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The effect of metformin on the motor activity and hippocampal dopamine metabolism in rats subjected to repeated immobilization stress

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Abstract: The present study investigates the impact of metformin treatment on motor activity and hippocampal dopamine metabolism in rats subjected to recurring immobilization stress. The experiment was conducted on male Albino Wistar rats, which have been exposed to repeated immobilization stress (2hrs; for 6 days) and administered with metformin on the dose of 50mg/kg. Motor activities were assessed in open field, dopamine and its metabolites were estimated in the hippocampal samples by high performance liquid chromatography with electrochemical detection (HPLC-EC). Biochemical parameters were monitored in serum (serum leptin, serum tryptophan, serum glucose, serum corticosterone, serum cholesterol) and brain samples (hippocampal tryptophan). The results revealed that metformin management mitigated the deleterious consequences of repeated immobilization stress on motor activities of the rats. Furthermore, metformin treatment was associated with alterations in dopamine metabolites in the hippocampus, suggesting possible modulatory role of dopaminergic neurotransmission in response to immobilization stress. These findings contribute to the knowledge of neurobiological mechanisms underlying the therapeutic effects of metformin in stress-associated situations, underscoring its capability as a pharmacological intervention for stress-triggered alterations in motor function and dopaminergic neurotransmission.

Keywords: Metformin; immobilization stress; dopamine; dopamine metabolites; hippocampus

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INTRODUCTION

Stress is a complex physiological and mental reaction to outer/ inner challenges. It leads to several behavioral and psychological manifestations including cognitive impairment, anxiety and depression (Shin *et al*., 2024). The brain has a central role in stress and adaptation. It initiates a cascade of neuroendocrine and neurotransmitter responses when an individual is exposed to stressful stimuli targeting adaptation to stress (McEwen & Gianaros, 2010). However, exposure to chronic stress/ stressors could result in adverse effects on brain anatomy and physiology originating from the dysregulation of immune function, metabolism and metabolic functions as well as key brain circuits (Roberts & Karatsoreos, 2021). Alterations in the brain regions involved in regulation of behavior, cognition and emotion such as amygdala, prefrontal cortex and hippocampus has been associated with the repeated/ chronic exposure to stress (Vaidya *et al*., 2024) resulting in increased susceptibility to psychiatric illnesses such as depression and anxiety, impaired learning and memory and mood disorders.

Apart from reward effects manifestations, dopamine is also involved in mediating the body's response to stress. Early life events have substantial impact on neurobiological stress systems and mesolimbic dopamine release in humans is reported to be associated with such stressful events (Pruessner *et al*., 2024). The fluctuations of dopamine levels in various brain regions during stress affect cognitive function, emotional regulation, and motivation (Zhou *et al*., 2024; Badgaiyan *et al*., 2024; Grogan *et al*., 2024). Hypothalamic-pituitary-adrenal (HPA) axis, which enables body to respond to stress, intricately releases dopamine. Adaptive responses to stress involve motor function, attention, and decision-making which is achieved by the modulation of dopaminergic neurotransmission via brain circuits in response to stress (Xu *et al*., 2024). Stress-related psychiatric disorders such as depression and addiction involve aberrant dopamine signaling via the dysregulation of the mesolimbic dopaminergic system (Kukucka *et al*., 2024).

Metformin, a widely prescribed antidiabetic medicine, has gained interest for its marked effects on stress. Emerging evidences show that metformin may also exert neuroprotective effects and modulate the brain's response to stress due to its antioxidant attributes (Buczyńska *et al*., 2024). Studies in animal models have shown that

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metformin administration can mitigate the adverse results of stress on various brain regions, including the hippocampus, through regulating neuroinflammation, oxidative stress, and synaptic plasticity (Li *et al*., 2022). Neuroprotective effects of metformin could also be attributed to its capability to enhance insulin sensitivity and regulate energy metabolism.

The purpose for investigating metformin in the context of stress-brought about dopamine alterations stems from its multifaceted pharmacological profile and its capability to modulate neurobiological pathways implicated in stress response (Isop *et al*., 2023). Dopamine is crucial for adaptation to stress management and modulation of dopaminergic neurotransmission could possibly be involved in metformin-induced neuroprotective effects (Alrouji *et al*., 2024). Given its potential to mitigate stress-induced neuroinflammation, oxidative stress, and synaptic plasticity, metformin represents a promising candidate for ameliorating stress-induced alterations in dopamine neurotransmission. Findings from the present study would help understand the neuroprotective effects of metformin in the context of stress. This would increase our understanding of its pharmacological profile and result in the improvement of focused interventions for stress-associated brain dysfunctions.

MATERIALS AND METHODS

Animals

Experimental design was carried out in strict accordance with the guidelines by the Institutional Animal Ethics Committee (IAEC). Male Albino-Wistar rats (weighing 200 ± 20 grams) provided by the HEJ Research Institute of Chemistry, University of Karachi were housed individually in perspex cages at room temperature (25 ± 2°C) under a 12:12 h light/ dark cycle (lights on at 6:00 hr) with a three day acquisition phase. The animal study protocol (ASP# 2018-0006) was approved by the institutional committee for animal care and use and conducted according to the guidelines of the National Institute of Health (NIH) for the care and use of laboratory animals.

Drug and doses

Metformin-HCl (Sigma, St. Louis, USA) dissolved in saline (0.9% NaCl) was freshly prepared before starting the experiment and injected intra peritoneally at 50mg/kg dose to the respective animals. Saline (0.9% NaCl solution; 1ml/kg) was injected to control animals. This dosage was selected based on the findings from a previous study conducted in our lab (Afroz *et al*., 2024).

Experimental protocol

Thirty-two male Albino Wistar rats were randomly divided into four e\qual treatment groups, each containing 8 rats: (i) saline-unstressed, (ii) saline-stressed (iii) metformin-unstressed, (ii) metformin-stressed treated rats. Metformin-treated groups were given metformin intraperitoneally at a dose of 50 mg/kg daily for 6 days. After 1 hour of drug administration, animals of the stressed group were immobilized on wire grids for two hours (11:40-1:40) daily for 6 days. Unstressed animals remained in their home cages throughout the experiment. A behavioral test for the assessment of motor activity was performed using an open field on days 2 and 6, to determine the effect of metformin treatment after single and repeated immobilization stress. On day 6 of the experiment, after monitoring behavior metformin and vehicle were administered and after 1 hour of drug or vehicle administration, animals were immobilized for two hours. Animals were decapitated immediately after the termination of stress and the brain was isolated from the skull and micro-dissected to collect the hippocampus samples for the analysis of tryptophan and dopamine and its metabolites. Blood samples were also collected to isolate the serum for the analysis of tryptophan, glucose, cholesterol, corticosterone, and leptin. Drug administration and open field activity were carried out in a balanced design to evade the effects of environmental changes.

Immobilization stress

The animals were immobilized as described before (Haleem and Ikram, 2013). Wire grids of 10″ × 9″ fitted with a Perspex plate of 9″ × 6.5″ were used. Zinc oxide plaster tape was used to tape the fore- and hindpaws pressed through the gaps in the metal grid, for 2 hrs.

Exploratory activity in a novel environment

A square area described earlier (Ikram *et al*., 2023) was used to monitor activity. Values of squares crossed with all four paws were recorded and reported for a period of 5min.

Decapitation of rat brain

Dissection procedure was essentially same as described earlier (Ikram *et al*., 2012; Ikram *et al*., 2024). After decapitation, a fresh brain was dipped in ice-cold saline and placed with its ventral side up in the molded cavity of the brain slicer. A fine razor/blade was inserted between the respective slots to give brain slices containing the hippocampus. The collected brain regions were stored at -70°C until analysis by HPLC-EC (High-Performance Liquid Chromatography with Electrochemical detector).

HPLC-EC estimation of dopamine and metabolites

Dopamine, DOPAC and HVA levels in hippocampus were analyzed in "Waters Alliance e2695 High-performance Liquid Chromatography (HPLC) system" with a "Waters 2465 electrochemical (EC) detector" (Salman *et al*., 2021). Hippocampus samples were subjected to homogenization in five volumes of the extraction medium (composed of 0.4 M perchloric acid, 0.1% sodium metabisulphite, 0.01% Ethylenediaminetetraacetic acid/EDTA, 0.01% cysteine). This homogenate was centrifuged for 15 minutes in "Heraeus Megafuge 8R" at 12000 rpm speed and at 4°C.

Table 1: Values of F as analyzed by two-way ANOVA (*p>0.05; **p<0.05; ***p<0.01).

1. Open field activity			
		Single stress	Repeated stress
Metformin		F= 21.32***	F= 50.22***
Stress		F= 167.31***	F= 32.39***
Metformin x stress		F= 1.52*	F= 22.68***
2. Tryptophan levels			
		Serum	Hippocampal
Metformin		F= 0.861*	F= 0.398*
Stress		F= 8.891***	F= 1.002*
Metformin x stress		F= 1.776*	F= 0.200*
3. Dopamine and metabolites in hippocampus			
	Dopamine	DOPAC	HVA
Metformin	F= 0.21*	F= 5.72***	F= 14.49***
Stress	F= 0.85*	F= 15.15***	F= 15.49***
Metformin x stress	F= 0.08*	F= 0.018*	F= 82.41***
4. Biochemical parameters			
	Corticosterone	Leptin	Cholesterol
Metformin	F= 49.97***	F= 8.66***	F= 0.839*
Stress	F= 13.11***	F= 5.89**	F= 14.0***
Metformin x stress	F= 121.94***	F= 19.17***	F= 0.66*

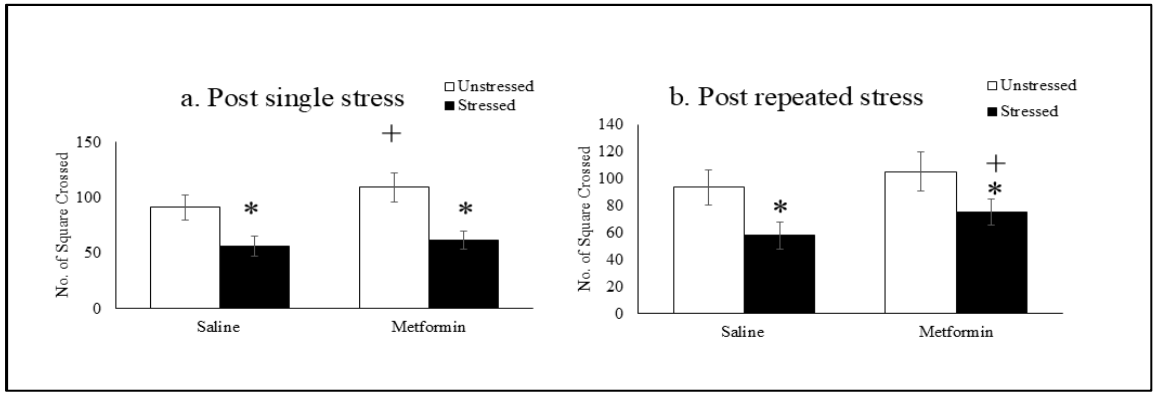

Fig. 1: Effects of post single and repeated (2 hours per day for 5 days) immobilization stress effects on motor activity in the open field in animals treated with/without metformin. Values (n=8) are presented as means ± SD. Significant differences: *p<0.01 from values of respective unstressed rats, +p<0.01 from values of the metformin treated animal group by Tukey's test following two-way ANOVA.

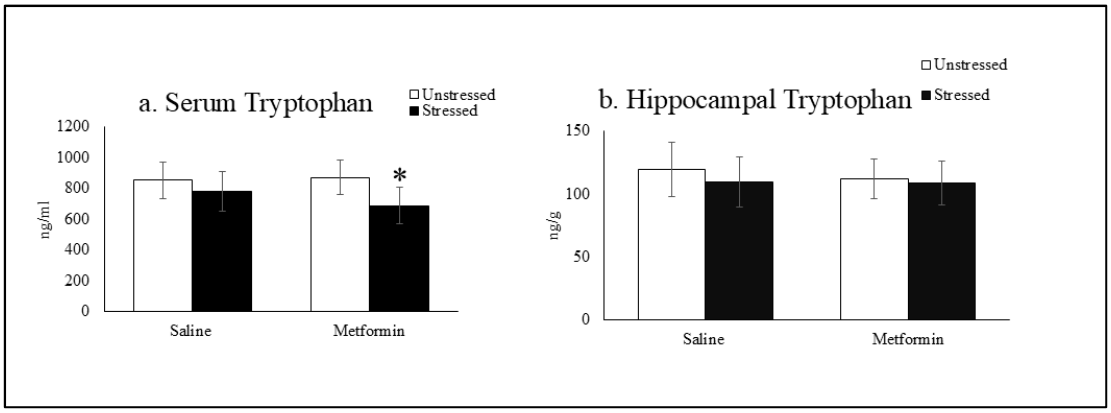

Fig. 2: Effects of repeated (2 hours per day for 6 days) immobilization stress on concentration of tryptophan in the serum and hippocampus. Values (n=8) are presented as means ±SD. Significant differences: *p<0.05 from values of respective unstressed rats by Tukey's test following a two-way ANOVA.

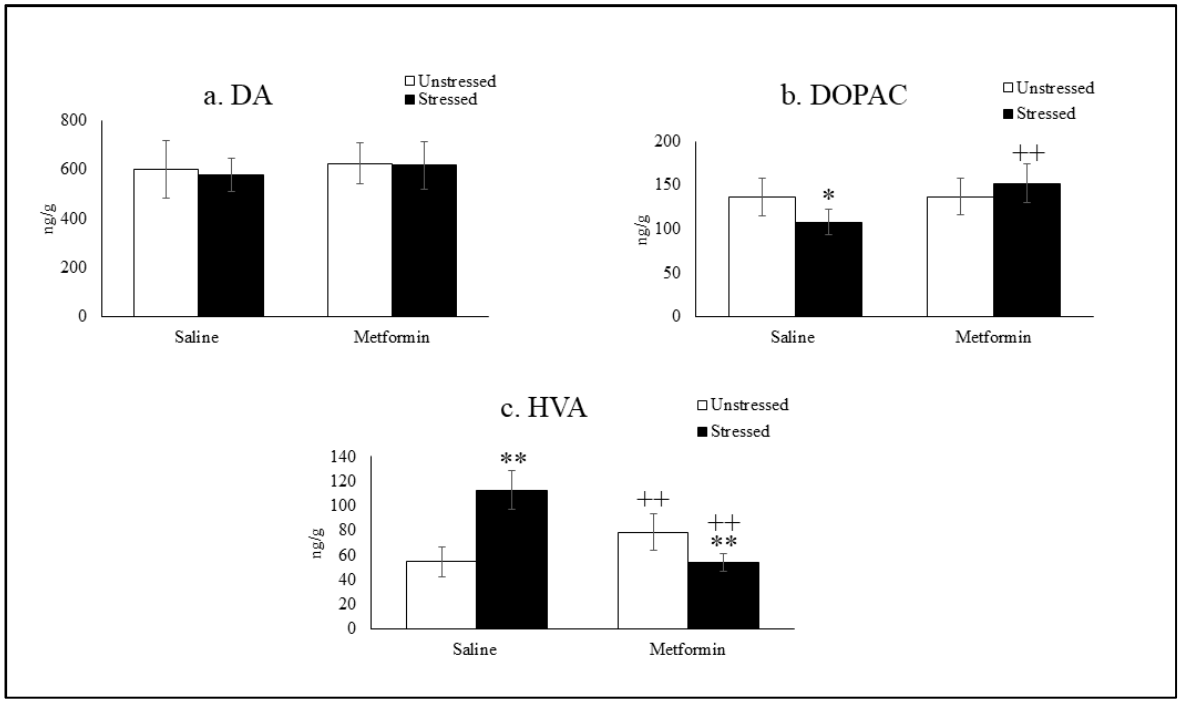

Fig. 3: Effects of repeated (2 hours per day for 6 days) immobilization stress effects on levels of dopamine and its metabolites in the hippocampus. Values (n=8) are presented as means ± SD. Significant differences: **p<0.01, *p<0.05 from values of respective unstressed rats, ++<0.01 from values of the metformin-treated animal group by Tukey's test following a two-way ANOVA.

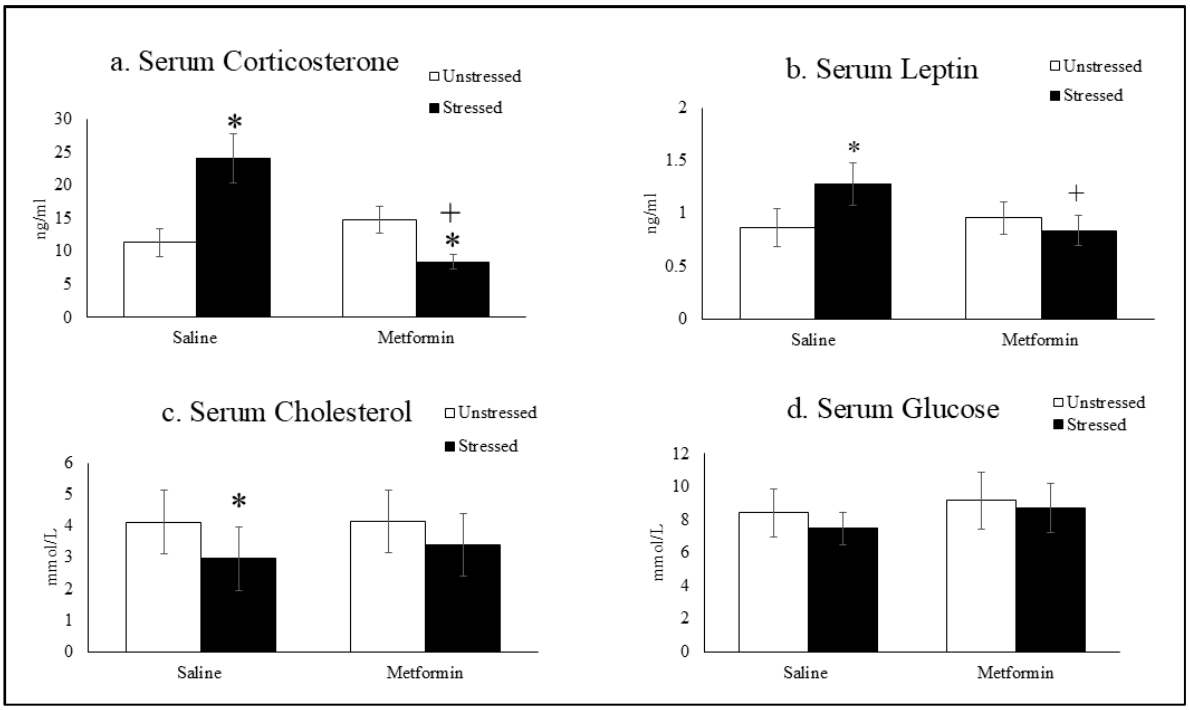

Fig. 4: Effects of repeated (2 hours per day for 6 days) immobilization stress effects on levels of biochemical parameters; serum corticosterone, leptin, cholesterol and glucose. Values (n=8) are presented as means ± SD. Significant differences: *p<0.05 from values of respective unstressed rats, +p<0.05 from values of the metformin-treated animal group by Tukey's test following a two-way ANOVA.

The collected supernatant was further centrifuged for 5 minutes at the same conditions and this supernatant was used for detection after filtration. The "Spherisorb© ODS2 (C18) analytical column of 4.6mm internal diameter, 150mm length, and 5mm particle size was used as a stationary phase, and for a mobile phase 0.1 M phosphate buffer was used containing: 10% Methanol, 0.023% Sodium Octyl Sulfate, and 0.005% EDTA with maintained pH at 2.9 and functioning pressure of 1500-2000 psi and +0.8 to +1.0 Volts potential. The detected data was recorded and retrieved using Empower™3 software.

Estimation of serum and hippocampal tryptophan levels

10% methanol was used as a mobile phase to estimate tryptophan concentration in the hippocampus and serum using the HPLC with UV detector, particularly at a wavelength of 273 nm. (Saeed *et al*., 2022). The concentration was quantified by comparing its retention time and area under the peak with the standard using the Empower™3 software.

Estimation of serum glucose and cholesterol

The estimation of glucose and cholesterol was performed with commercially available kit using calorimetric method as reported earlier from our lab (Gohar *et al*., 2020)

Hormonal levels estimation

To analyze corticosterone concentrations in serum, Rat Corticosterone ELISA Kit by Glory Science Co., Ltd. (catalog #. 30590) was used and leptin in the serum samples, commercially available (EMD Millipore Corporation, Billerica, MA 01821, USA) kits were used and the protocol was performed as per the manufacturer's instruction (Haleem and Mahmood, 2021).

Ethical approval

The animal study protocol was approved by Institutional Animal Ethical Committee (IAEC; animal study protocol no. 2018-006) for animal care and use at the animal resource facility, ICCBS, University of Karachi.

STATISTICAL ANALYSIS

All data are presented as mean values ± S.D. The group variance was analyzed by ANOVA using SPSS software version 21. The data was analyzed using two-way ANOVA. Post hoc analyses were performed by Tukey's test. Values of $p<0.05$ were considered significant.

RESULTS

Fig 1. shows the effects of post single and repeated (2 hours per day for 5 days) immobilization stress effects on motor activity in the open field in animals treated with/without metformin (values of F mentioned in table 1). Post hoc analysis showed that single and repeated immobilization-stress exposure significantly reduced open field activity of saline-treated animals. Single and repeated immobilization-stress also significantly decreased the open field activity of metformin-treated animals. Metformin-treated animals showed a significant increase in open field activity as compared to the saline-treated animals monitored after repeated immobilization stress (fig. 1).

Fig 2. shows the effects of repeated (2 hours per day for 6 days) immobilization stress on concentration of tryptophan in the serum and hippocampus (values of F mentioned in Table 1). Post-hoc analysis showed that immobilization significantly decreased tryptophan levels in serum of animals injected with metformin.

Fig 3. shows the effects of repeated (2 hours per day for 6 days) immobilization stress effects on levels of dopamine and its metabolites in the hippocampus (values of F mentioned in table 1). ANOVA two analysis of immobilization-stress on hippocampal DA levels of animals injected with/without metformin revealed no significant effects, suggesting no changes occurred in levels of dopamine in the hippocampus of animals injected with saline and metformin after repeated immobilization-stress (fig. 3a). The post hoc test revealed immobilization significantly decreased DOPAC levels in saline-injected animals. DOPAC levels of animals treated with metformin and exposed to repeated immobilization-stress were significantly increase as compared to saline-treated stressed animals. The levels of DOPAC in metformin and saline injected unstressed animals were comparable (fig. 3b). Post hoc analysis showed that immobilization significantly increased in HVA levels of animals injected with saline, but these levels were significantly lower of animals treated with metformin and exposed to immobilization stress. These levels were also comparable to levels of HVA of saline-injected animals. Metformin treatment also showed increase level of HVA as compared to saline-injected animals (fig. 3c).

Fig 4. shows the effects of repeated (2 hours per day for 6 days) immobilization stress effects on levels of biochemical parameters; serum corticosterone, leptin, cholesterol and glucose. (values of F mentioned in Table 1). Post-hoc test showed repeated immobilization-stress exposure increased serum corticosterone level in saline treated animals. Serum corticosterone levels were comparable in metformin-treated unstressed animals. Corticosterone levels were significantly decreased in metformin treated stress animals as compare to saline treated stress animals and metformin injected unstress animals. The findings suggest attenuation of stress-induced increased of corticosterone levels by metformin treatment (fig. 4a). Post hoc test revealed that repeatedly exposure of immobilization-stress significantly caused leptin serum levels elevation in saline-treated animals in comparison to saline-treated unstressed animals and which were comparable in metformin-treated unstressed animals. The concentration of leptin was significantly decreased in serum samples of metformin-treated stress animals as

compared to saline-treated stress animals and metformin-injected unstressed animals. The results suggest that metformin treatment ameliorates stress-induced changes in serum leptin concentration (fig. 4b). Post hoc test analysis showed that repeated immobilization stress decreased cholesterol level in the serum of saline-treated animals (fig. 4c). The data on serum glucose levels analyzed by two way ANOVA showed effects of metformin ($F=3.850$) and metformin x stress interaction ($F=0.242$) and effects of immobilization ($F=2.020$) were not significant ($p>0.05$).

DISCUSSION

The decreased locomotor activity of stressed rats shows a potentially decreased ameliorative effect of the drug due to stress-induced behavioral alterations. The open field activity was decreased in rats following first episode of repeated exposure to immobilization stress which was attenuated following repeated exposure to immobilization stress (fig. 1). However, in metformin-treated stressed rats, the open field activity was greater than saline stressed rats, suggesting potential mechanisms underlying the increased activity monitored in open field. This could involve exploring changes in neurotransmitter levels, such as dopamine, serotonin, or their metabolites, in brain regions such as hippocampus.

Others have reported that the medial prefrontal cortex (mPFC) of the mice subjected to social defeat stress is prevented in terms of any transcriptional changes following metformin treatment. They further reported that metformin increases the BDNF promoter along with histone acetylation thereby upregulating the expression of the brain-derived neurotrophic factor (BDNF). This was further attributed to the activation of cAMP-response element binding protein (CREB) and AMP-activated protein kinase (Fang *et al*., 2020). It has also been reported that metformin not only regulates the balance of excitation and inhibition (E/I balance) in neural networks but also regulates the synaptic transmission or plasticity in pathological conditions. However, much is not known about the exact mechanism underlying it's neuroprotective profile and the neurotransmitters involved and needs further investigation (Li *et al*., 2022). An important role of dopamine could be there as dopamine performs a critical function in regulating motor functions, and adjustments in its levels have been related to stress-related conditions (Curtin *et al*., 2024; Leow *et al*., 2024). By restoring dopamine levels or improving its neurotransmission, metformin also can mitigate the stress-induced behavioral deficits on motor activity.

In the present study, levels of serum tryptophan in stressed-metformin treated rats were decreased (fig. 2) which could be due to its increased utilization for serotonin synthesis. Tryptophan, a precursor to serotonin, performs a critical function in regulating mood and stress reaction pathways (Correia & Vale, 2022). Metformin's modulation of tryptophan levels may additionally characterize its capability to steer serotonin synthesis and availability, doubtlessly contributing to its reported antidepressant-like effects (Ochi *et al*., 2024). In stressed rats treated with metformin, changes in serum and hippocampal tryptophan levels ought to reflect adaptations in serotoninergic neurotransmission, which can also underlie improvements in mood-related behaviors observed in preclinical research. Further elucidating the mechanisms by means of which metformin influences tryptophan metabolism should provide valuable insights into its healing ability for stress-related issues and mood dysregulation.

In stressed rats, the administration of metformin is anticipated to exert multifaceted effects on various metabolic parameters, including serum glucose, corticosterone, cholesterol, and leptin levels (fig. 4a). Firstly, metformin's well-established antihyperglycemic properties may lead to a reduction in serum glucose levels among stressed individuals. By enhancing insulin sensitivity and glucose uptake in peripheral tissues, metformin could counteract stress-induced hyperglycemia, thereby promoting glucose homeostasis (Buczyńska *et al*., 2024; Xie *et al*., 2024).

Concurrently, metformin can also modulate the hypothalamic-pituitary-adrenal (HPA) axis, ensuing in alterations in serum corticosterone levels. In the present study, levels of corticosterone were elevated in stressed rats but were attenuated in stressed metformin treated rats (fig. 4b), suggesting metformin induced modulation of corticosterone levels (Gasser *et al*., 2023). The HPA axis serves as a primary neuroendocrine machine worried in the physiological response to pressure, orchestrating the secretion of corticotropin-freeing hormone (CRH) from the hypothalamus, adrenocorticotropic hormone (ACTH) from the pituitary gland, and glucocorticoids, frequently cortisol in humans and corticosterone in rodents, from the adrenal cortex. Metformin's capacity to modulate HPA axis function could contain several mechanisms, inclusive of attenuation of hypothalamic CRH expression, inhibition of pituitary ACTH secretion, and changes in glucocorticoid receptor sensitivity (Sun *et al*., 2024). By dampening the hyperactivity of the HPA axis, metformin may additionally mitigate stress-induced corticosterone launch, thereby exerting beneficial results on stress-associated physiological responses and potentially ameliorating the deleterious results of chronic stress on physical and mental health.

In the present study, levels of serum cholesterol were decreased in stressed rats but attenuated in metformin cotreated rats (fig. 4c). Metformin's lipid-decreasing results could facilitate the modulation of serum cholesterol levels in harassed rats. By inhibiting hepatic gluconeogenesis and improving peripheral glucose usage, metformin may additionally indirectly have an impact on cholesterol metabolism, leading to reduced serum LDL cholesterol concentrations (Xiao *et al*., 2022). In stressed

rats, metformin treatment may exert beneficial consequences on LDL cholesterol metabolism by influencing key regulatory pathways involved in lipid homeostasis (Chou *et al*., 2024). One possible mechanism via which metformin might also impact serum levels of cholesterol is through activation of ATP-activated protein kinase (AMPK), a cell strength sensor implicated in lipid metabolism. Activation of AMPK via metformin ends in phosphorylation and inactivation of key enzymes concerned in LDL cholesterol biosynthesis, along with three - hydroxyl - three - methyl - glutaryl - coenzyme A reductase (HMGCR), the rate-proscribing enzyme inside the mevalonate pathway (Xing *et al*., 2022). Consequently, decreased HMGCR activity results in reduced LDL cholesterol synthesis and circulating levels of cholesterol. Additionally, metformin may also enhance LDL cholesterol clearance from circulate by means of upregulating the expression and activity of hepatic low-density lipoprotein receptors (LDLRs), facilitating the uptake of cholesterol-rich LDL debris from circulation and promoting their degradation within hepatocytes.

Additionally, metformin's impact on serum leptin levels (fig. 4d) shows that it attenuated stress induced increase in serum leptin levels. This may offer insights into metformin’s role in energy homeostasis and appetite regulation. As a hormone predominantly secreted by adipocytes, leptin plays a pivotal role in signaling satiety and regulating energy expenditure (Picó *et al*., 2022). Metformin's effects on leptin levels could reflect its broader metabolic actions, potentially influencing food intake, energy balance, and body weight regulation in stressed animals. By restoring leptin homeostasis, metformin could mitigate stress-induced alterations in appetite regulation, energy expenditure, and adipose tissue metabolism (Casado *et al*., 2023). Moreover, the modulation of leptin levels by metformin may contribute to its overall metabolic benefits, including improvements in insulin sensitivity, glucose metabolism, and lipid profiles.

Hippocampal dopamine (fig. 3a) levels were found to be unaffected in stressed rats. Levels of DOPAC (fig. 3b) were decreased in stressed rats, which were attenuated in metformin cotreated rats. Metformin decreased HVA (fig. 3c) levels in unstressed rats which were potentiated in stressed rats. Levels of HVA increased in stressed rats which were attenuated in metformin cotreated rats. The decreased HVA levels represent increased utilization of dopamine / binding to receptors and produced its function rather than being available in synapse for degradation. Research has shown that metformin administration in stressed rats can influence synaptic neurotransmission and metabolism of dopamine in hippocampus. Metformin's ability to regulate dopamine metabolism in the hippocampus may contribute to its reported effects on stress resilience and cognitive function. By restoring dopamine homeostasis, metformin could mitigate the detrimental effects of stress on hippocampal structure and function, potentially preserving cognitive performance under stress conditions (Aderinto *et al*., 2024). Moreover, alterations in hippocampal dopamine metabolism may mediate metformin's effects on neuroplasticity and synaptic transmission (Ahmadi *et al*., 2024), which are critical for adaptive responses to stress. This mechanism could also explain metformin's potential role in reducing stress-related cognitive decline and enhancing memory formation. Additionally, further research may uncover how these dopaminergic effects interact with other neurochemical systems to promote overall brain health.

Understanding the mechanisms underlying metformin's modulation of hippocampal dopamine metabolism in stressed rats is vital for elucidating its therapeutic potential in stress-related psychiatric disorders. Further investigations into the molecular pathways concerned in metformin's movements on hippocampal dopamine neurotransmission may also find novel goals for pharmacological intervention. Ultimately, elucidating the outcomes of metformin on hippocampal dopamine metabolism in stressed rats holds promise for developing focused healing strategies geared toward mitigating the neurobiological outcomes of chronic stress and enhancing mental health outcomes.

CONCLUSION

In conclusion, this study sheds light on the capability therapeutic options of metformin in mitigating the adverse results of repeated immobilization stress on neurobehavioral and neurochemical parameters. Through comprehensive experimental investigations, we established that metformin treatment exerted beneficial consequences on motor activity and hippocampal dopamine metabolism in rats exposed to repeated immobilization stress. Our findings found that metformin management ameliorated the stress-induced decline in a motor hobby, as evidenced via extended locomotor pastime within the open area take a look at as compared to saline-treated harassed rats. Moreover, metformin treatment mitigated changes in hippocampal dopamine metabolism brought about by means of repeated immobilization stress, suggesting its capacity neuroprotective outcomes against stress-precipitated neurochemical dysregulation. Findings may help in extending therapeutics for stress, depression, anxiety, panic attack and related disorders.

Conflict of interest

There is no conflict of interest.

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