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Original Article

Exploring the antidepressant potential of raspberry ketone: Behavioural and neurochemical insights in a chronic unpredictable mild stress-induced depression model

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ABSTRACT

Objectives: This research investigates the potential antidepressant properties of raspberry ketone (RK) using a chronic unpredictable mild stress-induced depression model in mice.

Materials and Methods: Through a comprehensive examination encompassing behavioural and neurochemical assessments, the study reveals promising outcomes. RK administration results in significant enhancements in locomotor activity, reductions in immobility time and improvements in sucrose preference, indicative of antidepressant-like effects.

Results: RK treatment leads to elevated levels of dopamine and serotonin, coupled with a decrease in inflammatory cytokines, suggesting a multifaceted mechanism underlying its therapeutic potential.

Conclusion: These findings underscore the promise of RK as a novel antidepressant agent, with implications for developing alternative and potentially more tolerable treatments for depression. Further, exploration into its mechanisms and clinical applicability is warranted.

Keywords: Raspberry ketone, Depression, Chronic unpredictable mild stress, Neuroinflammation, Dopamine, Serotonin

INTRODUCTION

Major depressive disorder is an extreme mental issue, usually considered the most common type of depression. This condition is marked by enduring feelings of sadness and diminished interest or enjoyment in activities that were once pleasurable or rewarding.[1] According to the World Health Organization, depression is a prevalent mental illness affecting approximately 5% of adults worldwide. It is defined by enduring feelings of sadness and a reduced interest or pleasure in activities that were once enjoyable or fulfilling. The traditional monoamine hypothesis proposes that depression stems from a reduction in either the quantity or effectiveness of serotonin (5-HT), norepinephrine and dopamine (DA) in the cortical and limbic regions of the brain.[2] However, some recent studies have shown that the monoamine hypothesis is not solely responsible for depression but different factors such as hypothalamic-pituitary-adrenal (HPA) axis dysfunction, decreased levels of brain-derived neurotrophic factor in cerebrospinal

fluid, increase in oxidative stress due to neuroinflammation, glutamatergic excitotoxicity and increased cytokine production in the hippocampus can collectively cause the depression. Data from some recent findings suggest that the role of the production of cytokines (interleukin [IL]-17A) through the T-helper 17 (Th-17) cell pathway may have a link with depression.[3]

This pathway is based on the neuroinflammatory hypothesis of depression, which says that an increase in levels of cytokines may lead to trigger different pathways that collectively cause depression. Th-17 cells when entering the hippocampus region, and there is an increase in the expression of retinoic-acid-receptor-related orphan nuclear receptor gamma (RORγt) due to increased signal transducer and activator of transcription 3 (STAT3), leading to the production of IL-17A and other inflammatory cytokines.[4,5] It has been discovered that peroxisome proliferator-activated receptor-alpha (PPARα) and PPAR gamma inhibit the RORγt expression and STAT3, respectively, and along with it increase Foxp3 levels which suppress the proinflammatory cytokines.[6,7] Hence, we hypothesise that using PPARα agonist – raspberry ketone [4-(4-Hydroxyphenyl)-2-butanone], we can inhibit the production of inflammatory cytokines which may inhibit the subsequent activation of neurotransmitter depletion pathway, neuroendocrine pathway and neuroplasticity pathway.[8,9] The flow is depicted in Figure 1.

MATERIALS AND METHODS

Animals

Male Swiss Albino 8-week-old male mice weighing 20–28 g were procured from the National Institute of Bioscience, Pune, Maharashtra. The animals were kept in Makrolon cages at SVKM'S Animal Facility in Mumbai, following standard conditions: A 12-h light and dark cycle with lights on at 07:00 am and a room temperature maintained between 20°C and 22°C. They were provided with ad libitum access to food and water. Before the commencement of experiments, the animals were given 1 week to acclimate to their new surroundings. The protocol was approved by the Institutional Animal Ethics Committee (IAEC) for the use of animal subjects (Approval number CPCSEA/IAEC/P-77/2022 Dt. 08 October 2022).

Drug solutions

Before administration to the animals, both RK and fluoxetine were dissolved in distilled water. Fresh solutions of the drugs were prepared right before the dosing session. Both RK and fluoxetine were given orally (p.o.) to the animals.

Animal model (chronic unpredictable mild stress [CUMS] model for depression)

Male Swiss Albino 8-week-old mice, weighing between 20 and 28 g, were obtained following approval from the IAEC. They were given 1 week to adjust to the new surroundings before the experiment commenced. Following acclimatisation, the mice were divided into seven groups, each consisting of eight mice. The mice were then placed in groups of 4 in perspex cages at SVKM's Animal Facility in Mumbai, adhering to standard conditions: a 12-h light and dark cycle with lights turning on at 07:00 am, and a room temperature maintained between 20°C and 22°C. Depression was induced using the CUMS model, where the mice were consistently exposed to various unpredictable stressors

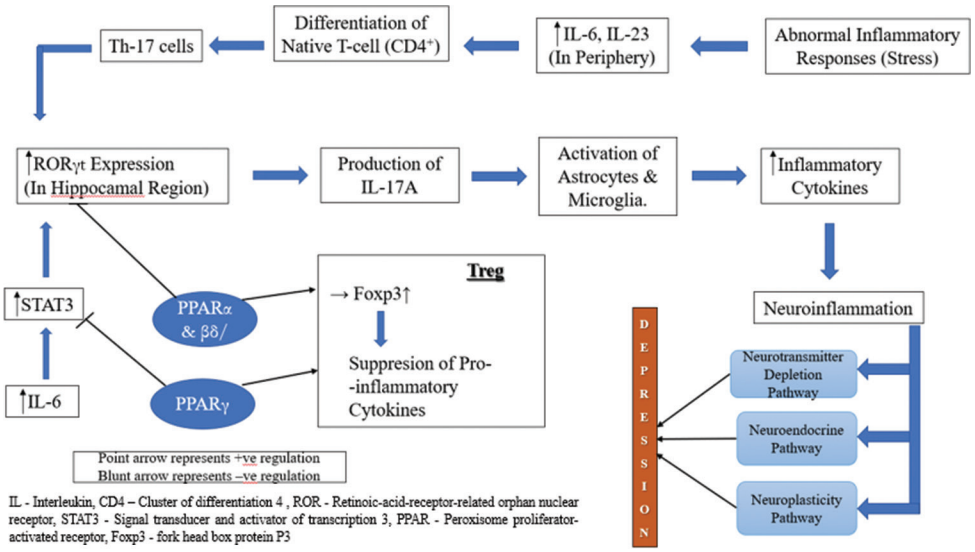

Figure 1: Flow chart of neurotransmitter depletion pathway, neuroendocrine pathway and neuroplasticity pathway. Th-17: T-helper 17 cells, Treg: Regulatory T cells.

over time. Throughout the experiment, the animals in the control (non-stressed) group remained undisturbed in their cages, except for routine care activities like cage cleaning. Conversely, each mouse in the CUMS-exposed groups was housed individually and exposed to various stressors once daily for 6 consecutive weeks. To prevent habituation and maintain unpredictability, the stressors were randomly scheduled each week for 6 weeks. Simultaneously treatment was given in the past 3 weeks (21 days). Then, mice were subjected to various evaluation parameters after the 6th week, which is a total of 6 weeks of stressors and 3 weeks of treatment. The protocol of chronic mild stress[10] and grouping of animals used in studies are shown in Figure 2 and Table 1 respectively. The grouping of animals used in the study is focused in Table 2.

Behavioural assessment

Sucrose preference test (SPT)

The SPT is a valuable tool in studying depression in mice. In this test, mice are given a choice between water and a sucrose solution, with a reduced preference for the sweetened solution, indicating anhedonia, a symptom associated with depression. The significance of this test lies in its ability to gauge hedonic responses, providing insights into the impact of depression on pleasure-seeking behaviours. Researchers use sucrose preference (SP) as a measurable indicator, aiding in the assessment of antidepressant effects and contributing to a comprehensive understanding of depressive-like behaviours in preclinical studies with mice. Before the assessment, each mouse was housed individually and presented with two bottles concurrently: one filled with a 1% sucrose solution (w/v) and the other with tap water. After 24 h, one of the bottles containing the sucrose solution was substituted with tap water for an additional 24 h. After this adjustment period, a 24-h period without access to water and food was started. To avoid any preference for a specific location, the distance between the mouse and the two bottles in the cage was maintained equally, and their positions were interchanged every 12 h. The SPT was carried out during the dark phase (7:00 p.m. to 9:00 p.m.), during which mice were presented with two bottles containing either a 1% sucrose solution (w/v) or tap water. The consumption of each solution was measured after 2 h of exposure. Baseline SPT measurements were taken before and at the conclusion of the 0-, 3- and 5-week CUMS procedures. SP was calculated through the sucrose intake rate by the following formula: SP = (sucrose intake [g]/(sucrose intake [g] + water intake [g]) × 100%.[11]

Actophotometer test

The actophotometer test is employed in depression studies with mice to assess their locomotor activity. This test involves monitoring the animal's movement within an enclosed space. Significantly, alterations in locomotor activity can be indicative of changes in mood and motivation, offering researchers valuable insights into depressive-like behaviours. The actophotometer test's significance lies in its ability to complement other behavioural assays, providing a comprehensive understanding of the impact of interventions on the overall activity levels of mice, thereby contributing to the broader investigation of potential treatments for depression. The animal was placed in the actophotometer chamber and was acclimatised for 120 s if needed. The total number of times the animal obstructs the light beam was counted for 300 s.[12]

Tail suspension test (TST)

The TST is another crucial tool in depression studies involving mice. In this test, mice are suspended by their tails, and their immobility is monitored to assess despair-related behaviour. This method is significant in depression research as it provides a standardised and reliable approach to evaluate antidepressant effects. By quantifying immobility duration,

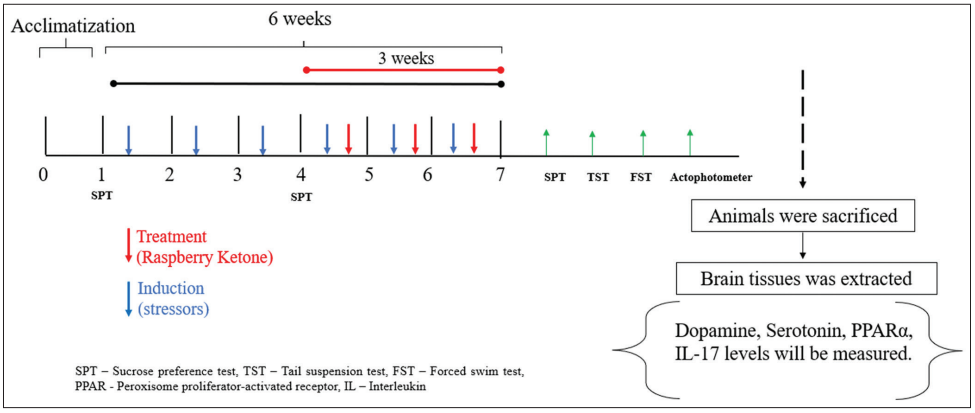

Figure 2: Chronic unpredictable mild stress protocol of studies.

Table 1: Stressors used in CUMS model.

Day	Task
Day 1	Tilting of the cage to 45° (for 4 h)
Day 2	Wet Bedding (for 4 h)
Day 3	Food and water deprivation (for 6 h)
Day 4	Swimming in cold water (4°) for 5 min
Day 5	Swimming in hot water (45°) for 5 min
Day 6	Disruption of light/dark cycle
Day 7	No stress

CUMS: Chronic unpredictable mild stress

Table 2: Grouping of animals used in studies.

Group number	Treatment and Dose	Number of animals
I	Control group (Saline)	08
II	Disease control (CUMS)- Stressors	08
III	Vehicle control (Tween 80) + Stressors	08
III	Fluoxetine (10 mg/kg oral gavage) (Standard drug) + Stressors	08
IV	RK (50 mg/kg) + Stressors	08
V	RK + (100 mg/kg) + Stressors	08
VI	RK + (200 mg/kg) + Stressors	08

RK: Raspberry ketone, CUMS: Chronic unpredictable mild stress

researchers gain insights into the efficacy of potential treatments, aiding in the identification and development of novel therapeutic strategies for depression in mice, thereby enhancing our understanding of related mechanisms and potential interventions. Mice were kept in four to five per cage in a room with a 12 h light/dark cycle and were given access to food and water. Behavioural testing was performed between 10 am and 2 pm. All the cages containing rats were then transferred to the behaviour testing room 1 h before the first trial began. Adhesive tape was used to suspend each rat by its tail. The test was conducted for 5 min and video recording was done. The time spent immobile by the animal during 5 min will be interpreted as a measure of depression-like behaviour.[13,14]

Forced swim test (FST)

The FST is a widely used method in depression studies involving mice. In this test, mice are placed in a water container, and their behaviour is observed to assess despair-like responses. The test's significance lies in its ability to model aspects of depressive behaviour, aiding researchers in understanding potential antidepressant effects. By measuring parameters like immobility time, scientists can evaluate the impact of various interventions on mood-related behaviours, contributing to the development of novel treatments for depression in both mice and potentially extrapolated to humans. Mice were kept in four to five per cage in a room with a 12 h light/dark cycle and were given access to food and water. Behavioural testing will be performed between 10 am and 2 pm. All the cages containing mice will be transferred to the behaviour testing room 1 h before the first trial begins. A plexiglass cylinder (25 cm height × 10 cm diameter) will be used for the test. All mice will be subjected to the pre-test session. Each mouse will receive 30-minute treatments before the test session and will be allowed to swim for 6 min, and the last 5 min will be evaluated for the duration of immobility.[15]

Biochemical assessment

Determination of PPARα receptor, IL-17 receptor, DA and Serotonin levels in the brain

The brains were washed with phosphate-buffered saline with a pH of 7.4 to eliminate any remaining blood. Subsequently, the brains were diced for homogenisation. Following homogenisation, the resulting homogenates were centrifuged at −4°C and 3000 revolutions/min for 15 min.[16-19] The remaining procedure was carried out as per the instructions given in the commercially available enzyme-linked immunosorbent assay (ELISA) kit by Krishgen Biosystems. The optical density of the ELISA plate was determined using a microplate reader set at 450 nm.

Statistical analysis

GraphPad Prism (8.0.1) software was used for statistical analysis. The one-way analysis of variance (ANOVA) test was applied along with Tukey's multiple comparison tests. The data are represented as mean ± standard error of the mean (SEM) values and $n = 8$ per group.

RESULTS

Behavioural tests

FST

The immobility time was significantly higher in the diseased and vehicle control group as compared to the normal control group. The standard control group also showed decreased immobility time as compared to the diseased control group, while RK-treated groups showed a significant decrease in immobility time [Figure 3].

TST

The negative control group exhibited a noteworthy rise in immobility duration compared to both the normal control and standard control groups. Conversely, the treatment group

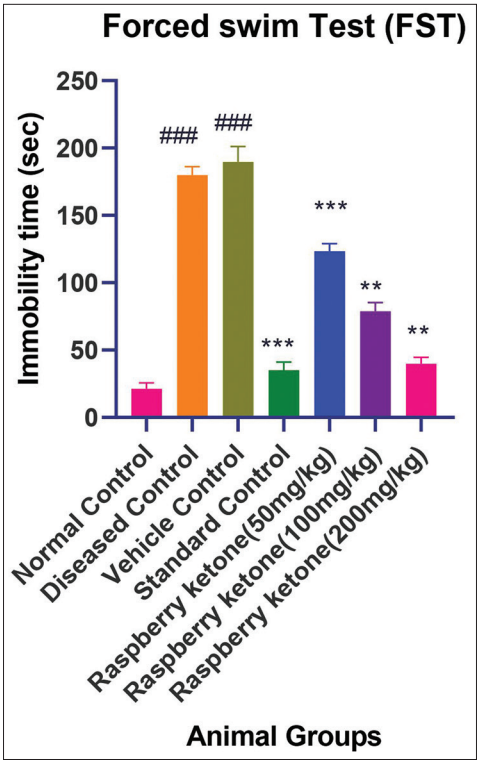

Figure 3: Forced swim test interpretation of different groups. The values are expressed as mean ± standard error of the mean. Data were analysed by one-way analysis of variance followed by *post hoc* Tukey Honestly Significant Difference test. ($^{###}P < 0.001$ vs. normal control, $^{***}P < 0.001$ vs. diseased control and $^{**}P < 0.01$ vs. diseased control).

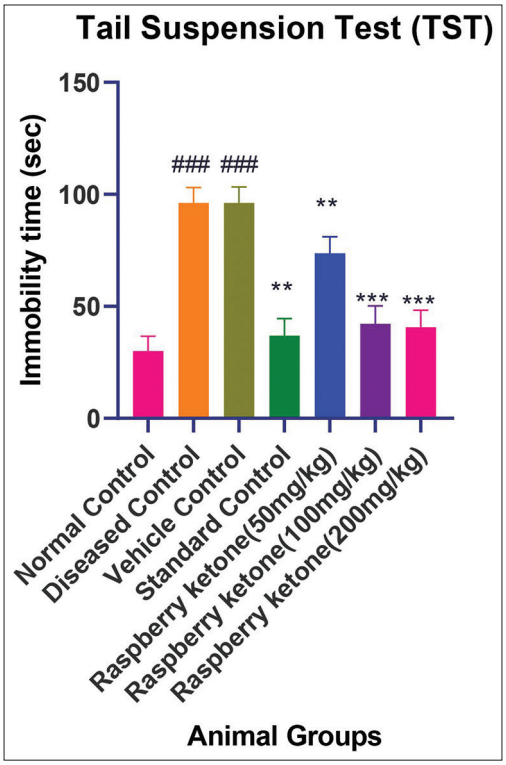

Figure 4: TST interpretation of different groups. The values are expressed as mean ± standard error of the mean. Data were analysed by one-way analysis of variance followed by a *post hoc* Tukey Honestly Significant Difference test. ($^{###}P < 0.001$ vs. normal control, $^{***}P < 0.001$ vs. diseased control and $^{**}P < 0.01$ vs. diseased control).

demonstrated a marked decrease in immobility time, with groups 2 (RK 100 mg/kg) and 3 (RK 200 mg/kg) displaying closely comparable outcomes [Figure 4].

Actophotometer test

The diseased control group and the vehicle control group showed significantly less light beam crossing value than the normal control group. The fluoxetine-treated group, that is standard control group, showed significantly more light beam crossing value, which was closer to the normal control group. Test group 2 (RK 100 mg/kg) and test group 3 (RK 200 mg/kg) had somewhat similar effects. However, test group 1 (RK 50 mg/kg) showed the least no of beam crossing across all treatment groups [Figure 5].

SPT

The values are expressed as mean ± SEM. Data were analysed by one-way ANOVA followed by *post hoc* Tukey Honestly Significant Difference test. $^{###}P < 0.001$ versus normal control, $^{***}P < 0.001$ versus diseased control, $^{***}P < 0.001$ versus diseased control [Figures 6-8].

Biochemical tests

DA levels

The DA levels were significantly high in the normal control group and treatment groups as compared to the diseased and vehicle control group indicating loss of dopaminergic neurons in the diseased and vehicle control group. However, all three test groups do not significantly increase DA levels as compared to the standard group [Figure 9].

Serotonin Levels

The diseased and vehicle control group showed significantly lower serotonin levels as compared to the normal control group. The standard control group showed significantly higher levels of serotonin. Test group 2 (RK 100mg/kg) and test group 3 (RK 200mg/kg) showed a dose-dependent effect. However, test group 1(RK 50mg/kg) showed a lower level of serotonin across all treatment groups [Figure 10].

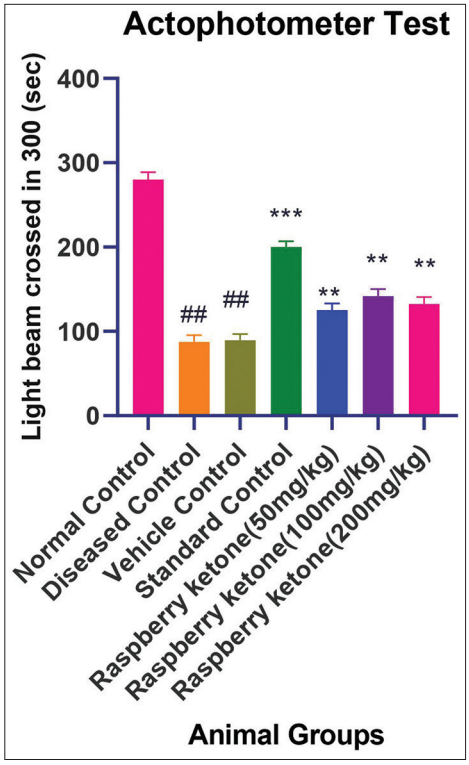

Figure 5: Actophotometer interpretation of different groups. The values are expressed as mean ± standard error of the mean. Data were analysed by one-way analysis of variance followed by *post hoc* Tukey Honestly Significant Difference test. ($^{##}P < 0.01$ vs. normal control, $^{***}P < 0.001$ vs. diseased control and $^{**}P < 0.0001$ vs. diseased control).

PPAR-α levels

The levels of PPARα were minimal in the diseased and vehicle control group as compared to the normal control group. Test group 1 (RK 50 mg/kg), test group 2 (RK 100 mg/kg), and test group 3 (RK 200 mg/kg) show a dose-dependent increase in PPARα levels, which are near to normal control [Figure 11].

IL-17 levels

The levels of IL-17 were maximum in diseased control and vehicle control groups as compared to the normal control group. Test group 1 (RK 50 mg/kg), test group 2 (RK 100 mg/kg), and test group 3 (RK 200 mg/kg) show a dose-dependent decrease in IL-17 levels which are near to normal control [Figure 12].

DISCUSSION

The experimental model of CUMS-induced depression has gained prominence in research studies as a robust and reliable model for evaluating potential antidepressant activities of various compounds. In this study, we have proposed the utilisation of this CUMS-induced depression model to assess

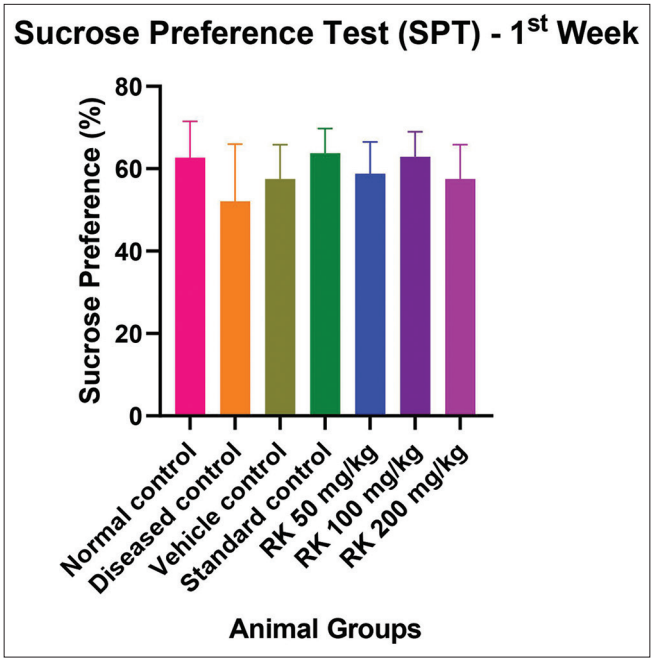

Figure 6: Sucrose preference test interpretation of different groups (1st week). RK: Raspberry ketone

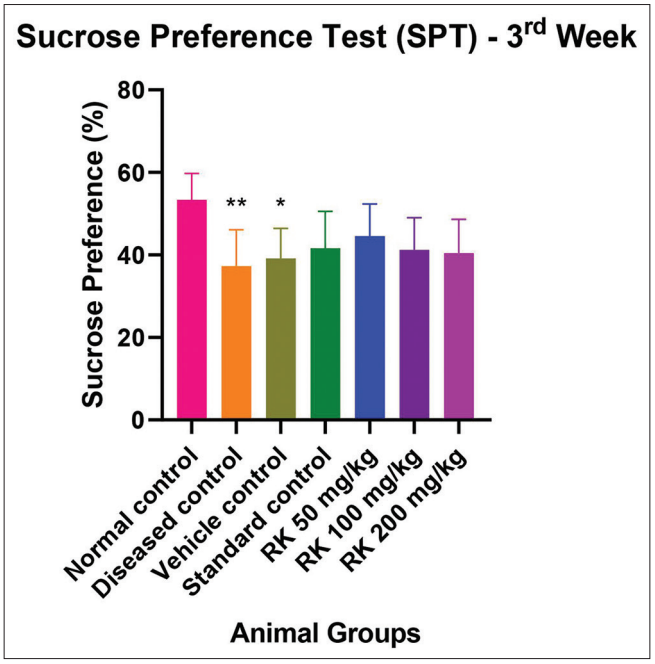

Figure 7: Sucrose preference test interpretation of different groups (3rd week). The values are expressed as mean ± standard error of the mean. Data were analysed by one-way analysis of variance followed by post hoc Tukey Honestly Significant Difference test. (**P < 0.01 vs. normal control, *P < 0.005 vs. normal control). RK: Raspberry ketone.

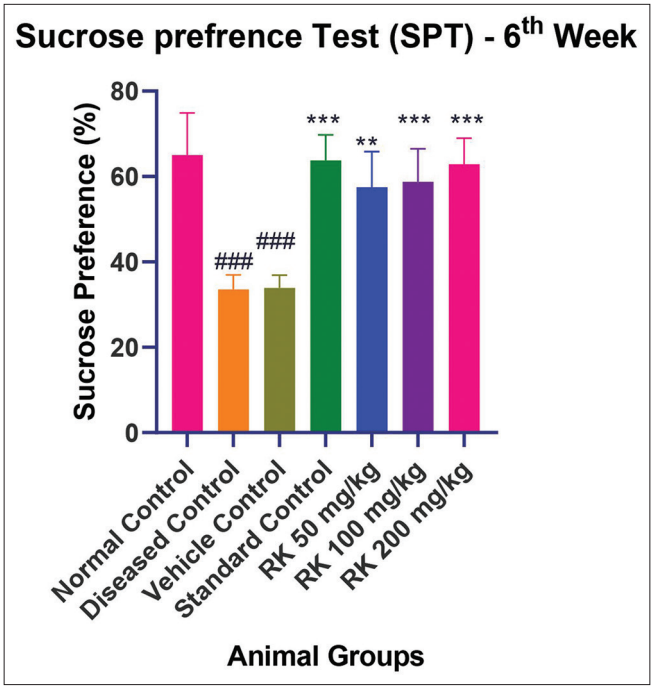

Figure 8: Sucrose preference test interpretation of different groups (6th week). The values are expressed as mean ± standard error of the mean. Data were analysed by one-way analysis of variance followed by *post hoc* Tukey Honestly Significant Difference test. (###$P < 0.001$ vs. normal control, ***$P < 0.001$ vs. diseased control and **$P < 0.01$ vs. diseased control). RK: Raspberry ketone

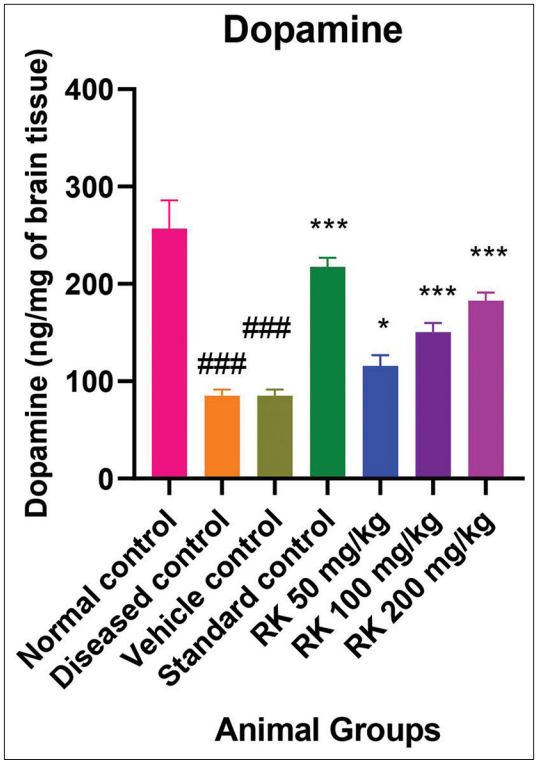

Figure 9: Dopamine levels of different groups. The values are expressed as mean ± standard error of the mean. Data were analysed by one-way analysis of variance followed by *post hoc* Tukey Honestly Significant Difference test. (###$P < 0.001$ vs. normal control, ***$P < 0.001$ vs. negative control, *$P < 0.005$ vs. negative control). RK: Raspberry ketone

the effectiveness of RK as an antidepressant agent. This model involves subjecting animals to a series of unpredictable mild stressors over an extended period, leading to behavioural and neurochemical alterations reminiscent of depressive symptoms in humans. The choice of this model underscores its ability to induce consistent and reproducible depressive-like behaviours, making it a valuable tool for evaluating antidepressant intervention.[10]

The rationale behind employing RK lies in its potential pharmacological properties and the need for novel antidepressant agents. RK, a natural phenolic compound found in red raspberries, has demonstrated antioxidant and anti-inflammatory effects in preclinical studies.[8,9] These properties suggest its potential to modulate neurobiological pathways implicated in depression. The weight management (anti-obesity) and anti-inflammatory benefits of RK do not necessarily imply effectiveness in treating depression. While RK has been researched for its potential in managing weight and reducing inflammation, the causes of depression are somewhat complex and involve disruptions in neurotransmitter systems such as serotonin, DA and NE, as well as factors such as neuroinflammation and oxidative stress. Current studies on RK primarily explore its metabolic and anti-inflammatory properties rather than its effects on depression. Therefore, while RK's overall health advantages may indirectly enhance well-being, its specific impact on depression remains uncertain and necessitates further clinical investigation focused on mental health outcomes.[20,21] Previous research on the antidepressant effects of natural compounds has paved the way for exploring the therapeutic potential of RK in mitigating the detrimental effects of chronic stress-induced depression. This research endeavour aligns with the growing interest in natural products as alternative or adjunctive treatments for depression. The proposal to evaluate RK in the context of CUMS-induced depression aims to contribute to the expanding body of knowledge regarding the antidepressant properties of phytochemicals.

Recent studies have implicated the Th17 cell differentiation pathway as a significant contributor to neuroinflammation, a key factor in the development of depression. The reported findings suggest that dysregulation of Th17 cells and their associated cytokines may play a pivotal role in the inflammatory processes observed in depressive disorders.[4,5] In response to this hypothesis, our research

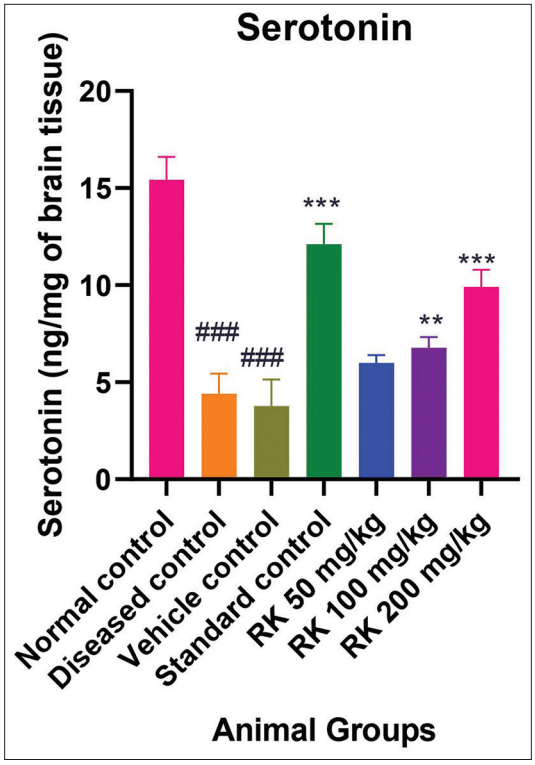

Figure 10: Serotonin levels of different groups**.** The values are expressed as mean ± standard error of the mean. Data were analysed by one-way analysis of variance followed by *post hoc* Tukey Honestly Significant Difference test. ($^{###}P < 0.001$ vs. normal control, $^{***}P < 0.001$ vs. negative control and $^{**}P < 0.01$ vs. negative control). RK: Raspberry ketone

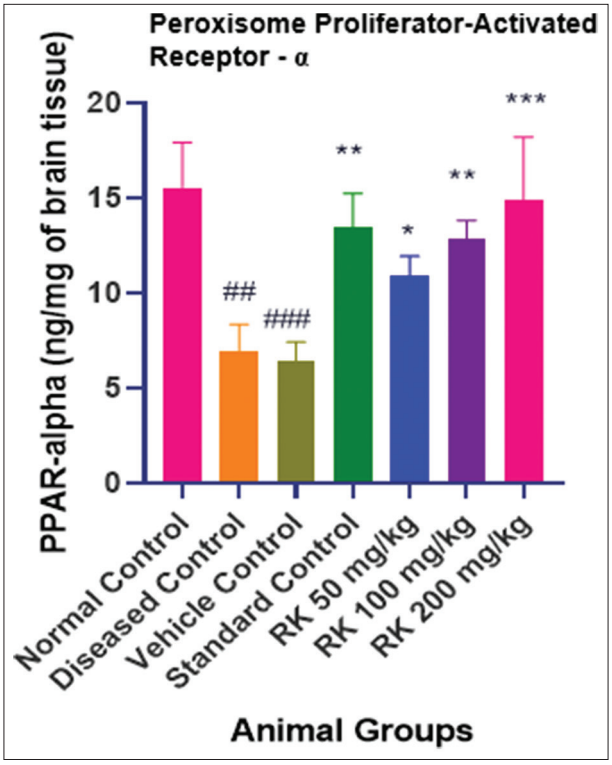

Figure 11: Proliferator-activated receptor alpha levels of different groups. The values are expressed as mean ± standard error of the mean. Data were analysed by one-way analysis of variance followed by *post hoc* Tukey Honestly Significant Difference test. ($^{##}P < 0.01$,$^{###}P < 0.001$ vs. normal control, $^{***}P < 0.001$ vs. diseased control and $^{*}P < 0.005$ vs. diseased control). RK: Raspberry ketone

sought to investigate the potential of RK as a modulator of the Th17 cell differentiation pathway, with the aim of mitigating neuroinflammation and inducing antidepressant-like effects. RK, a natural phenolic compound found in red raspberries, has demonstrated anti-inflammatory properties in various experimental models.[8,9] This study aligns with the emerging interest in understanding the immune system's role in mental health and exploring novel therapeutic interventions that target immune-mediated pathways implicated in depression. Our research design involved evaluating the impact of RK on Th17 cell differentiation and associated cytokine production. The rationale behind this investigation stems from the hypothesis that by modulating the Th17 pathway, RK may exert anti-inflammatory effects and, subsequently, alleviate depressive symptoms. Preliminary data from our study suggest a potential regulatory role of RK in Th17 cell differentiation, warranting further exploration into its underlying mechanisms and therapeutic implications for depression. Research on the long-term efficacy and safety of RK is sparse, with most studies focusing on short-term effects. RK has shown promise for weight management and anti-inflammatory benefits in the short term, but its prolonged use and associated risks are not well understood. Concerns exist about potential side effects and interactions with medications due to RK's metabolic impact. Future research should emphasise long-term clinical trials to determine its extended safety and effectiveness before recommending it for health management.[22]

Our comprehensive study not only investigated the potential modulation of the Th17 cell differentiation pathway but also assessed the behavioural outcomes associated with RK administration in mice. The results revealed promising improvements in key indicators of depressive-like behaviour. RK demonstrated a positive impact on locomotor activity, suggesting an enhancement in overall mobility. In the actophotometer test, light beam crossing values exhibited notable distinctions among the normal control group, diseased control group and vehicle control group. Both the diseased and vehicle control groups demonstrated significantly lower values compared to the normal control group. In contrast, the fluoxetine-treated group (standard control group) displayed significantly higher light beam crossing values, closely resembling those of the normal control group. Test

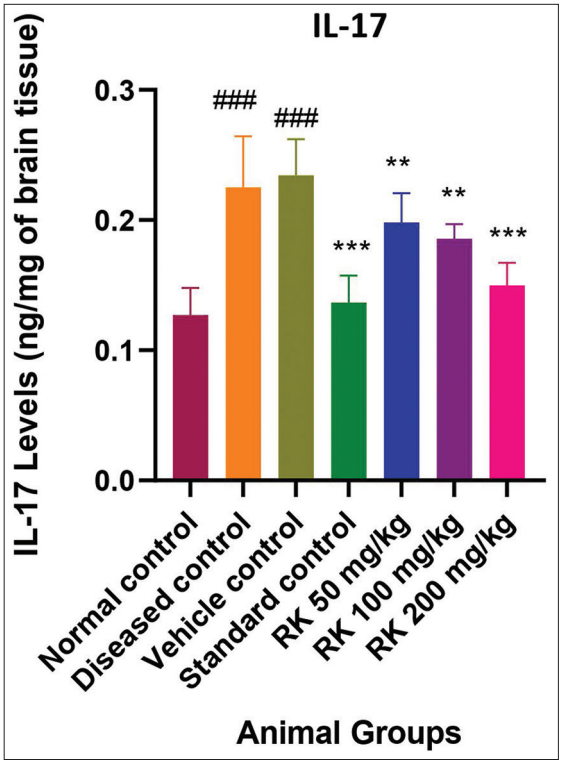

Figure 12: Interleukin-17 levels of different groups. The values are expressed as mean ± standard deviation. Data were analysed by one-way analysis of variance followed by *post hoc* Tukey Honestly Significant Difference test. ($^{###}P < 0.001$ vs. normal control, $^{***}P < 0.001$ vs. diseased control and $^{**}P < 0.01$ vs. diseased control). RK: Raspberry ketone

groups 2 (RK 100 mg/kg) and 3 (RK 200 mg/kg) exhibited somewhat similar effects, while test group 1 (RK 50 mg/kg) demonstrated the fewest beam crossings among all treatment groups. Furthermore, a significant reduction in immobility time in both FST and TST for standard and test drug groups was observed, which is a commonly used measure in assessing despair-like behaviour, indicating a potential antidepressant effect. In FST, the normal control group exhibited significantly lower immobility times compared to both the diseased and vehicle control groups. In the standard control group, immobility time was also decreased compared to the diseased control group. Notably, the RK-treated groups demonstrated a significant reduction in immobility time, highlighting their potential impact on this parameter. In TST, the negative control group displayed a noteworthy increase in immobility time when compared to both the normal control and standard control groups. In contrast, the treatment group exhibited a significant reduction in immobility time. Notably, groups 2 (RK 100 mg/kg) and 3 (RK 200 mg/kg) demonstrated nearly identical results in terms of immobility time.

Another noteworthy finding was the improvement in SP, a measure linked to anhedonia, a core symptom of depression. We have performed SPT for 3 weeks. For 1st week test, results were stagnant as stressors were not started. For weeks 3rd and 6t, results were impressive as the groups, such as negative control and vehicle control, showed significantly lesser SP, while standard and other test drug groups showed relatively higher SP. A standard group among all groups showed the highest SP. These behavioural outcomes collectively support the notion that RK may exert a beneficial influence on depressive-like behaviours in the experimental mouse model. These findings align with the growing interest in natural compounds as potential alternatives for treating depression. The observed improvements in locomotor activity, immobility time and SP underscore the multifaceted nature of RK's potential antidepressant effects. Our research monitored body weight and food intake patterns in mice subjected to CUMS, revealing significant changes indicative of an anhedonic state seen in depressive disorders. Studies have consistently shown that CUMS can reduce body weight and disrupt eating habits due to HPA axis dysregulation.[23] Our findings support these observations, highlighting stress-induced metabolic and feeding behaviour disruptions leading to weight loss. Future studies should explore biochemical pathways further to deepen our understanding of stress-related changes in depression models.

In addition to the observed behavioural improvements, our study investigated the neurochemical impact of RK treatment in mice subjected to the Th17-induced depression model. Notably, the administration of RK led to a significant increase in the levels of key neurotransmitters, namely, DA and serotonin. These findings align with the well-established role of DA and serotonin in mood regulation, suggesting a potential neurochemical basis for the antidepressant-like effects of RK. In DA ELISA, both the normal control group and the treatment groups displayed significantly elevated DA levels when compared to the diseased and vehicle control groups. This suggests a notable loss of DArgic neurons in the diseased and vehicle control group. Interestingly, while the treatment groups exhibited higher DA levels, none of the three test groups showed a statistically significant increase compared to the standard control group. In serotonin, ELISA, the normal control group demonstrated significantly higher serotonin levels compared to both the diseased and vehicle control groups. The standard control group exhibited notably elevated serotonin levels. Test groups 2 (RK 100 mg/kg) and 3 (RK 200 mg/kg) displayed a dose-dependent effect, with serotonin levels increasing accordingly. In contrast, test group 1 (RK 50 mg/kg) consistently exhibited lower serotonin levels compared to all other treatment groups.

Moreover, the study revealed a concurrent reduction in inflammatory cytokines, providing further evidence of the compound's anti-inflammatory properties. In IL-17 ELISA, the diseased control and vehicle control groups displayed the highest levels of IL-17 in comparison to the normal control

group. Conversely, test groups 1 (RK 50 mg/kg), 2 (RK 100 mg/kg) and 3 (RK 200 mg/kg) exhibited a dose-dependent reduction in IL-17 levels. Notably, the IL-17 levels in these test groups approached those observed in the normal control group. Furthermore, PPARα ELISA results were promising, as in the diseased and vehicle control groups, the levels of PPARα were notably lower compared to the normal control group. Conversely, test groups 1 (RK 50 mg/kg), 2 (RK 100 mg/kg) and 3 (RK 200 mg/kg) exhibited a dose-dependent increase in PPARα levels. Importantly, the PPARα levels in these test groups approached those observed in the normal control group. The modulation of neurotransmitter levels and the attenuation of inflammatory markers collectively suggest a multifaceted mechanism through which RK may exert its therapeutic effects in depression. These neurochemical findings contribute to the broader understanding of the potential mechanisms underlying the antidepressant properties of RK. The observed elevation in DA and serotonin levels aligns with established theories of depression, implicating imbalances in these neurotransmitters. In addition, the reduction in inflammatory cytokines underscores the potential anti-inflammatory role of RK in ameliorating neuroinflammation associated with depressive disorders.

The amalgamation of positive outcomes from both behavioural and biochemical assessments in our study suggests that RK holds promise as an antidepressant in the CUMS-induced depression model. The observed improvements in locomotor activity, immobility time and SP, coupled with elevated levels of neurotransmitters such as DA and serotonin, point toward its potential therapeutic efficacy. Furthermore, the concurrent reduction in inflammatory cytokines underscores its anti-inflammatory properties, adding to the multifaceted nature of its antidepressant effects. Importantly, the potential of RK as an antidepressant introduces the prospect of mitigating the side effects associated with currently available antidepressant drugs, offering a novel and potentially more tolerable treatment option. In addition, the economic viability of this approach, in comparison to other antidepressant medications, adds a practical dimension to its potential clinical application. However, the realisation of its clinical utility hinges on a more profound understanding of its mechanisms and further rigorous investigation. Hence, it is essential to conduct further investigations to validate the efficacy of RK and its underlying mechanisms in ameliorating depressive-like symptoms in this experimental model. While further research is warranted to elucidate the precise mechanisms underlying these behavioural changes, our study contributes valuable insights into the behavioural aspects of RK as a potential therapeutic agent for depression.

The limitation of the study was tailoring the effects of RK only in male mice. The exclusive use of male mice was due to considerations related to hormonal variations in female mice, which can introduce additional variables and complicate the interpretation of results. However, we recognise the importance of including both sexes to fully understand RK's antidepressant potential. Future studies will incorporate female mice to provide a more comprehensive analysis and ensure the generalizability of our findings across the sexes. While our study provides valuable insights into RK's potential mechanisms in alleviating depression through the modulation of DA, serotonin and inflammatory cytokines, we acknowledge that our biochemical data could be more comprehensive due to limited resources and tools that restricted our exploration of complex biochemical pathways. A recent study offers a detailed examination of these mechanisms, showing that RK can mitigate depression-like behaviours in LPS-induced mice by targeting the TLR-4/NF-κB signalling pathway through the gut-brain axis.[24] These findings highlight the significance of the gut-brain axis in depression and suggest that RK's antidepressant effects may be mediated through its anti-inflammatory properties. Despite these limitations, our research contributes to understanding RK's role in depression and sets the stage for future investigations that could build on our findings and explore these biochemical pathways in greater detail with advanced techniques and broader resources.

CONCLUSION

Our study on the preclinical properties of RK in the CUMS-induced depression model has revealed promising anti-depressant activity. The administration of RK demonstrated notable improvements in various behavioural parameters, including locomotor activity, immobility time and SP, indicating a comprehensive impact on depressive-like behaviours. These behavioural outcomes were accompanied by favourable changes in neurochemical profiles, with increased levels of neurotransmitters such as DA and serotonin. Notably, the study highlights the potential pharmacological mechanisms underlying RK's anti-depressant effects, primarily attributed to its ability to decrease elevated inflammatory cytokine levels. Importantly, these beneficial effects were achieved at a low dose, suggesting a favourable safety profile with minimal or no side effects. Collectively, our findings support the notion that RK may serve as a promising and well-tolerated intervention for depression, warranting further exploration in clinical settings.

Data availability

It will made available by the corresponding author on request.

Ethical approval

The research/ study was approved by Institutional Animal Ethics Committee at SVKM's Animal House Facility,

Approval number CPCSEA/IAEC/P-77/2022, dated 08 th October 2022

Declaration of patient consent

Patient's consent was not required as there are no patients in this study.

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Conflicts of interest

There are no conflicts of interest.

Use of artificial intelligence (AI)-assisted technology for manuscript preparation

The authors confirm that there was no use of artificial intelligence (AI)-assisted technology for assisting in the writing or editing of the manuscript and no images were manipulated using AI.

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