Khadeejah A, J Brain Neuros Res 2024, 8: 028
DOI: 10.24966/BNR-HSOA/100028

HSOA Journal of
Brain & Neuroscience Research

Research Article

Metformin Improves Neurobehavioural Responses, Prevented Memory Dysfunctions and Oxidative Damage in the Brain of A Phychoemotionally Stressed Wistar Rats

Akanji Khadeejah*

University of Technology, Ogbomoso, Oyo State Nigeria

Abstract

The removal of the rat whiskers has been shown to produce significant Psycho-Emotional Stress (PES). Metformin (MET) has shown to exert different biological effect outside its antidiabetic effects as it ameliorated the brain and behavioural deficits in rat model of restrain stress. However it is not known if this property of metformin is model specific and if metformin could also be protective in other animal model of PES. This study is however designed to examine the effects of MET on brain activities and behavioural alteration in an animal model of PES. Thirty (30) adults male Wistar rats (170-190g) were randomized and used for the study. Rats were restrained and twenty of these rats were dewhiskered to induce Psychoemotional Stress with the help of scissors. Animals were grouped into three groups (n=10) each as follows: Control, PES and PES + MET groups treated with 100mg/kg of metformin P.O, a day after the dewhiskering for 21 days. Following MET administration, behavioural studies was conducted. The animals were then sacrificed using synergistic effects of I.P administration of 90mg ketamine/kg and 10mg xylazine/kg, to examine the brain markers of oxidative stress, acetylcholinesterase activities, Protein and Nitrite concentration.

The Result showed that PES in rats significantly (P = 0.05) increased the percentage time spent in the closed arm compartment of the Elevated Plus Maze test (EPM) but significantly (P =0.05) decreased: the percentage bias score for Novel Object Recognition test (NOR), the percentage spontaneous alterations in Y- maze test and the distance covered in centimeter within 5 minutes in open field box test.

PES worsened spatial and recognition memory in rats. However, the PES + Metformin group can discriminate novel locations in NOR tests, suggesting that metformin can prevent and improve these impairments. Metformin prevented memory dysfunctions and oxidative damage (MDA levels) in the brain tissue and improved the antioxidant enzymes activities (CAT, GPx, and SOD) in psycho-emotionally stressed rats. Metformin administration also improved the locomotive function via improvement of the cholinergic neurotransmission.

Introduction

Rats rely on the movable set of whiskers organized in five horizontal rows along their snots for tactile perception, whereas humans move the tips of their fingers for this purpose [1].Rats' facial vibrissae, or whiskers, are essential for detecting their immediate surroundings. Since many rats and other rodents are nocturnal or crepuscular and cannot fully utilize the sources of visual information, they use their whiskers to navigate their environment, learn new routes, and distinguish between the textural qualities of objects' surfaces [2]. Rats' whisker system is similar to primates' fingertip palpation system in that, it can detect minute variations in object surface roughness [3]. When compared to other body parts, the rat's cortical representation of its whiskers is unusually large [3]. According to Afarinesh and Behzadi [1], the main somatosensory (barrel) cortex plays a crucial role in roughness discrimination using whiskers.

According to Afarinesh and Behzadi [1], the barrel cortex, also known as the whisker section of the somatosensory cortex, receives the representation of tactile information from vibrissae on the contralateral snout. In layer IV of the somatosensory cortex, the barrel is home to unique cellular aggregates [1]. According to [4], the motor cortex's whisker portion is a region in the frontal cortex's agranular medial field that regulates both sides' whisking movements. According to studies, experience-dependent plasticity, the loss of peripheral sensory information inputs from the whiskers causes cortical whisker map modifications [5], which altered tactile discrimination abilities [6].

Different environmental stimuli have impact on brain development [1]. Some of these stimuli works through the sensory inputs [6], while others works through hormonal inputs [7]. The progressive emergence of various defensive reflexes is a hallmark of the evolution of protective behaviour in rats [8]. This depends on the total amount of sensory afferent information as well as the age of the animal and the stimulus potential for harm [9].

According to Caldji [10], sensory deprivation causes an increase in emotionality, anxiety, aggression and decreases the ability to learn pain stimuli avoidance behaviour. In Rats, the whisker supplied this

***Corresponding author:** Akanji Khadeejah, University of Technology, Ogbomoso, Oyo State Nigeria, Email:- akanjikhadeejah@gmail.com

Citation: Khadeejah A (2024) Metformin Improves Neurobehavioural Responses, Prevented Memory Dysfunctions and Oxidative Damage in the Brain of A Phychoemotionally Stressed Wistar Rats. J Brain Neursci Res 8: 028.

Received: August 23, 2024; **Accepted:** September 12, 2024; **Published:** September 13, 2024

crucial sensory information and it has been observed that shaving rat whiskers virtually alters their neuro-behavioural reactions [11, 12]. With the development of behavioural capacities and naturally occurring whisker-mediated actions already established, the understanding of barrel cortex physiology development [13]. In particular, researchers have shown that, in a texture discriminating task, whisker removal causes changes in brain responses as well as reduction in tactile acuity [11, 12], and behavioral alteration is significantly impacted by the unilateral shaving of the whiskers.

Metformin has been shown to exert different biological effects outside of its antidiabetic effects. It has been shown to ameliorate brain and behavioral deficits in a rat model of restrain stress [14]. However, it is not known if the positive effects of metformin is model specific and in particular, it is not known if metformin could also be protective in an animal model of Psychoemotional stress. Thus, the aim of this work is to examine the possible effect of metformin on brain and behavioural alterations in an animal model of Psychoemotional stress.

Materials and Methods

Study Area

The study was carried out at Department of Physiology Laboratory of Ladoke Akintola University of Technology, Ogbomoso, Oyo State Nigeria.

Animals

Thirty (30) adults male Wistar rats (9 weeks old) average weight 150g-170 g used for this study were gotten from the animal house of the Department of Physiology, Ladoke Akintola University, Ogbomoso. The animals were housed in plastic cages located in a well-ventilated vivarium, under standard laboratory conditions and provided with standard rat pellets (Ladokun Feeds Limited) and water ad libitum. The rats were acclimatized for two weeks before exposure to the various treatments. Adequate amount of wood shavings which cover the whole floor of the cages was provided as bedding. Animal care and experimental procedure were done in accordance to the Principles of Laboratory Animal Care.

Ethical Approval

The protocol were approved by the Ethical Research Committee of Faculty of Basic Medical Sciences, Ladoke Akintola university of Technology, Ogbomosho with the approval number: ERCFBMSLAUTECH:025/02/2024.

Drug

Metformin was manufactured by Impulse Pharma limited and marketed by EDEN U.K Pharmaceutical limited, was purchased from a pharmaceutical shop.

Experimental Design

The rats were randomly allocated to three (3) groups of ten (10) rats each and were treated for twenty-one (21) consecutive days as follows: Control rats exposed to normal drinking water, Psycho-emotional Stress (PES) rats exposed to drinking water, and Psycho-emotional Stress (PES), Rats exposed to 100mg/kg of metformin [15]. Pyscho-emotional stress was induced by cutting off the whiskers of rats [16].

Behavioural Test

Novel Object Recognition Test

The methodology for this test was based on Bevins and Besheer [17]. This test depends on innate curiosity and the capacity to identify an object that has been seen before. This test relies on three sessions: one habituation session, one training session, and one test session. The rats were acclimated to the empty arena with no objects for ten minutes a day before the test (Habituation session). Every rat in the training phase spent ten minutes in a box containing two identical rectangular objects (Training session). Training simply involves the rat visual exploration, of two identical objects. Two (2) hours later, one of the identical rectangular object was replaced by a triangular object (Novel object). The rats were allowed to investigate and explore both the new (triangular novel object) and familiar objects (rectangular object) for five minutes before the object recognition test was conducted. Test session involves replacing one of previously explored object with a novel object. The novel object recognition test was evaluated by the differences in the rat exploration time with the novel object and the familiar object.

Y-maze Spontaneous Alternation Test

The Y-maze test was carried out following the Arai [18] method, spatial recognition memory was assessed using an apparatus with three identical arms. Every rat was given one trial during the test, giving it eight minutes to explore the equipment. The entry of each arm was recorded; one alternation was defined as the rat entering three arms in a row; alternations were only considered valid selections when the rat entered three distinct arms in a row. The percentage of spontaneous alternations is used to express this, and it was computed using the formula [numbers of alternations/number of entries-2] × 100. The more the mice's memory is intact, the higher the alternation percentage.

Light and Dark Box (Light–dark (LD) exploration)

The light-dark box was comprised of two compartments: a light compartment measuring 27 cm by 27 cm by 27 cm and a dark compartment measuring 27 cm by 18 cm by 27 cm. The two compartments were divided by a partition featuring a single opening measuring 7 cm by 7 cm, which allowed passage from one compartment to the other, as previously described. The device was placed in a screen-enclosed section of the behavior core facility room, and as previously mentioned in there was only one experimenter / observer present in the room during the experiment. Standard lighting conditions in the room were approximately 700 lx. Time was measured using the previously mentioned manual scoring method. An observer who was blind to the treatment measured how much time was spent in the illuminated area overall for five minutes. A rat is exposed to a novel environment with protected (dark) and unprotected(light)areas.

Open Field Box

After being chosen at random, the rats were positioned in the middle of the open space and given five minutes to explore. An overhead camera observed the animal's behavior in the open field and recorded its total distance traveled, number of rearings, and amount of time spent in the field's center. Following the recording, the animal was taken out, and the open field's surface was scrubbed with a swab to get rid of any dirt left by earlier animals [19].

The Elevated Plus Maze Test (EPM)

Based on rats' dislike of open areas, the elevated-plus maze model was created. Thigmotaxis, or avoiding open areas by limiting movement to enclosed spaces or the edges of a confined space, is a behavior that results from this aversion. The EPM was situated in a room with soft lighting (50 lx) and was composed of two open arms (30 x 5 cm), two enclosed arms (30 x 5 cm), and a central platform (5 x 5 cm) that was raised 38.5 cm above the floor. The number of head dips over the edge of the open arms and the percentage of time spent in the open and central arms were measured during a 5-minute session.

Biochemical Analyses

After the neurobehavioral evaluation was finished, the rats were sacrificed using synergistic effects of I.P administration of 90mg ketamine/kg and 10mg xylazine/kg. The entire brain was extracted right away and refrigerated. After homogenizing the entire brain with 50 mM Tris–HCl buffer (pH 7.4), the sample was centrifuged at 12,000 g for 15 minutes at 4 °C. Utilizing the resultant supernatant, biochemical analyses were conducted.

Assessment of Neurotoxicity and Oxidative Stress Indices

Using ELISA kit, the SOD and CAT activities were assessed with the aid of 752SUV-VIS Spectrophotometer (Ningbo,China), all biochemical assays were analyzed using a SpectraMaxplate reader (MolecularDevices, CA, USA).

Protein Concentration

Protein concentration was measured using the method, with bovine serum albumin serving as the reference. In short, the reagents were used exactly as supplied. Coomassie Brilliant Blue G-250 was acquired from Sigma. Also Mercaptoethanol was purchased from Sigma. Triton X- 100 was acquired from Schwartz/Mann. sodium dodecyl sulfate was purchased from BDH Chemicals Ltd. in Poole, England. The source of hemosol was Scientific Products. The remaining reagents were all analytical grade or the highest grade that was offered. For the protein preparation, cytochrome (horse heart), chymotrypsinogen A, and bovine serum albumin (2x crystallized) were acquired from Schwartz/Mann. Nutritional Biochemical Corporation provided human serum albumin and hemoglobin. Protein concentration was assayed according to Bradley and Markwell, 2007, using bovine serum albumin as standard. Briefly, the reagents, Coomassie Brilliant Blue G-250 was obtained from Sigma, and used assupplied.2-Mercap to ethanol was obtained from Sigma.TritonX-100 was obtained from Schwartz/Mann. Sodium dodecyl sulfate was obtained from BDH Chemicals Ltd., Poole, England. Hemosol was obtained from Scientific Products. All other reagents were of analytical grade or the best grade available. For the protein preparation, bovine serum albumin (2xcrystallized), chymotrypsinogen A ,and cytochrome c (horseheart) were obtained from Schwartz/Mann. Hemoglobin and human serum albumin were obtained from Nutritional Biochemicals Corporation.

Acetylcholinesterase (AChE) activity

The assay for acetylcholinesterase (AChE) was conducted in accordance with Misra [20]. The general procedure involved adding concentrated reagent solutions using micropipettes to the photocell after filling it with buffer. Blowing through the pipettes and moving them around the bottom of the photometer cells continued to stir the mixture. In this manner, in ten to fifteen seconds, the reagents were added, combined, and the cell compartment cover was replaced. The solutions that are utilized are pH 8.0, 0.1 M buffer phosphate. Acetylthiocholine iodide, 0.075 M (21.67 mg/ml), is a component of the substrate. Successful use of this solution was observed for 0–15 days when refrigerated. The reagent includes: Dithiobisnitrobenzoic acid (DTNB) 0.01M of the 5:5-dithiobis-2- nitrobenzoic acid (Prepared by dissolving 39.6mg in 10 ml pH 7.0 phosphate buffer (0.1M) with 15mg of sodium bicarbonate added). These agent was made up in buffer of pH 7 in which it was more stable than in that of pH 8. The Enzymes are: Bovine erythrocyte cholinesterase (Nutritional Biochem.Corp.,20,000units) was dissolved in 20 ml of 1% gelatin. This solution was diluted 1:200 with water for use, yielding a solution of 5units/ml. 0.4 ml aliquot of the brain homogenate was added to a cuvette containing 2.6 ml of phosphate buffer (pH 8.0, 0.1 M). 100 μlDTNB reagent were added to the photocell and the absorbance was measured at 412$m\mu$; when this had stopped increasing, the photometer slit was opened so that the absorbance was set to zero. 20 $\mu l$of the substrate were added and Changes in absorbance were recorded and the change in absorbance per min. was then calculated as follows:

$$R = \frac{\Delta A}{1.36(10^4)} \times \frac{1}{(400/3120)C_0} = 5.74(10^{-4})\,\Delta A/C_0$$

where R= rate ,in moles substrate hydrolyzed per min per g of tissue;

ΔA= change in absorbance per min;

C_0= original concentration of tissue (mg/ml).

Superoxide Dismutase Activity (SOD)

The activities of superoxide dismutase (SOD) were measured in accordance with Claiborne, 1995. The basis for the SOD assay is the ability of superoxide dismutase to inhibit the auto-oxidation of adrenaline at pH 10.2. Adrenaline is known to be oxidized to 87adrenochrome by the superoxide anion (O2) produced by the xanthine oxidase reaction. Both an increase in adrenaline concentration and pH caused an increase in the amount of adrenochrome generated per superoxide anion [21]. These gave rise to the theory that adrenaline undergoes auto-oxidation through at least two different pathways, one of which involves superoxide radical and is therefore susceptible to inhibition by SOD. 17 milliliters of distilled water with 0.05M carbonate buffer (pH 10.2) were used to dissolve 0.01 of adrenaline (Sigma Aldrich, Germany). 14.32 g of $Na_2Co_3.10H_20$ and 4.20 g of $NaHCO_3$ were dissolved in distilled water and madeupof1000 ml with distilled water and the pH adjusted to 10.2.

A volume (0.2ml) of each of the tissue homogenates was added to 2.5 ml of 0.05 carbonate buffer (pH10.2) to equilibrate in the spectrophotometer and the reaction was started by the additionof0.3ml of freshly prepared 0.3mM adrenaline to the mixture. The absorbance of the sample was measured at 450 nm using spectrophotometer. Change in ab/min=A5-A1/2.5% inhibitionincrease in abs of sample/increase in abs of blank*1001unit SOD=amount that cause 50%inhibition.

Catalase Assay (CAT)

The assessment of Catalase (CAT) activities was conducted in accordance with Claiborne [21]. The measurement of the hydrogen

peroxide substrate left over after catalase action served as the foundation for this assay technique. First, hydrogen peroxide is converted by catalase into water and oxygen (a catalytic pathway). Sodium azide is then used to stop this enzymatic reaction. The amount of hydrogen peroxide left in an aliquot of the reaction mixture was then measured using a colorimetric technique. In the presence of hydrogen peroxide and horseradish peroxidase (HRP), a substituted phenol (3, 5- dichloro-2-hydroxybenzenesulfonic acid) couples oxidatively to 4-aminoantipyrine to produce a red quinoneimine dye (N-(4-antipyryl)-3-chloro-5-sulfonatep-benzoquinone-monoimine) that absorbs at 520 nm. This is how the colorimetric method works.

Reduced Glutathione (GSH) Concentration

Glutathione (GSH) levels were measured in accordance with Claiborne [1]. In short, an equal volume of 4% sulfosalicylic acid was added to an aliquot of the sample to deproteinize it, and the sample was centrifuged for five minutes at 4,000 rpm. Next, 4.5 ml of Ellman's reagent was combined with 0.5 ml of the supernatant. 0.5 ml of the diluted precipitating agent and 4.5 ml of Ellman's reagent were used to create a blank. The absorbance at 412 nm is equal to reduced glutathione, or GSH.

Lipid Peroxidation

The lipid peroxidation was measured using the Adedara [22], method of measuring the formation of thiobarbituric acid reacting substances (TBARS). In short, a shaking water bath was used to incubate 1 mg/ml of the microsomal fraction in isotonic phosphate buffer (pH 7.4) for six hours at 37°C. To a 0.5 ml incubation mixture containing 0.01 ml 5% (w/v) butylated hydroxytoluene (BHT) that had previously been quenched with 0.5 ml 10% TCA, 0.5 ml 0.75% thiobarbituric acid in 0.1 M HCl was added. After 20 minutes of heating at 90±95oC, the mixture was centrifuged at 780 g for 10 minutes to cool it down. After being moved into an acid-resistant tube, the supernatant was centrifuged for ten minutes at 32,000g. The absorbance of the resulting clear solution was determined at 532nm using a Cecil CE599 automatic scanning spectrophotometer. Malondialdehyde (MDA) was quantitated by using $\Sigma = 1.56\times10^{5}M/cm$ [23].

Evaluation of reactive nitrogen (Nitrite) species (RONS) level

Using a well-established method based on the RONS-dependent oxidation of 2′,7′-dichlorodihydrofluoresc in diacetate (DCFH-DA) to DCF, the amount of RONS generation in the brain was assessed [22].

In summary, 10 µL of the fresh sample, 150 μL of 0.1 M potassium phosphate buffer (pH 7.4), 35 μL of distilled water, and 5 μL of DCFH-DA (200 μM, final concentration 5 μM) with minimal air contact made up the reaction mixture. Using a SpectraMax plate reader (Molecular Devices, CA, USA), the fluorescence emission of DCF resulting from DCFHDA oxidation was examined for 10 min (30 s intervals) at 488 nm excitation and 525 nm emission wavelengths. The percentage of the control value was used to express the rate of DCF generation.

Statistical analyses

Data were analyzed using one-way analysis of variance (ANOVA) and Tukey's post-hoc test using GRAPH PAD PRISM 7 software (Version 7; Graph Pad Software, La Jolla, California,USA).Values of P <0.05 were considered significant.

Results

Behavioural Studies

Effect of Metformin on Activity of Psychoemotional Stress (PES) Rats in Light-DarkBox. Psychoemotional stress in the rats does not significantly affect the time spent with movement in the light compartment compared to the control rat's movement in the light compartment. Psychoemotional stress rats treated with Metformin significant increase the time spent in the light phase compared to the control and the untreated Psychoemotional stressed rats (Figure 1).

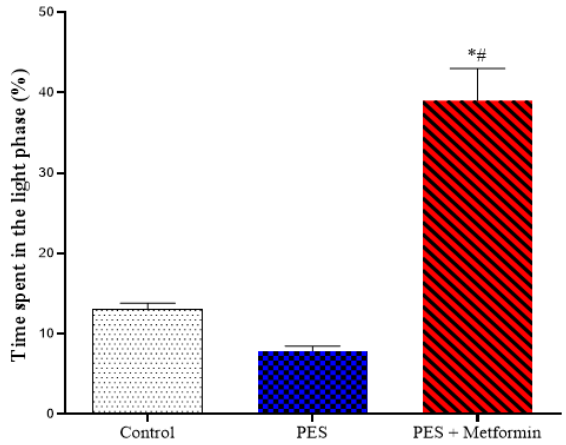

Figure 1: Chart showing the percentage time spent in the light phase. Data are expressed as mean ± S.D.* = significant (P < 0.05) compared to control, # = significant (P < 0.05) compared to PES.

Effect of Metformin on Activity of Psychoemotional stress (PES) rats in the Elevated-Plus Maze

The percentage time spent in the closed arm compartment of the elevated plus maze was significantly increased in the Psychoemotional stress rats when compared to the control rat's time spent in the closed arm compartment. Metformin treatment of the Psychoemotional stress rats caused a significant increase in the percentage time spent in the closed arm compartment compared to the control rats (Figure 2).

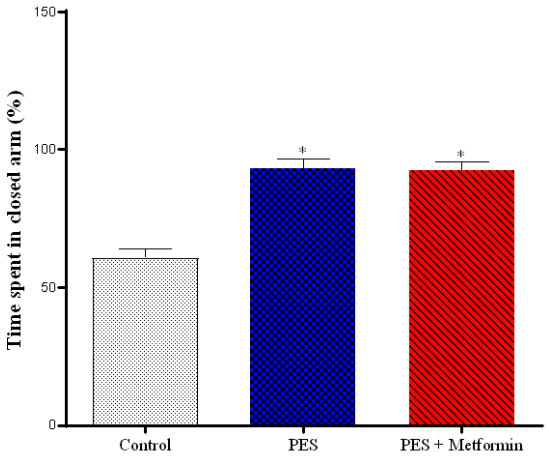

Figure 2: Chart showing the percentage time spent in closed arm.Data are expressed as mean ± S.D. * = significant (P < 0.05) compared to control.

Effect of Metformin on Activity of Psychoemotional stress (PES) rats on the Novel Object Recognition

Psychoemotional stress significantly decrease the percentage bias score in rats when compared to the control rat (Figure 3). Metformin treatment in the psychoemotional stress rats significant increase in the percentage bias score compared to the untreated psychoemotional stress rats (Figure 3).

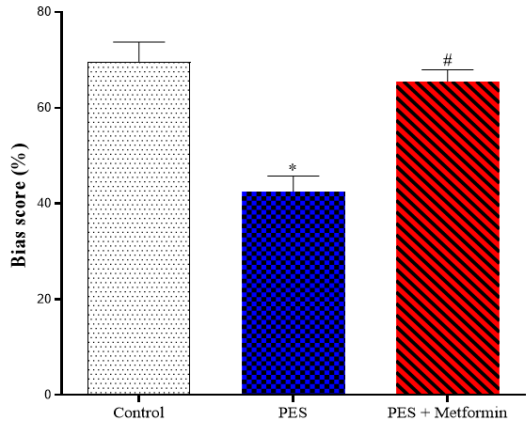

Figure 3: Chart showing the percentage bias score.Data are expressed as mean ± S.D. * = significant (P < 0.05) compared to control, # = significant (P < 0.05) compared to PES.

Effect of Metformin on Activity of Psychoemotional Stress (PES) rats on the Y-maze spontaneous alterations

Psychoemotional stress in the rats significantly decrease the percentage spontaneous alterations when compared to the control rat (Figure 4). However, metformin treatment of the in the psychoemotional stress rats caused a significant increase in the percentage spontaneous alterations compared to the psychoemotional stress rats (Figure 4).

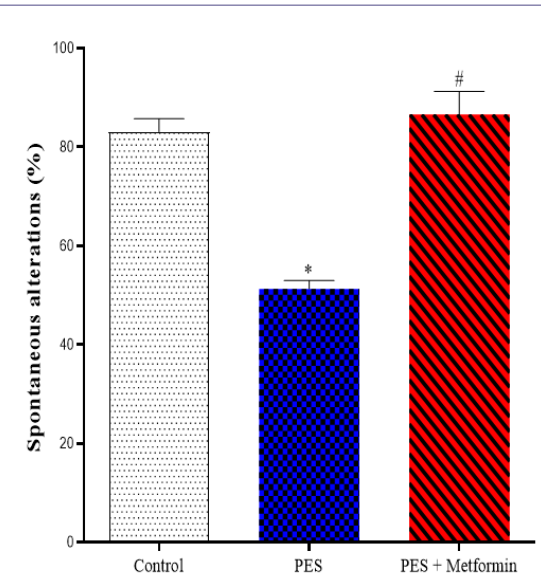

Figure 4: Chart showing the percentage spontaneous alterations.Data are expressed as mean ± S.D. * = significant (P < 0.05) compared to control,# = significant (P < 0.05) compared to PES.

Effect of Metformin on Activity of Psychoemotional Stress (PES) rats on the Open Field Box Test

The distance covered in centimeter within 5 minutes by the psychoemotional stress rats significantly decrease when compared to the control rat (Figure 5). Also, the metformin treatment in the psychoemotional stress rat's significant decrease in the distance covered in 5 minutes compared to the control rats (Figure 4.5). However, no significant change in the distance covered in 5 minutes (cm) in the psychoemotional stress rats and the metformin treated psychoemotional stress rats (Figure 5).

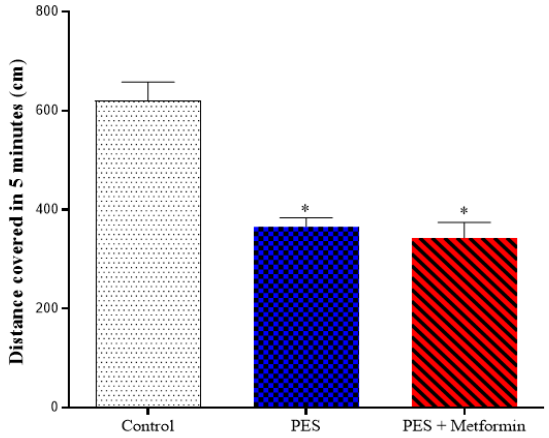

Figure 5: Chart showing the distance covered within 5 minutes in an open field box test.Data are expressed as mean ± S.D. * = significant (P < 0.05) compared to control.

Biochemical Studies

Effect of Metformin on the Lipid Peroxidation Leveland Reduced Glutathione (GSH) concentration of Psychoemotional Stress (PES) rats

There was significant increase in the malonaldehyde (MDA) concentration in the psychoemotional stress rats when compared to the control rat (Figure 6). Metformin treatment in the psychoemotional stress rats caused a significant decrease in the MDA concentration compared to the psychoemotional stress rats (Figure 6).

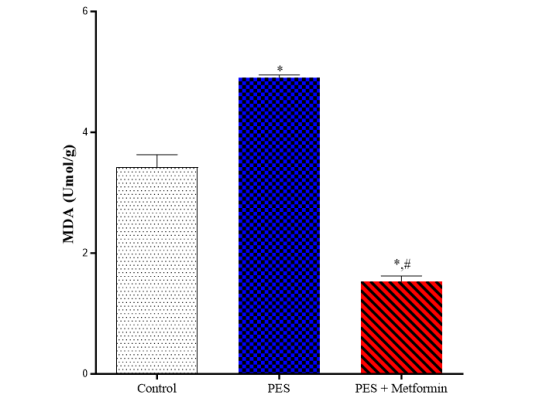

Figure 6: Chart showing the MDA concentration in the brain.Data are expressed as mean ± S.D. * = significant (P < 0.05) compared to control,# = significant (P < 0.05) compared to PES.

There is significant decrease in the brain GSH concentration in the psychoemotional stress rats when compared to the control rat (Figure 7). Metformin treatment in the psychoemotional stress rats caused a significant increase in the GSH concentration compared to the untreated psychoemotional stress rats (Figure7).

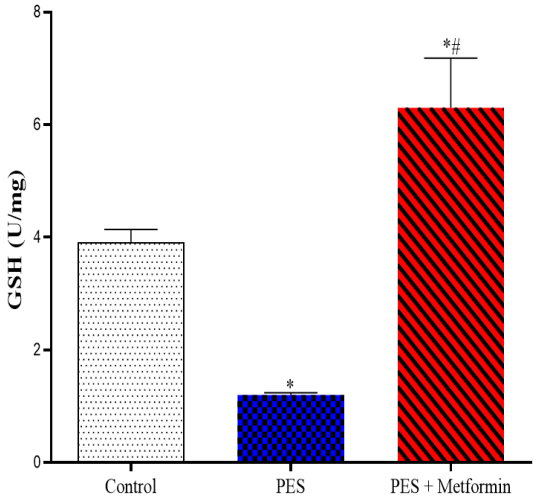

Figure7: Chart showing the GSH concentration in the brain. Data are expressed as mean ± S.D. * = significant (P < 0.05) compared to control,# = significant (P < 0.05) compared to PES.

Effect of Metformin on the Brain Superoxide Dismutase (SOD) and Catalase Activities of Psychoemotional Stress (PES) rats

There is significant decrease in the SOD and catalase activities in the psychoemotional stress (PES) rats when compared to the control rat (Figure 8A and B). Metformin treatment of the psychoemotional stress rats caused a significant increase in the activities of SOD and catalase when compared to the untreated psychoemotional stress rats (Figure 8A and B).

Effect of Metformin on the Brain Acetylcholinesterase Enzymes activities of Psychoemotional Stress (PES) Rats

There is significant decrease in the Acetylcholinesterase enzymes concentration in the Psychoemotional stress rats when compared to the control rat (Figure 9). Metformin treatment however causes a significant increase concentration of acetylcholinesterase enzymes in the psychoemotional stress rats when compared to the control and the untreated psychoemotional stress rats (Figure 9).

Effect of Metformin on the Total Brain Protein and Nitrite concentration of Psychoemotional Stress (PES) Rats

There is significant increase and decrease in the total brain protein and nitrite concentration respectively in the psychoemotional stress rats when compared to the control rat (Figure 10A and B). Metformin treatment in the psychoemotional stress rats does not caused a significant decrease in the total brain protein concentration compared to the untreated psychoemotional stress rats (Figure 10A) but significantly increased the brain nitrite concentration when compared to the untreated Psychoemotional stress rats (Figure 10B).

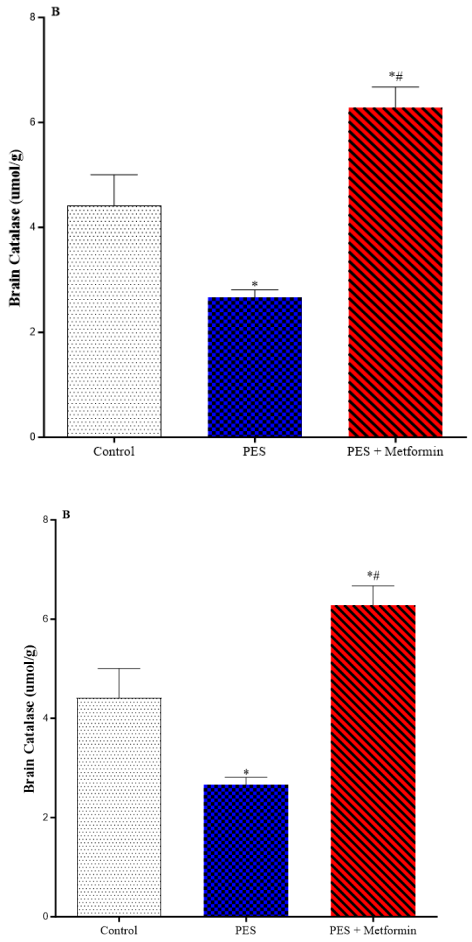

Figure 8: Chart showing the (A) SOD and (B) Catalase activities in the brain. Data are expressed as mean ± S.D. * = significant (P < 0.05) compared to control # = significant (P < 0.05) compared to PES.

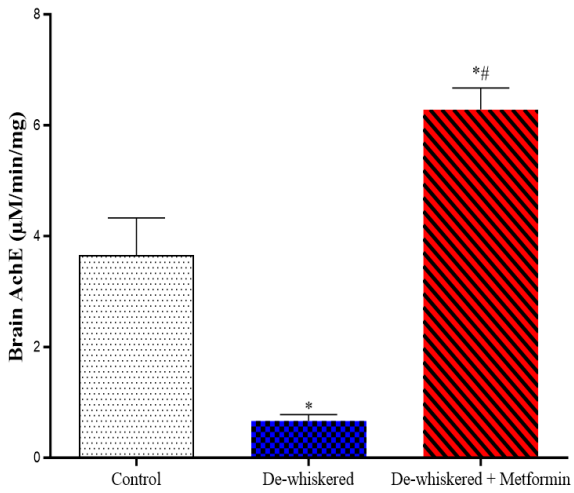

Figure 9: Chart showing the acetylcholine activities in the brain. Data are expressed as mean ± S.D. * = significant (P < 0.05) compared to control, # = significant (P < 0.05) compared to PES.

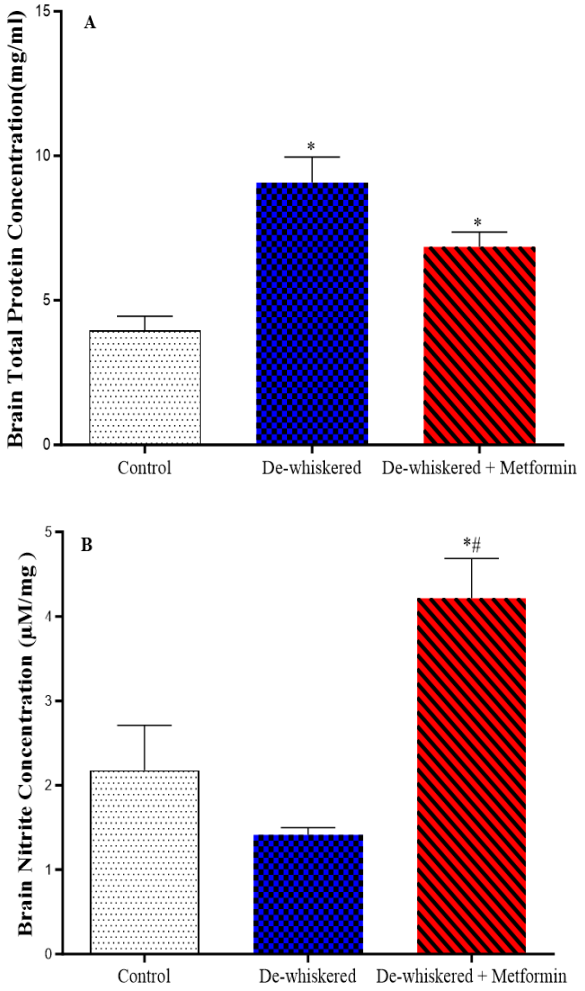

Figure 10: Chart showing the (A) protein and (B) nitrite concentration in the brain. Data are expressed as mean ± S.D. * = significant ($P < 0.05$) compared to control, # = significant ($P < 0.05$) compared to PES.

Discussion

Metformin (MET) is an antidiabetic medication used to treat type 2 diabetes [24]. Research has shown that MET reduces gluconeogenesis. [25, 26]. Studies have shown that the drug has neuroprotective effects in a variety of CNS pathological conditions, such as Alzheimer's disease, Parkinson's disease, and aging [27,28]. In the meantime, a number of studies have shown that the mechanisms underlying these protective effects may involve both an increase in brain-derived neurotrophic factor (BDNF) levels in the central nervous system and an antioxidative mechanism [24].According to studies, experience-dependent plasticity—the deprivation of peripheral sensory information inputs from the whiskers—caused cortical whisker map changes [5], which altered tactile discrimination abilities [6].According to Caldji [10], sensory deprivation causes an increase in emotionality, anxiety, aggression, and a decrease in the ability to learn pain stimulus avoidance reactions. In rats, the whiskers supplied this crucial sensory information. It has been shown that shaving rats' whiskers almost completely alters their neuro-behavioral reactions. Conversely, anxiolytics are frequently used in clinical practice to reduce anxiety when an unusual or typical circumstance causes an excessively intense and non-adaptive anxiety state.

In psychoemotional stress rats, the current study examined the impact of metformin on neurobehavioural performance and brain redox assessment. Through de-whiskering, which can cause fear or anxiety, experimental animal models used in this study showed complex behavioral alterations during exposure to Psychoemotional stress. The increased anxiolytic effect is demonstrated by the declining percentage of time spent in the light compartment of the light-dark box and the rising percentage of time spent in the elevated plus maze's closed arm compartment. The reduction in the percentage of time spent on novel object recognition, spontaneous changes in the Y-maze, and the distance traveled in the open field test within the allotted time confirms that the psychoemotional stress rats experience anxiety-like effects.

This is consistent with previous study showing that shaving rats and other rodents virtually eliminates their ability to respond neuro-behaviorily [3,11,12]. On the other hand, the psychoemotional stress rats' neurobehavioural activities were enhanced by metformin. This is consistent with earlier research by Ebokaiwe [29] and Fatemi [24], which found that metformin treatment enhanced neurobehavioral functions, thereby improving behavioral disorders. Rats' whisker system is similar to primates' finger-tip palpation system in that it can detect minute variations in object surface roughness [30]. The rodent has a disproportionately large cortical representation for the whiskers compared with other body areas [25].Roughness discrimination using whiskers has been found to be critically dependent on the primary somatosensory (barrel) cortex [31]. However, understanding the vibrating nature of the whiskers is crucial to comprehend their behavior in object localization, particularly in collision, as well as during exploration and texture discrimination [32-34]. Quist [35], the inertial phenomena due to the vibrissae mass is relevant only during the collision with an object.

Reactive oxygen species (ROS) generation, oxidative stress, and altered levels of inflammatory mediators have been strongly implicated in tissue damage due to hyperglycemia [36, 37]. Estimation of antioxidant defense along with LPO provide insight into the brain redox status [38] since membranes within the brain are known to be rich in peroxidizable fatty acids, thus they undergo peroxidation under oxidative insult [39]. The loss of tactile stimulation as a result of the loss of whiskers in rats disrupts the brain redox status as shown by the gross impairment to the lipid peroxidation and antioxidant activities in the psychoemotional stress rats suggesting brain oxidative damaged. This is in line with other studies that had reported that loss of tactile stimulation resulted in brain damage [40-42].

Acetylcholinesterase (AChE) is the neurotransmitter enzymes that terminate the neurotransmission at cholinergic synapses by splitting the neurotransmitter acetylcholine (Ach) to choline and acetate [43]. Ach plays an important role in sending signals from neuron to the next when it is released from vesicles in the axon terminus, across the synapse, and onto receptors in the dendrites of the next neuron [44]. Acetylcholinesterase hydrolyzes acetylcholine, an essential neurotransmitter in the regulation of motor function and locomotion [45]. The brain cholinergic system modulated the cognitive functions. In this psychoemotional stress rats, significant fall in the AChE showed the cognitive functions is impaired as disruption of the cholinergic neurotransmission and abolishment of locomotive functions [29]. Metformin administration improves the locomotive function via improvement of the cholinergic neurotransmission. This is in line with previous study [29]. Loss of tactile stimulation in rats does not impair

the total protein concentration thus showing that there are no inflammatory responses in the brain cells of the Psychoemotional Stress (PES) rats.

Conclusively, the rat de-whiskered, experienced psychoemotional stress due to the lack of tactile stimulation, which drastically changed their neurobehavioral patterns. This might be because the brain cells are experiencing oxidative damage. However, the administration of metformin repaired the brain cell's oxidative damage, which lessened the impairment to neurobehavioral activities. This is most likely caused by the medication metformin's antioxidant qualities.

This study recommends that the drug metformin should be encouraged as an intervention in psychoemotional stress management and in the treatment of diabetes neuropathy and other mechanisms in which the loss of tactile stimulation causes neurobehavioural disruption.

References

1. Afarinesh MR, Behzadi G (2018) The Effects of De-Whiskering and Congenital Hypothyroidism on The Development of Nitrergic Neurons in Rat Primary Somatosensory and Motor Cortices. Cell Journal 20: 157-167.
2. Brecht M (2007) Barrel cortex and whisker-mediated behaviors. Current opinion in neurobiology 17: 408-416.
3. Aggestam F, Cahusac PM (2007) Behavioural lateralization of tactile performance intherat. Physiology and behavior 91: 335-339.
4. Bradford M (1976) A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry 72: 248-254.
5. Alonso-Bde C, Lowe AS, Dear JP, Lee KC, Williams SC, et al. (2008) Sensory inputs from whisking movements modify cortical whisker maps visualized with functional magnetic resonance imaging. CerebralCortex 18: 1314-1325.
6. Nicolelis MA, Lin RC, Chapin JK (1997) Neonatal whisker removal reduces the discrimination of tactile stimuli by thalamic ensembles in adult rats. Journal of Neurophysiology 78: 1691-1706.
7. Bernal J (2007) Thyroid hormone receptors in brain development and function. Nature Clinical Practice Endocrinology and Metabolism 3: 249-259.
8. Shishelova AY (2006) Effect of whisker removal on defensive behavior in rats during early ontogenesis. Neuroscience and Behavioral Physiology 36; 883-888.
9. Wiedenmayer CP, Barr GA (2001) Developmental changes in responsivity to threat are stimulus-specific in rats. Developmental Psychobiology 39: 1-7.
10. Caldji C, Tannenbaum B, Sharma S, Francis D, Plotsky PM, et al. (1998) Maternal care during infancy regulates the development of neural systems mediating the expression of fearfulness in the rat. Proceedings of the National Academy of Sciences of the United States of America 95: 5335-5340.
11. Carvell GE, Simons DJ (1995) Task- and subject-related differences in sensory-motor behavior during active touch. Somatosensory and motor research 12: 1-9.
12. Carvell GE, Simons DJ (1996) Abnormal tactile experience early in life disrupts active touch. The Journal of neuroscience 16: 2750-2757.
13. Brecht M, Krauss A, Muhammad S, Sinai-Esfahani L, Bellanca S, et al. (2004) Organization of rat vibrissa motor cortex and adjacent areas according to cytoarchitectonics, microstimulation, and intracellular stimulation of identified cells. Journalof Comparative Neurology 479: 360-373.
14. Wang YW, He SJ, Feng X, Cheng J, Luo YT, et al. (2017) Metformin: a review of its potential indications. Drug design, development and therap 11: 2421-2429.
15. Gai-Fen L, Ming XZ, Tong Z, Xiang C, Ming F, et al (2019) Effect of metformin on depressive behaviour in chronic stress rats. Chinese journal of physiology 35: 245-249.
16. Ibironke GF, Fasanmade AA (2016) Neuroprotective effects of kolaviron against psycho-emotional stress induced oxidative brain injury in rats: The whisker removal model. African journal of medicine and medical sciences 45: 253-260.
17. Bevins RA, Besheer J (2006) Object recognition in rats and mice: a one-trial non-matching-to-sample learning task to study 'recognition memory'. Nature protocols 1: 1306-1311.
18. Arai K, Matsuki N, Ikegaya Y, Nishiyama N (2001) Deterioration of spatial learning performances in lipopolysaccharide-treated mice. Japanese journal of pharmacology 87: 195-201.
19. Seibenhener ML, Wooten MC (2015) Use of the Open Field Maze to measure locomotor and anxiety-like behavior in mice. Journal of visualized experiments 96: 52434.
20. Misra HP, Fridovich I (1972) The Role of Superoxide Anion in the Autoxidation of Epinephrine and a Simple Assay for Superoxide Dismutase. Journal of Biological Chemistry 247: 3170-3175.
21. Claiborne AL (1995) Catalase activity. Handbook Methods For Oxygen Radical Research 283-284.
22. Adedara IA, Abolaji AO, Rocha JBT, Farombi EO (2016) Diphenyl Diselenide Protects Against Mortality, Locomotor Deficits and Oxidative Stress in Drosophilamelanogaster Model of Manganese-Induced Neurotoxicity. Neurochem Res 41**:** 1430-1438.
23. Buege JA, Aust SD (1978) Microsomal lipid peroxidation. Methods in enzymology 52: 302-310.
24. Fatemi I, Saeed-Askari P, Hakimizadeh E, Kaeidi A, Esmaeil-Moghaddam S, et al. (2020) Long-term metformin therapy improves neurobehavioral functions and antioxidative activity after cerebral ischemia/reperfusion injury in rats. Brain Research Bulletin 163: 65-71.
25. Woolsey TA, Vander LH (1970) Structural organization of layer IV in somatosensory region, SI, of mouse cerebral cortex: The description of a cortical field composed of discrete cytoarchitectonic units. Brain Res 17: 205-242.
26. Jollow DJ, Mitchell JR, Zampaglione N, Gillette JR (1974) Bromobenzene-Induced Liver Necrosis. Protective Role of Glutathione and Evidence for 3,4-Bromobenzene Oxide as the Hepatotoxic Metabolite. Pharmacology 11: 151-169.
27. Zakeri M, Fatemi I, Kaeidi A, Zakeri MA, Hakimizadeh E, et al. (2019) Pro-neurocognitive and anti-sarcopenic benefits of one-year metformin therapy in ovariectomized aged mice. Clinical and experimental pharmacology & physiology 46: 1133-1140.
28. Wahlqvist LM, Meei-Shyuan L, Chih-Cheng H, Shao-Yuan C, Jiunn-Tay L, et al. (2012) Metformin-inclusive sulfonylurea therapy reduces the risk of Parkinson's disease occurring with Type 2 diabetes in a Taiwanese population cohort. Parkinsonism & Related Disorders 18: 753-758.
29. Ebokaiwe AP, Okori S, Nwankwo JO, Ejike C, Osawe SO (2021) Selenium nanoparticles and metformin ameliorate streptozotocin-instigated brain oxidative-inflammatory stress and neurobehavioral alterations in rats. Naunyn-Schmiedeberg's archives of pharmacology 394: 591-602.
30. Carvell GE, Simons DJ (1990) Biometric analyses of vibrissal tactile discrimination in the rat. JNeurosci 10: 2638-2648.
31. Guicrobles E, Jenkins WM, Bravo H (1992) Vibrissal roughness discrimination isbarrelcortex-dependent. Behav Brain Res 48: 145-152.
32. Neimark MA, Andermann ML, Hopfield JJ, Moore CI (2003) Vibrissa resonance as a transduction mechanism for tactile encoding. J Neurosci 23: 6499-6509.
33. Boubenec Y, Shulz DE, Debrégeas G (2012) Whisker encoding of mechanical events during active tactile exploration. Front Behav Neurosci 6: 74.

34. Yan W, Kan Q, Kergrene K, Kang G, Feng XQ, et al. (2013) A truncated conical beam model for analysis of the vibration of rat whiskers. J Biomech 46: 1987-1995.

35. Quist WB, Vlad S, Lucie AH, Todd DM, Mitra JZH (2014) Modeling Forces and Moments at the Base of a Rat Vibrissa during Noncontact Whisking and Whisking against an Object. Journal of Neuroscience 34: 9828-9844.

36. Ellman GL, Courtney KD, Andres V, Featherstone RM (1961) A new and rapid colorimetric determination of acetylcholinesterase activity. Biochemical Pharmacology 7: 88-95.

37. Newsholme P, Cruzat VF, Keane KN, Carlessi R, de Bittencourt PI, (2016) Molecular mechanisms of ROS production and oxidative stress in diabetes. The Biochemical journal 473: 4527-4550.

38. Valko M, Jomova K, Rhodes CJ, Kuča K, Musílek K (2016) Redox- and non-redox-metal-induced formation of free radicals and their role in human disease. Archives of toxicology 90: 1-37.

39. Shichiri M (2014) The role of lipid peroxidation in neurological disorders. Journal of Clinical Biochemistry and Nutrition 54: 151-160.

40. Crapo JD, McCord JM, Fridovich I (1978) [41] Preparation and assay of superioxide dismutases. Methods in enzymology 53: 382-393.

41. Doyle S, Bennett S, Fasoli SE, McKenna KT (2010) Interventions for sensory impairment in the upper limb after stroke. The Cochrane database of systematic reviews 6: 6331.

42. Mc Donagh B, Scullion SM, Vasilaki A, Pollock N, McArdle A, et al. (2016) Ageing-induced changes in the redox status of peripheral motor nerve supply, an effect on redox signalling rather than oxidative damage. Free radical biology and medicine 94: 27-35.

43. Tripathi A, Srivastava UC (2008) Acetylcholinesterase: A versatile enzyme of nervous system. Ann Neurosci 15: 106-110.

44. Habila N, Inuwa H, Aimola I, Lasisi O, Chechet D, et al. (2012) Correlation of acetylcholinesterase activity in the brain and blood of wistar rats acutely infected with Trypanosoma congolense. Journal of Acute Disease 1: 26-30.

45. Walls R, Kumar KS, Hoechstein P (1976) Ageing of human erythrocytes. Archives of Biochemistry and Biophysics 100: 114-128.

HERALD

Advances In Industrial Biotechnology | ISSN: 2639-5665

Advances In Microbiology Research | ISSN: 2689-694X

Archives Of Surgery And Surgical Education | ISSN: 2689-3126

Archives Of Urology

Archives Of Zoological Studies | ISSN: 2640-7779

Current Trends Medical And Biological Engineering

International Journal Of Case Reports And Therapeutic Studies | ISSN: 2689-310X

Journal Of Addiction & Addictive Disorders | ISSN: 2578-7276

Journal Of Agronomy & Agricultural Science | ISSN: 2689-8292

Journal Of AIDS Clinical Research & STDs | ISSN: 2572-7370

Journal Of Alcoholism Drug Abuse & Substance Dependence | ISSN: 2572-9594

Journal Of Allergy Disorders & Therapy | ISSN: 2470-749X

Journal Of Alternative Complementary & Integrative Medicine | ISSN: 2470-7562

Journal Of Alzheimers & Neurodegenerative Diseases | ISSN: 2572-9608

Journal Of Anesthesia & Clinical Care | ISSN: 2378-8879

Journal Of Angiology & Vascular Surgery | ISSN: 2572-7397

Journal Of Animal Research & Veterinary Science | ISSN: 2639-3751

Journal Of Aquaculture & Fisheries | ISSN: 2576-5523

Journal Of Atmospheric & Earth Sciences | ISSN: 2689-8780

Journal Of Biotech Research & Biochemistry

Journal Of Brain & Neuroscience Research

Journal Of Cancer Biology & Treatment | ISSN: 2470-7546

Journal Of Cardiology Study & Research | ISSN: 2640-768X

Journal Of Cell Biology & Cell Metabolism | ISSN: 2381-1943

Journal Of Clinical Dermatology & Therapy | ISSN: 2378-8771

Journal Of Clinical Immunology & Immunotherapy | ISSN: 2378-8844

Journal Of Clinical Studies & Medical Case Reports | ISSN: 2378-8801

Journal Of Community Medicine & Public Health Care | ISSN: 2381-1978

Journal Of Cytology & Tissue Biology | ISSN: 2378-9107

Journal Of Dairy Research & Technology | ISSN: 2688-9315

Journal Of Dentistry Oral Health & Cosmesis | ISSN: 2473-6783

Journal Of Diabetes & Metabolic Disorders | ISSN: 2381-201X

Journal Of Emergency Medicine Trauma & Surgical Care | ISSN: 2378-8798

Journal Of Environmental Science Current Research | ISSN: 2643-5020

Journal Of Food Science & Nutrition | ISSN: 2470-1076

Journal Of Forensic Legal & Investigative Sciences | ISSN: 2473-733X

Journal Of Gastroenterology & Hepatology Research | ISSN: 2574-2566

Journal Of Genetics & Genomic Sciences | ISSN: 2574-2485

Journal Of Gerontology & Geriatric Medicine | ISSN: 2381-8662

Journal Of Hematology Blood Transfusion & Disorders | ISSN: 2572-2999

Journal Of Hospice & Palliative Medical Care

Journal Of Human Endocrinology | ISSN: 2572-9640

Journal Of Infectious & Non Infectious Diseases | ISSN: 2381-8654

Journal Of Internal Medicine & Primary Healthcare | ISSN: 2574-2493

Journal Of Light & Laser Current Trends

Journal Of Medicine Study & Research | ISSN: 2639-5657

Journal Of Modern Chemical Sciences

Journal Of Nanotechnology Nanomedicine & Nanobiotechnology | ISSN: 2381-2044

Journal Of Neonatology & Clinical Pediatrics | ISSN: 2378-878X

Journal Of Nephrology & Renal Therapy | ISSN: 2473-7313

Journal Of Non Invasive Vascular Investigation | ISSN: 2572-7400

Journal Of Nuclear Medicine Radiology & Radiation Therapy | ISSN: 2572-7419

Journal Of Obesity & Weight Loss | ISSN: 2473-7372

Journal Of Ophthalmology & Clinical Research | ISSN: 2378-8887

Journal Of Orthopedic Research & Physiotherapy | ISSN: 2381-2052

Journal Of Otolaryngology Head & Neck Surgery | ISSN: 2573-010X

Journal Of Pathology Clinical & Medical Research

Journal Of Pharmacology Pharmaceutics & Pharmacovigilance | ISSN: 2639-5649

Journal Of Physical Medicine Rehabilitation & Disabilities | ISSN: 2381-8670

Journal Of Plant Science Current Research | ISSN: 2639-3743

Journal Of Practical & Professional Nursing | ISSN: 2639-5681

Journal Of Protein Research & Bioinformatics

Journal Of Psychiatry Depression & Anxiety | ISSN: 2573-0150

Journal Of Pulmonary Medicine & Respiratory Research | ISSN: 2573-0177

Journal Of Reproductive Medicine Gynaecology & Obstetrics | ISSN: 2574-2574

Journal Of Stem Cells Research Development & Therapy | ISSN: 2381-2060

Journal Of Surgery Current Trends & Innovations | ISSN: 2578-7284

Journal Of Toxicology Current Research | ISSN: 2639-3735

Journal Of Translational Science And Research

Journal Of Vaccines Research & Vaccination | ISSN: 2573-0193

Journal Of Virology & Antivirals

Sports Medicine And Injury Care Journal | ISSN: 2689-8829

Trends In Anatomy & Physiology | ISSN: 2640-7752