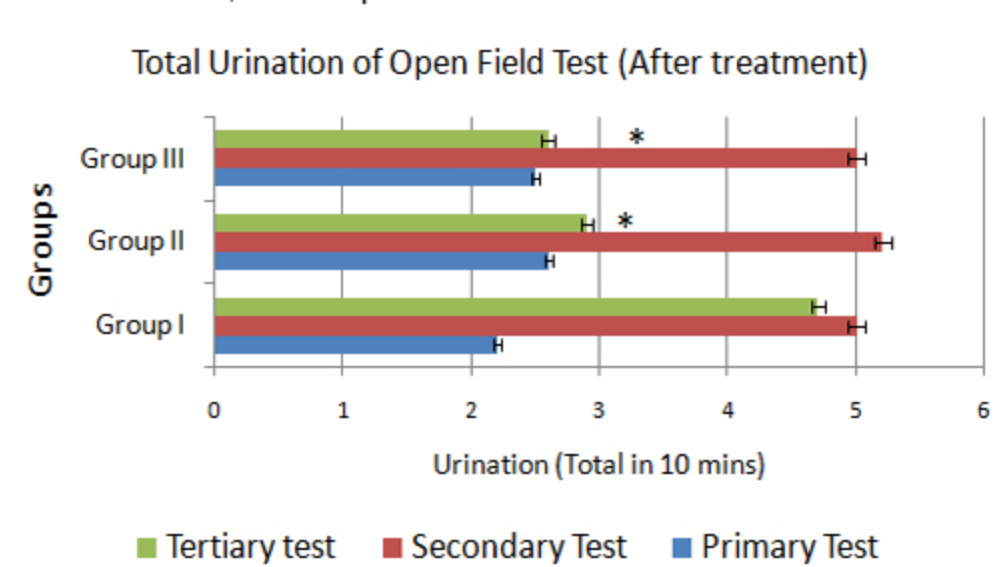


Fig.8. Open field urination rate by the negative and positive control and experimental (for depression treatment) mice (M±S); # -  $p < 0.05$  compared to the primary test, \* -  $p < 0.05$  compared to the secondary test

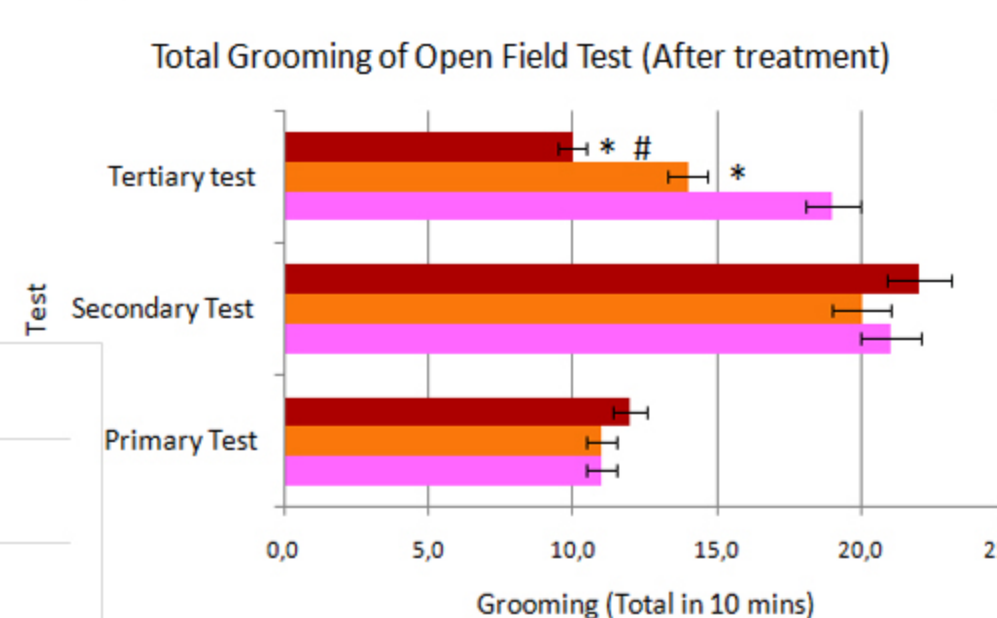


Fig.9. Open field grooming rate in the control and experimental (M±S)

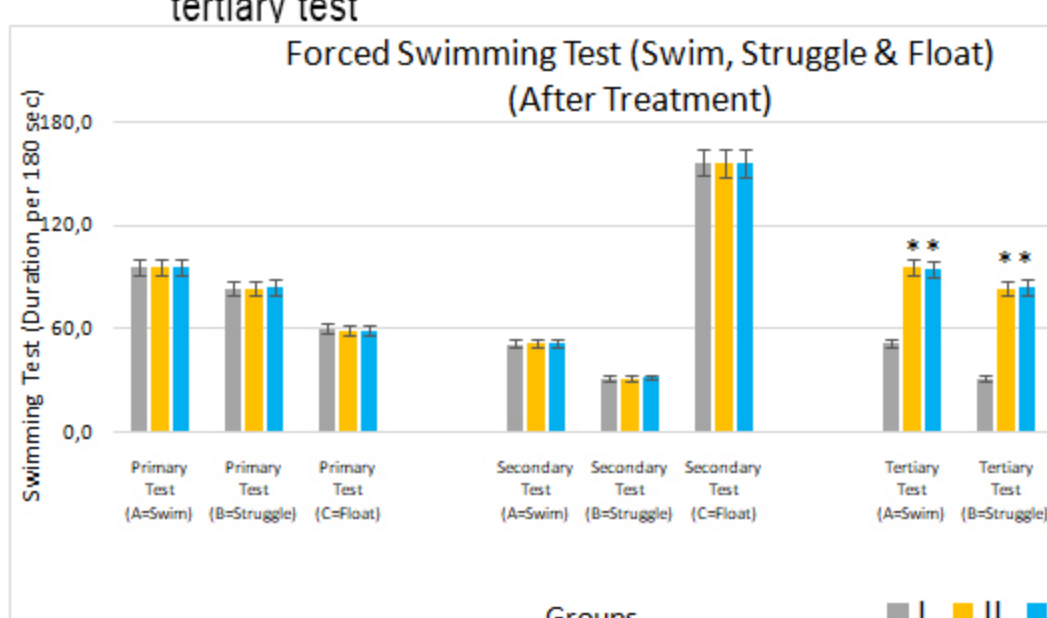


Fig.10. Forced swimming test results in experimental (for depression treatment), positive control and negative control groups, (sec, M±S)

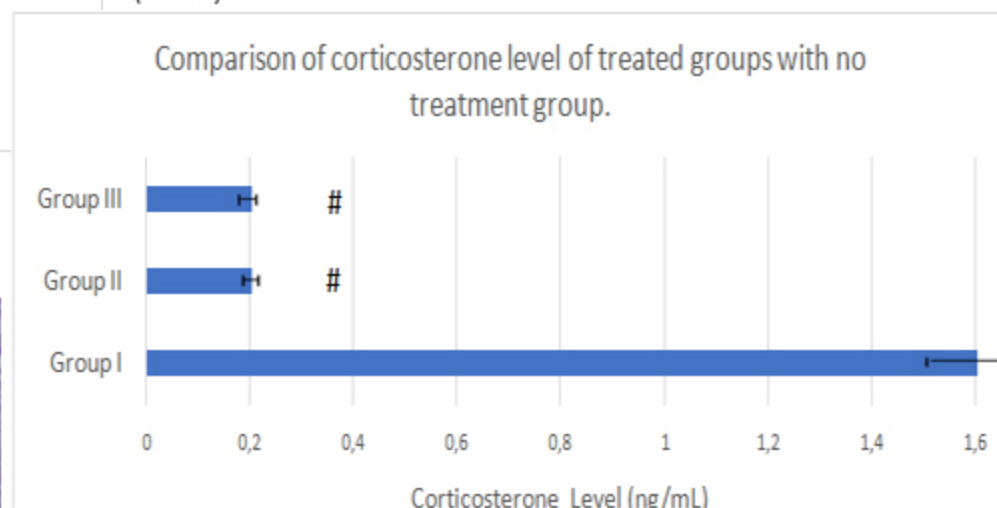


Fig.11. Forced swimming test results in experimental (for depression treatment), positive control and negative control groups, (sec, M±S)

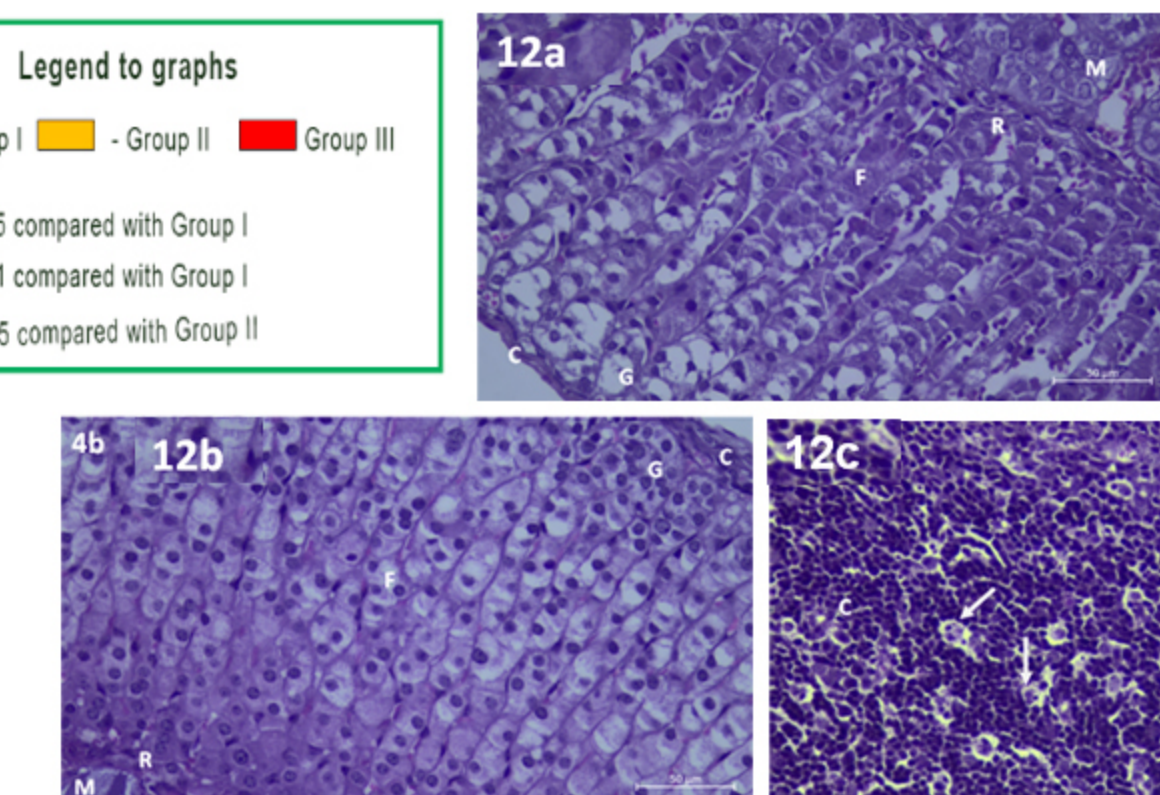


Fig.12. Adrenal gland (12a, 12b) and thymus (12c, 12d) of Group I (12a, 12c) and Group III (12b, 12d) animals. Micrographs. Hematoxylin-eosin staining. x40. Co - cortex, M - medulla, arrow - macrophage with apoptotic bodies, C - capsule, G,F,R - zone glomerulosa, fasciculata, reticularis

**Results:** The results of the sorption test revealed the efficiency of Polysorbate 80 as a surfactant for BDNF transport was lower (80%), indicating a reduced success rate in facilitating BDNF delivery to the target brain region. (Fig.5).

Animals of Group II and Group III showed significant positive changes in behavior in the open field test (Fig.6-9), significantly longer duration of the active phase (struggling, swimming) and shorter passive phase (floating) in the forced swimming test (Fig.10), and a significantly lower level of corticosterone in the blood ( $p < 0.01$ ) (Fig.11) than the Group I animals in the tertiary test. Besides, "Alarm" grooming frequency was significantly higher in Group II than in Group III, indicating that nano-BDNF was more efficient in reverting the depressive changes in the behaviour than fluoxetine. In animals of Group I, the microscopic picture (Fig.12) of zona glomerulosa and reticularis of the adrenal glands did not differ significantly from that of Groups III. However, the zona fasciculata was hyperplastic; its cells were larger in size, the cytoplasm was foamy, and adenocytes with dark cytoplasm were scarce in Group I animals (Fig.12a,b) compared to Group III. Thymus of the Group I animals had a microstructural picture indicating immunosuppressive changes (reduced cellularity, washed-out border between the cortex and medulla, numerous tangible-body macrophages with apoptotic bodies) (Fig.12c,d).

Image analysis a significant decrease in the volume density of the zona fasciculata of the adrenal cortex, as well as of the area of its spongiocytes and their nuclei compared in Group III compared to Group I, while in Group II these changes did not show statistically meaningful difference with Group I. (Fig.13). In the thymus a significantly higher cortex-medulla ratio and lower volume density of tingibile body macrophages was seen in Groups II and III compared to Group I, with a significant difference between Groups II and III (Fig.14). In contrast, the Group II showed no significant difference in the volume density of the tingibile body macrophages compared to Group I, while Group III was significantly different from both Group I and II, indicating that nano-BDNF was more effective in reverting hyperplasia of the adrenal cortex and immunosuppressive changes than fluoxetine.

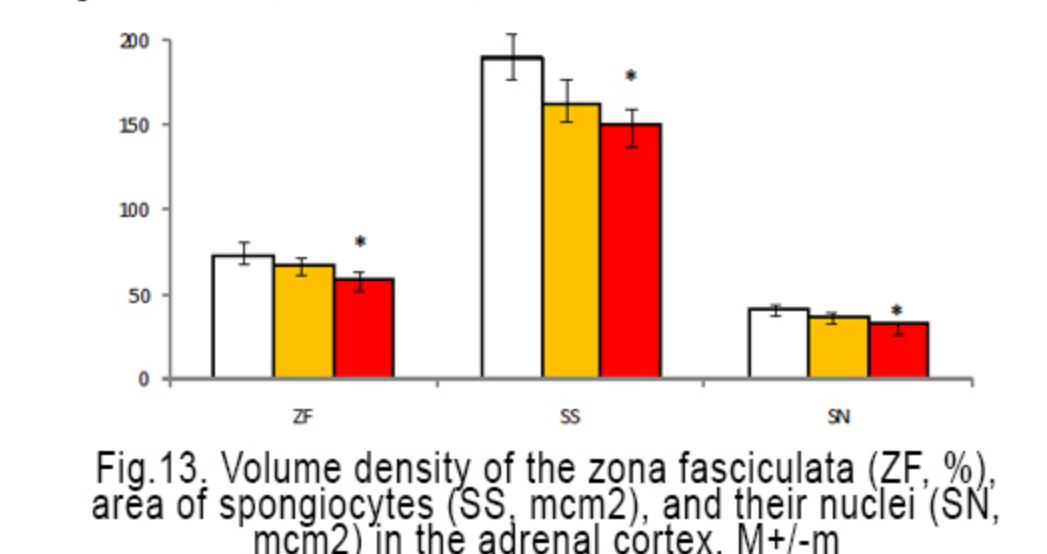


Fig.13. Volume density of the zona fasciculata (ZF, %), area of spongiocytes (SS, mcm2), and their nuclei (SN, mcm2) in the adrenal cortex, M+/-m

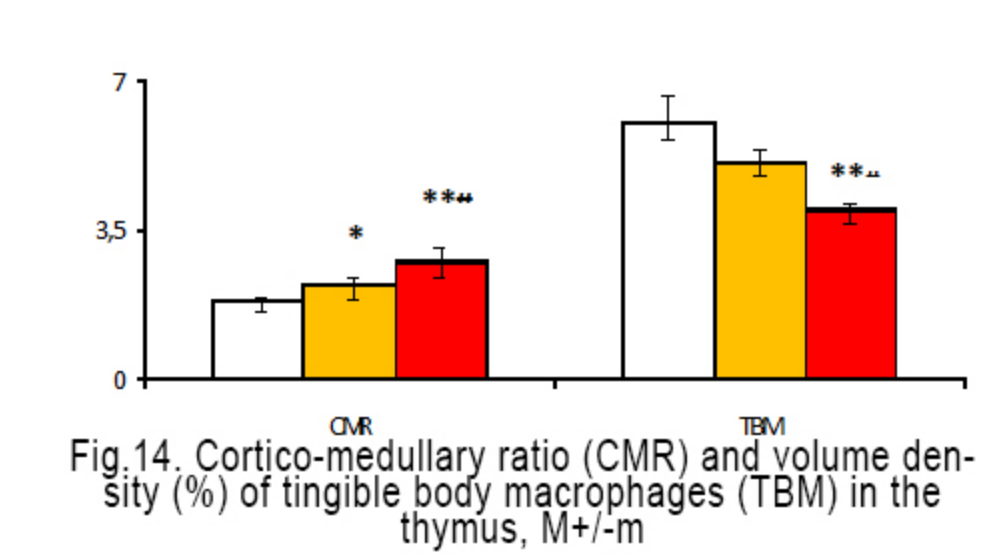


Fig.14. Cortico-medullary ratio (CMR) and volume density of tingibile body macrophages (TBM) in the thymus, M+/-m

**Conclusion:** 1. Nano-BDNF shows higher efficacy in depression treatment comparable to the best traditional antidepressants (fluoxetine), without any side effects of the latter, as evidenced by behavioral tests in mice. 2. Nano-BDNF reduced level of activation of the HHAA, manifested by reversed hyperplasia of the zona fasciculata of the adrenal cortex and hypertrophy of its spongiocytes, as well as reduced immunosuppressive changes in the thymus, indicating a reduced level of immunosuppression more effectively than the traditional antidepressant fluoxetine. 3. PLGA-BDNF with a surfactant (Poloxamer 188) may be considered a potential medication for the treatment of depression in human patients.

**References.**  
 1. Liu, Q., He, H., Yang, J., Feng, X., Zhao, F. & Lyu, J. (2020). Changes in the global burden of depression from 1990 to 2017: Findings from the Global Burden of Disease study. *Journal of Psychiatric Research*, 126, 134-140.  
 2. Li, C.T. (2023). Overview of treatment-resistant depression. *Progress in Brain Research*, 278:1-23. doi: 10.1016/bs.pbr.2023.03.007.  
 3. Mikulska, J., Juszczak, G., Gawrońska-Grzywacz, M. & Herbet, M. (2021). HPA Axis in the Pathomechanism of Depression and Schizophrenia: New Therapeutic Strategies Based on Its Participation. *Brain Sciences*, 11(10), 1298.  
 4. Leistner, C. & Menke, A. (2020). Hypothalamic-pituitary-adrenal axis and stress. *Handbook of Clinical Neurology*, 175, 55-64

**Acknowledgment.** The authors would like to thank the Sarawak Research and Development Council (SRDC) and the Government of Sarawak State for providing funding for this study under grant number RDCRG/CAT/2019/17