Protective effects of Baicalein against haloperidol and high fructose-induced behavioural changes *in mice* via stimulation of antioxidant pathways

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ABSTRACT

Background: Previous research has indicated that neurodegenerative disorders are connected to metabolic alterations, which triggers their progression or onset. Haloperidol and high-fructose diet have also been found to induce neurobehavioural abnormalities via induction of oxidative stress. Baicalein has been shown to exhibit neuroprotective effect.

Objective: This study was conducted to investigate the modulatory effects of Baicalein against high-fructose and haloperidol-induced behavioural alterations, and antioxidant levels in mice.

Methods: Male Swiss mice (20-25 g) were randomly distributed into 7 groups (n = 6) and fed with high fructose diet (HFD) ad libitum for two weeks. Baicalein (BAC) was given at low (10 mg/kg), moderate (50 mg/kg) and high doses (100 mg/kg). The treatment schedule were as follows: Group 1: control (Normal Saline: NS, 10 mL/kg i.p.); Group 2: High Fructose Diet (HFD, 20 % p.o.); Group 3: Haloperidol (HAL, 2 mg/kg i.p.); Group 4: Baicalein (BAC2, 50 mg/kg p.o.); Group 5: HFD (20 % p.o.) + HAL (2 mg/kg i.p.) + BAC (10 mg/kg p.o.); Group 6: HFD (20 % p.o.) + HAL (2 mg/kg i.p.) + BAC (50 mg/kg p.o.); Group 7: HFD (20 % p.o.) + HAL (2 mg/kg i.p.) + BAC (100 mg/kg p.o.) for 14 days. Behavioural parameters were observed and recorded. Blood glucose levels were taken and recorded on days 1, 8 and 14. Afterwards, the animals were sacrificed and the brain was extracted for further assays.

Results: Baicalein improved locomotion, mitigated cognitive deficits and improved head dips in the open field, Y-maze and hole board tests respectively. Also, Baicalein treatment reduced the blood glucose levels increased by haloperidol and HFD exposure, as well as, increasing the levels of antioxidants glutathione, catalase and superoxide dismutase that was reduced by HFD and haloperidol.

Conclusion: Findings suggest that Baicalein possess mitigatory effects on haloperidol and high fructose-induced alterations in mice.

Keywords: Baicalein, Haloperidol, Catalepsy, Motor Dysfunction, blood glucose, antioxidants.

Effets protecteurs de la baicaléine contre les changements comportementaux induits par l'halopéridol et un taux élevé de fructose chez les souris via la stimulation des voies antioxydantes

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RÉSUMÉ

Contexte: Des recherches antérieures ont indiqué que les troubles neurodégénératifs sont liés à altérations métaboliques, ce qui déclenche leur progression ou leur apparition. Il a également été démontré que l'halopéridol et un régime riche en fructose induisent des anomalies neurocomportementales par l'induction d'un stress oxydatif. La baicaléine a démontré des effets neuroprotecteurs.

Objectif: Cette étude a été menée afin d'étudier les effets modulateurs de la baïcaléine sur les altérations comportementales induites par un régime riche en fructose et l'halopéridol, ainsi que les niveaux d'antioxydants chez les souris.

Méthodes: Des souris mâles Swiss (20-25 g) ont été réparties aléatoirement en 7 groupes (n = 6) et nourries à volonté avec un régime riche en fructose (HFD) pendant deux semaines. La baïcaléine (BAC) a été administrée à des doses faibles (10 mg/kg), modérées (50 mg/kg) et élevées (100 mg/kg). Le programme de traitement était le suivant : Groupe 1 : témoin (solution saline normale : NS, 10 ml/kg). i.p.); Groupe 2 : Régime riche en fructose (HFD, 20 % po) ; Groupe 3 : halopéridol (HAL, 2 mg/kg i.p.); Groupe 4 : baïcaléine (BAC2, 50 mg/kg p.o.) ; Groupe 5 : HFD (20 % p.o.) + HAL (2 mg/kg i.p.) + BAC (10 mg/kg po) ; Groupe 6 : HFD (20 % p.o.) + HAL (2 mg/kg i.p.) + BAC (50 mg/kg p.o.) ; Groupe 7 : HFD (20 % p.o.) + HAL (2 mg/kg i.p.) + BAC (100 mg/kg po) pendant 14 jours. Les paramètres comportementaux ont été observés et enregistrés. Les taux de glycémie ont été mesurés et enregistrés les jours 1, 8 et 14. Ensuite, les animaux ont été sacrifiés et le cerveau a été extrait pour des analyses complémentaires.

Résultats: La baicaléine a amélioré la locomotion, atténué les déficits cognitifs et amélioré les plongeons de la tête lors des tests en champ libre, du labyrinthe en Y et de la planche perforée, respectivement. De plus, le traitement à la baicaléine a réduit les taux de glycémie, augmentés par l'halopéridol et l'exposition au HFD, ainsi que les taux d'antioxydants glutathion, catalase et superoxyde dismutase, diminués par le HFD et l'halopéridol.

Conclusion: Les résultats suggèrent que la baicaléine possède des effets atténuants sur les altérations induites par l'halopéridol et le fructose élevé chez la souris.

Mots clés: Baïcaléine, halopéridol, catalepsie, dysfonctionnement moteur, glycémie, antioxydants.

INTRODUCTION

Haloperidol administration to mice induces a well-described condition of immobility characterized by muscle rigidity, diminished sensitivity to pain, and frozen posture usually termed catalepsy.[1] In rodents, the administration of the dopaminergic D2 antagonist, haloperidol affects the nigrostriatal pathway to induce catalepsy, a state of immobility similar to Parkinson's disease (PD), bradykinesia and akinesia.[2] This condition in mice resembles similar human conditions in disorders such as Parkinson's disease and schizophrenia. Haloperidol-induced catalepsy often serves as a useful animal model for the study of Parkinsonism, given its simplicity.[3] The degree of catalepsy is mostly evaluated by the catalepsy bar test, which quantifies the time it takes the rats to correct an externally imposed posture.[4] Metabolic alterations play an important role in cataleptic diseases such as Alzheimer's and Parkinson's Diseases on both the systemic and central nervous system level.

Fructose, a member of a group of carbohydrates known as simple sugars, or monosaccharides, occurs in fruits, honey, and syrups. When it is consumed in excess, it may be associated with increased risk of obesity, diabetes, and cardiovascular disorders that are part of metabolic syndrome.[5] In rodents, increased dietary fructose intake for 4-12 weeks recapitulates many aspects of metabolic syndrome.[6-8] Metabolic alterations, related to cerebral glucose metabolism, age-induced mitochondrial dysfunction, and brain insulin resistance, play an essential role in various diseases such as Alzheimer's disease (AD) on both the central and peripheral nervous system level.[9] Previous research has indicated that neurodegenerative disorders are connected to metabolic alterations, which triggers their progression or onset.[10] Metabolic alterations are linked to several adverse health outcomes, including poor cognitive function, anxiety and stress-related disorders as well as oxidative stress.[11] For instance, the impact of metabolic alterations have been shown to affect cognition and also increasing iron concentration in several brain areas, causing neurotoxicity, which can lead to multiple neuronal diseases such as Parkinson's disease (PD), Alzheimer's disease (AD), and Huntinghton's diseases (HD).[12]

Evidence suggests that oxidative stress contributes to development of different range of chronic conditions such as diabetes, Alzheimer's disease, asthma, Parkinson's disease, and cancer.[13] The brain particularly, is vulnerable to oxidative stress because the brain cells require enormous amount of oxygen. These brain cells use oxygen to perform intense metabolic activities that generate free radicals. During this, excess free radicals can damage structures inside the brain cells or even cause cell death, which leads to Parkinson's disease.

Baicalein (5,6,7-trihydroxyflavone) is a phenolic flavonoid compound derived mainly from the root of Scutellaria baicalensis Georgi, a medicinal plant traditionally used in oriental medicine. Baicalein has been shown to exhibit neuroprotective properties and prevent dopaminergic neuronal loss.[14] It has been used in the treatment of hypertension, neurodegenerative diseases, inflammation, cardiovascular diseases, and respiratory infections.[15-16] An extensive literature search indicated that no study had been done to determine the effect of Baicalein on alterations caused by the combination of haloperidol and high fructose. Hence, this study was conducted to investigate the effects of Baicalein on high-fructosehaloperidol-induced catalepsy and alterations in various behavioural phenotypes and oxidative stress in mice.

MATERIALS AND METHODS

Drugs and chemicals

Baicalein was obtained from Sigma-Aldrich chemicals Co. (St Louis, Missouri, USA). Haloperidol hydrochloride was purchased from MANFES Pharm. Co. Nig Ltd. (Onitsha, Anambra State, Nigeria). D-Fructose was obtained from Burgoyne Reagents (India). Reduced glutathione (GSH), metaphosphoric acid, and trichloroacetic acid (TCA) were purchased from J.I. Baker (Center Valley, PA 18034 U.S.A.). Thiobarbituric acid (TBA) was purchased from Sigma Chemical Company (USA).

Experimental animals

Adult male mice (20-25 g) were used for this study. The mice were housed at room temperature in plastic cages with a 12:12 light-dark cycle and were allowed free access to commercial rodent pellet diet and water ad libitum. They were allowed to acclimatize before commencement of experiments and all experimental procedures were carried out in strict compliance with the NIH Ethical Guidelines for the Care and Use of Laboratory Animals.

Preparation of baicalein and treatments

Baicalein and Haloperidol were administered orally after being dissolved in distilled water prior to usage. The mice except control were fed with high fructose diet (HFD) ad libitum for two weeks.[17] Also, neurobehavioural abnormality (NA) was achieved during the week 2 (day 8 -

14) in the HF-fed mice. Baicalein was given at low (10 mg/kg), moderate (50 mg/kg) and high doses (100 mg/kg) following HFD administration and subjected to various tests.

Experimental procedures

The mice were randomly divided into 7 groups of 6 mice per group. In this present study, normal mice were fed high fructose diet (HFD) ad libitum for two weeks and neurobehavioural abnormality (NA) was observed during the period. Haloperidol and Baicalein were administered for one week. Baicalein (BAC) was given at low (10 mg/kg), moderate (50 mg/kg) and high doses (100 mg/kg). The treatment schedule was as follows: Group 1: control (Normal Saline: NS, 10 mL/kg i.p.); Group 2: HFD (20 %, p.o.); Group 3: Haloperidol (HAL: 2 mg/kg, i.p.); Group 4: Bacalein (BAC: 50 mg/kg, p.o.); Group 5: HFD (20 %, p.o.) + HAL (2 mg/kg, i.p.) + BAC (10 mg/kg, p.o.); Group 6: HFD (20 %, p.o.) + HAL (2 mg/kg, i.p.) + BAC (50 mg/kg, p.o.); Group 7: HFD (20 %, p.o.) + HAL (2 mg/kg, i.p.) + BAC (100 mg/kg, p.o.).

Test for blood glucose levels.

Fasting blood glucose levels (FBGLs) were measured at basal, day 8 and day 14 using a portable Accu-check glucometer (Gluco-Plus Inc., Quebec, Canada) in the blood collected via the tail vein.

Test for locomotor activity in the open-field test

This test was done using open field chamber on day 14 post treatment, each mouse was placed in the center of the box for 5 minutes while the locomotory frequency (horizontal activity counts) or exploratory activity (vertical activity counts) was measured in terms of the number of lines crossed.

Test for anxiety-like behavior using the hole board.

The hole-board is a golden white wooden board (40 x 40 cm) with four equidistant holes (1 cm diameter 2 cm depth).[18] The hole board was used to assess the effect of Baicalein on induced anxiety-like behaviour. The animal was placed in turn at one corner of the apparatus and the number of head dips and duration of head dips (in seconds) during 5-min duration was then estimated.

Y-Maze spontaneous alternation test

The Y-maze is a two-trial recognition memory test in which performance does not involve the learning of a rule because it taps on an innate tendency of mice to explore new (i.e. not-encountered-before) environments.[19] The apparatus for Y-maze test was made of three wooden arms (dimension 30 cm) at 120 degrees to each other. During the test, individual mice were placed on the intersection of three arms of the maze. The trial began immediately and ended when 5 min had elapsed. Scoring consisted of recording each arm entry (defined as all four paws entering arm). Entries into three different arms in succession (e.g. ABC or BCA or CBA or CAB) are defined as alternations. Arena was cleaned with ethanol (70 %) between trials.

Catalepsy test

Catalepsy was monitored using a horizontal bar placed 6 cm from the testing surface according to Hoffman and Donovan.[20] The forepaws of each mouse were placed gently on the bar, with the body positioned at an angle of 45^{o} to the testing surface. The latency for the mouse to move one or both hind paws towards the bar was measured manually using a stopwatch with a cut-off time of 30 s. Animals that failed in the pretest (inability to move the hind paws within 5 s) were disqualified in the process. Animals were allowed to rest and re-examined 30 s later until 240 s. A mouse which remained on the bar between 15 and 30 s was considered to be cataleptic.

Biochemical assessment

The animals were sacrificed after behavioral testing and brain was isolated, weighed and kept in 10 % w/v phosphate buffer (0.1 M, pH 7.4). The whole brains of the mice were homogenized with the 10 % w/v phosphate buffer and the supernatant stored at <20 ^{o}C until use for the different biochemical assay.

Determination of oxidative stress parameters

Biochemical tests were performed on brain tissues to determine the effects of Baicalein, high fructose and haloperidol on oxidative stress parameters. Malondialdehyde (MDA) levels were determined after tissue deproteinization with trichloroacetic acid (TCA) and incubation with thiobarbituric acid. Absorbance was measured at 532 nm, and MDA levels were evaluated in the tissues as described by Varshney and Kale.[21] The Griess procedure[22] was used to measure tissue nitrite as a nitrergic stress marker. The Ellman's procedure was used to detect reduced glutathione (GSH) in plasma and tissue samples after TCA deproteinisation.

Absorbance was measured at 412 nm while concentration was determined as well. The adrenaline auto-oxidation method described by 23 was used to determine the activity of superoxide dismutase (SOD) in the tissue samples. The method published by Goth 24 was used to

determine catalase (CAT) activity and represented as µmoles of hydrogen peroxide decomposed per min per milligram of protein (Unit/mg protein).

Statistical evaluation

The data obtained were expressed as mean ± S.E.M (standard error of mean) and analyzed with Graph Pad Prism software version 8.4.2. Statistical analysis of data was done using One-way Analysis of variance (ANOVA), followed by the Tukey post-hoc test. P-values less than 0.05 ($P < 0.05$) were considered statistically significant.

RESULTS

Effects of Baicalein on high fructose-haloperidol-induced locomotor deficit in open field test in mice

It shows that those fed with HFD resulted in decreased activity at days 1 to 4 followed by fluctuations when compared with control group that was not treated. The haloperidol (HAL) group shows a sharp decline in activity after day 1 with sustained low levels throughout the other days. Baicalein combinations of different doses with HAL showed consistent low levels of motor activity. HAL alone strongly reduces locomotor activity which is expected given its sedative properties. BAC when administered alone, slightly suppresses activity indicating mild sedation.

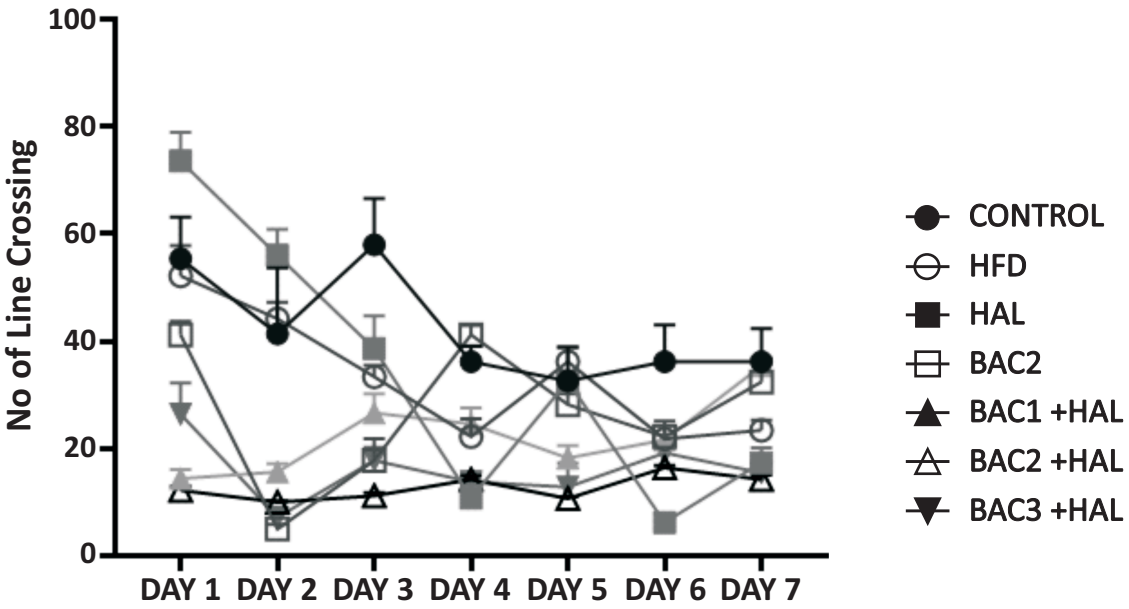

Figure 1: The result showing effect of haloperidol, HFD and Baicalein on locomotory activity in the open field test. The lines represent mean ± S.E.M for 6 animals per group. HFD- High fructose diet, BAC- Baicalein, HAL- Haloperidol

Effects of Baicalein on high fructose-haloperidol-induced memory deficit activity in y-maze test in mice

The control group showed higher number of alternations on day 1 with gradual decrease over the next days. The HFD shows fewer alternations when compared with control and later some fluctuations. HAP group exhibited a moderate performance initially but followed by fluctuations. BAC alone group showed an initial result mirroring that of control but then declined.

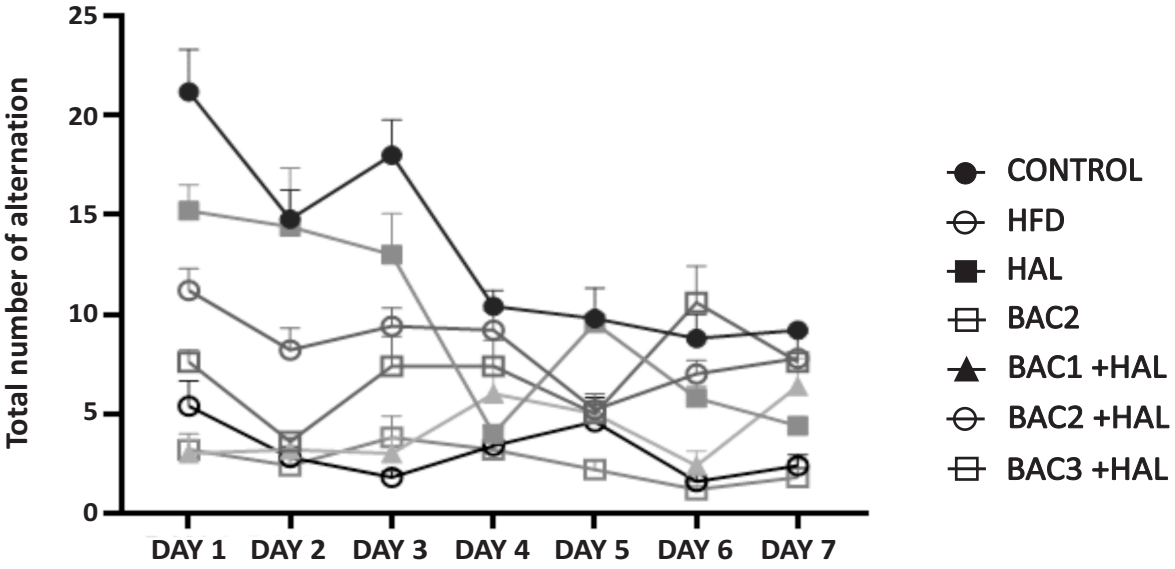

Figure 2: Result showing the effect of the various treatment groups on cognitive performance in the Y-maze test. The lines represent mean ± S.E.M for 6 animals per group. HFD- High fructose diet, BAC- Baicalein, HAL- Haloperidol.

Effects of Baicalein on high fructose-haloperidol-induced anxiety-like activity in hole-board test in mice.
The control group in the hole board test showed consistent head dipping behaviour throughout the 7-day period. HFD initially showed similar headcounts with control but showed a marked decline after day 1, with fluctuations afterwards at a lower level when compared with control. HAP showed decrease in head dips from day 3 and was consistently lower than control. BAC alone treated group showed an initial decline but significant recovery from day 4 later exceeding that of HFD and HAP groups.

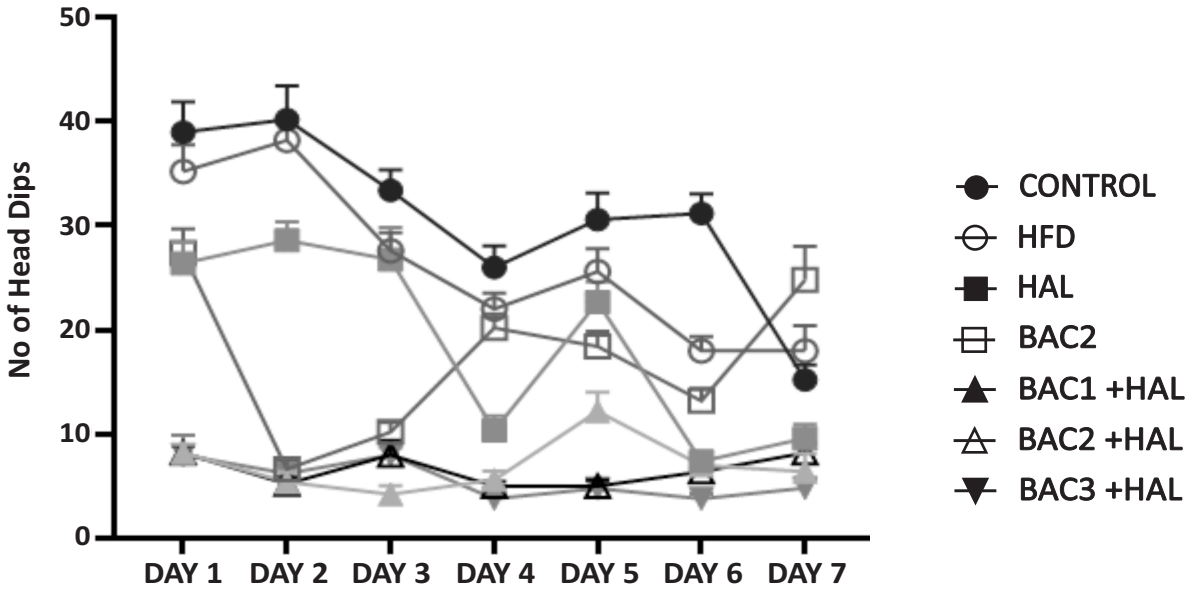

Figure 3: Result showing the effect of the various treatment groups on anxiety-like related behaviour in the hole-board test. The lines represent mean ± S.E.M for 6 animals per group. HFD- High fructose diet, BAC- Baicalein, HAL- Haloperidol

Effects of Baicalein on high fructose-haloperidol-induced catalepsy in bar test in mice
HFD showed increase in catalepsy on days 4 and 5 but returned to baseline. The haloperidol treated group showed a peak on day 5, with some persistence over the next days. BAC alone showed low levels of catalepsy compared to control and significantly lower than that of HAP, indicating that it does not induce catalepsy alone. The various treatment groups generally showed some reduction in catalepsy compared to haloperidol alone especially after day 5.

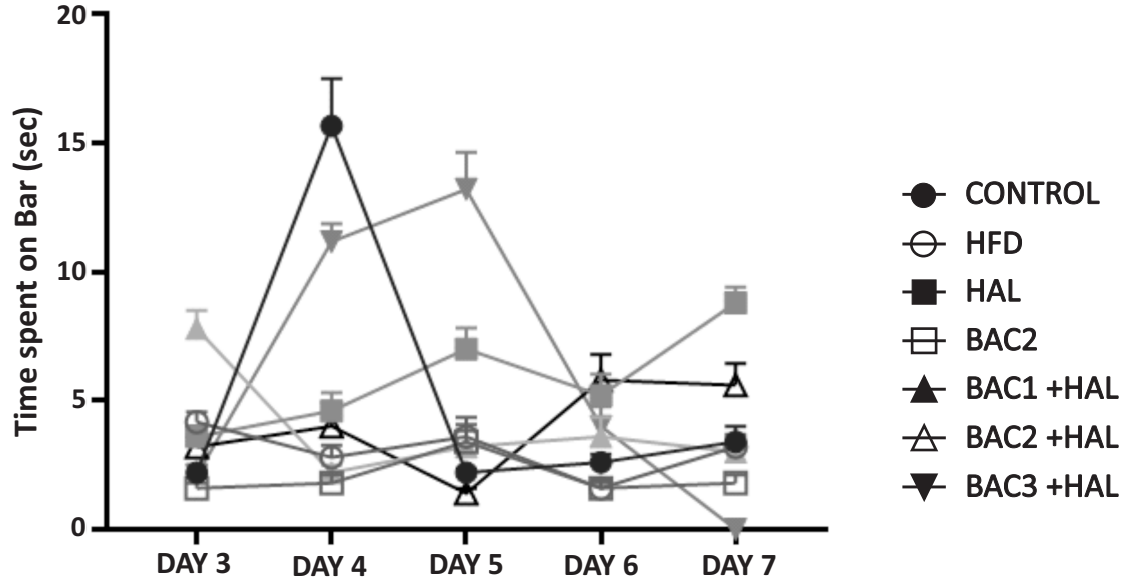

Figure 4: This result shows the effect of treatment groups on time spent on bar in the catalepsy test. The lines represent mean ± S.E.M for 6 animals per group. HFD- High fructose diet, BAC- Baicalein, HAL- Haloperidol.

Effects of Baicalein on blood glucose of mice fed with high fructose diet and haloperidol administration

On day 1, the group administered with Baicalein alone significantly reduced the blood glucose levels when compared with haloperidol and HFD. HFD increased glucose levels when compared with control though not significantly. Though BAC alone decreased glucose level, in combination with HAP, it may not have immediate effect or impact on glucose levels. On day 8, the HFD and HAP showed significant increase in blood glucose levels when compared with control. BAC alone plus BAC in combination with HAP significantly reduced the glucose levels when compared with HFD and HAL. On day 14, HFD and HAP showed similar elevation in glucose levels compared with control. Also, BAC 2 and BAC combined with HAP showed significant reduction in glucose levels compared to HFD and HAP.

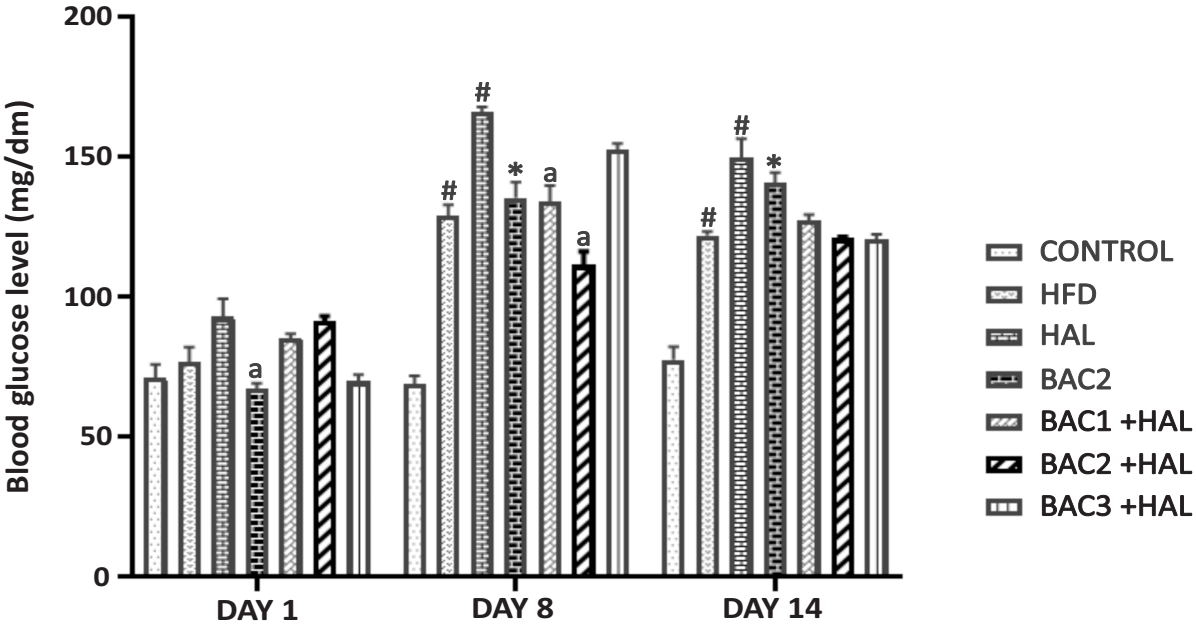

Figure 5: This result shows the effect of treatment groups on blood glucose level on basal, day 8 and day 14 in mice. The lines represent mean ± S.E.M for 6 animals per group. HFD- High fructose diet, BAC- Baicalein, HAL- Haloperidol

Effects of Baicalein on high fructose-haloperidol-induced oxidative damage in mice

The result showed that HFD and Haloperidol significantly reduced levels of SOD when compared with control while Baicalein alone group significantly elevated the SOD levels when compared with HFD and HAP groups. The combination of Baicalein with HAL didn't significantly elevate these levels though there was a slight increase (Figure 6). On Catalase level, The result showed that HFD and Haloperidol significantly reduced levels of CAT when compared with control while Baicalein at 50 mg/kg in combination with HAP group significantly decreased the CAT levels when compared with HFD and HAP groups. The combination of higher doses of Baicalein with HAL didn't significantly elevate these levels as well (Figure 7). On GSH level, the result showed that HFD and Haloperidol significantly reduced levels of GSH when compared with control while Baicalein alone and Bac 100 mg/kg plus HAL significantly elevated the GSH levels when compared with HFD and HAP groups (Figure 8).

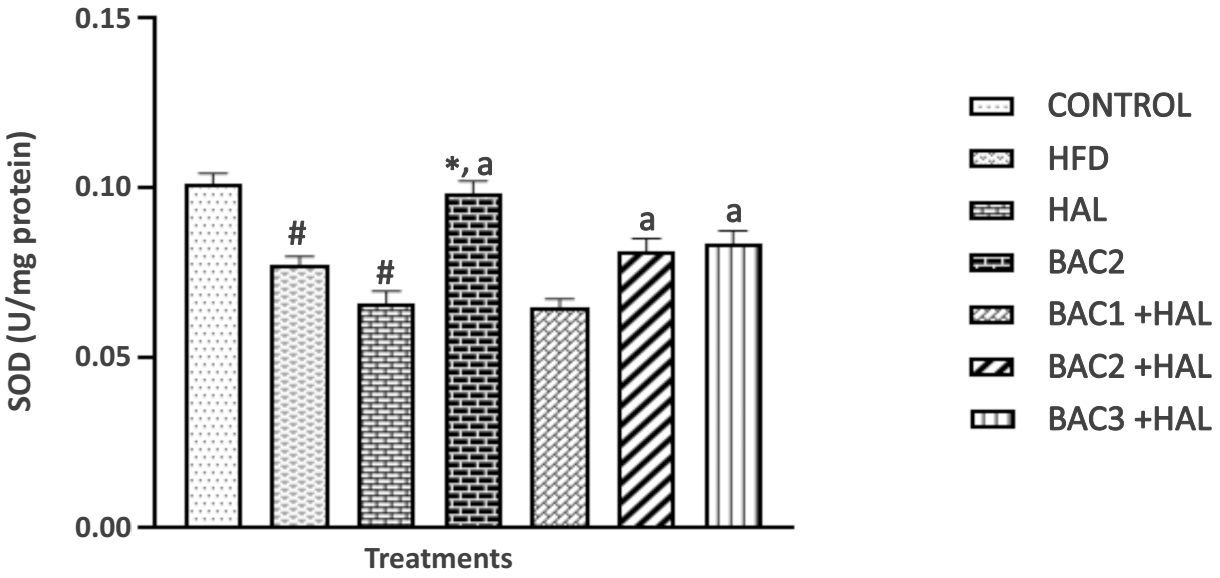

Figure 6: A figure showing the results of various treatment groups on the levels of SOD in mice. Values represent mean ± S.E.M for 6 animals per group. #P < 0.05 compared to normal control, *P < 0.05 compared to HFD and [a]P< 0.05 compared to HAP. HFD- High fructose diet, BAC- Baicalein, HAL- Haloperidol

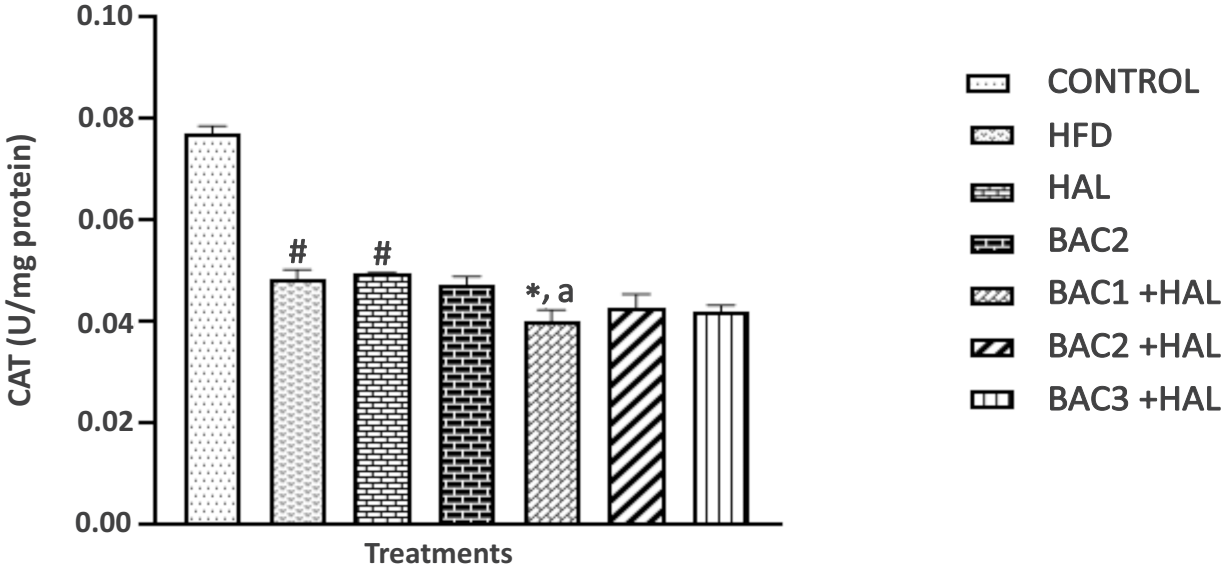

Figure 7: A figure showing the results of various treatment groups on the levels of CAT in mice. Values represent mean ± S.E.M for 6 animals per group. #P < 0.05 compared to normal control, *P < 0.05 compared to HFD and [a]P< 0.05 compared to HAP. HFD- High fructose diet, BAC- Baicalein, HAL- Haloperidol, CAT- Catalase

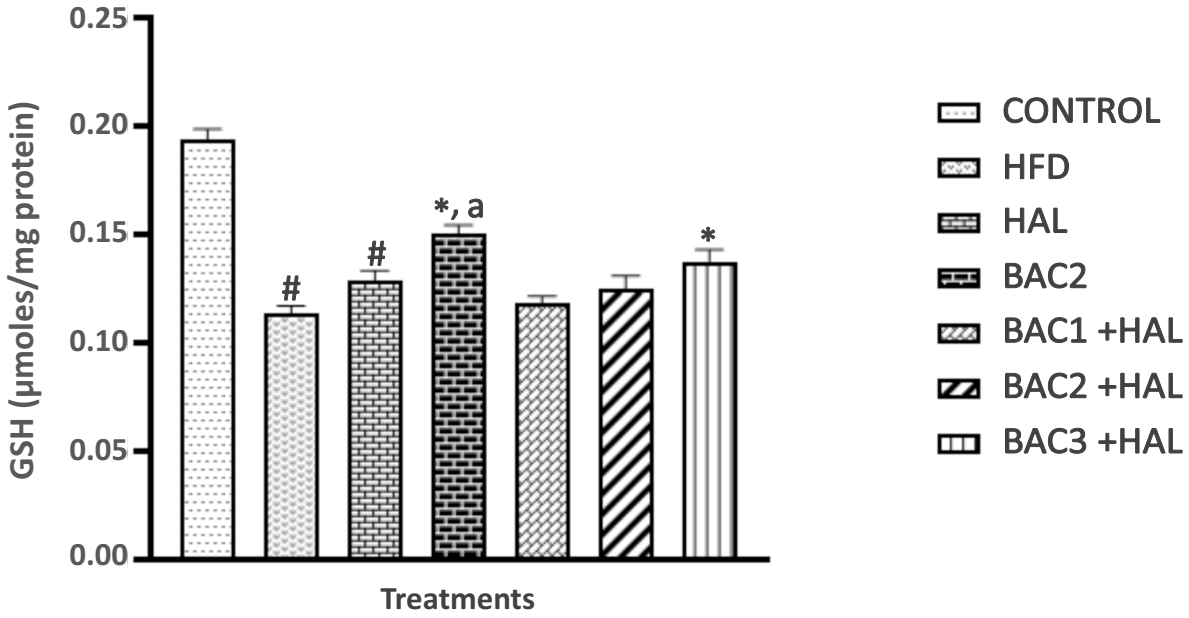

Figure 8: A figure showing the results of various treatment groups on the levels of GSH in mice. Values represent mean ± S.E.M for 6 animals per group. #P < 0.05 compared to normal control, *P < 0.05 compared to HFD and [a]P< 0.05 compared to HAP. HFD - High fructose diet, BAC - Baicalein, HAL - Haloperidol, GSH - Glutathione

DISCUSSION

Metabolic alterations are linked to several adverse health outcomes, including poor cognitive function, anxiety and stress-related disorders as well as oxidative stress.[11] Baicalein has been used in the treatment of hypertension, neurodegenerative diseases, inflammation, cardiovascular diseases, and respiratory infections.[15-16]

Generally, the overall results showed that Baicalein has some protective effects against elevated blood glucose levels induced by high fructose administration, reversed alterations in behavioural phenotypes in the Y-maze, hole board and open field tests. It also showed that Baicalein has protective roles in alterations caused by high-fructose diet (HFD) and haloperidol (HAL) in the antioxidant defense system as evidenced by elevation in the antioxidant activities in Baicalein treated groups.

In the open field test, high-fructose-diet (HFD) and haloperidol (HAL) decreased the number of lines crossed which is a measure of locomotion and motor activity. Low level of locomotor activity has been qualified as depressive state.[25] Haloperidol is known for its sedative and motor inhibiting effects.[26] The result implies that HFD and HAL have negative influence on motor activity. Baicalein (BAC) showed a slight initial decline but later increased which suggests some improvement in locomotion and that BAC may need to be administered for a longer time and probably higher doses to further explore its interaction with haloperidol in terms of locomotion. Baicalein combinations of different doses with HAL showed consistent low levels of motor activity. The non-significant rebound in activity across the days suggests that BAC may not counteract the sedative effects of HAL which strongly reduced locomotion as expected due to its sedative properties. HFD independently reduces activity which may be relevant in understanding how diet influences behaviour and metabolic health. While BAC did not counteract effect of Haloperidol, its mild effect on locomotion may suggest it may have therapeutic potential without significantly impairing movement.

Some recent studies suggest that intake of fructose, at doses ranging from 35 % to 60 % can induce impairments in spatial memory and learning in rodents.[27-29] The parameter indicative of memory in the Y-maze test is the ability of rodents to remember the sequence of arms entry commonly known as spontaneous alternation.30 In this study, the HFD showed fewer alternations and later some fluctuations which suggest that it could reduce memory function. The consistent but lower alternations suggest possible detrimental effect on memory due to the diet. Haloperidol group exhibited a moderate performance initially, but followed by fluctuations. This

reflects its known side effects which include cognitive dulling thus affecting ability to perform in the Y maze. The group administered with BAC alone showed an initial result mirroring that of control but then declined. However, on days 6 and 7, the increase in alternation compared to Haloperidol and HFD group confirms its neuroprotective effect and suggests its protective properties may occur with higher repeated dose administration. Also, the results of the various combinations of different doses of Baicalein suggest that the doses may not be strong enough to counteract the effect of Haloperidol in just seven days or it may not be effective at the doses tested. So, further dose optimization of BAC will be needed to understand its interaction with Haloperidol. Generally, it showed that HAL and HFD impaired cognition, while BAC alone exhibited some neuroprotection. The groups of BAC combined with Haloperidol may not show a promising outcome, possibly due to adverse drug interactions or due to overwhelming Haloperidol negative effects, or the dose administered may not be sufficient at that time in combination with Haloperidol.

It has been previously demonstrated that mice on a high-fat diet (HFD) developed significant insulin resistance (IR), and cognitive impairments.[31] This study revealed that HFD consistently raised blood glucose levels, indicating a potential diabetogenic effect, same as haloperidol on days 1,7, and 14. While the groups administered with Baicalein significantly decreased the blood glucose levels, which implies that Baicalein may help mitigate the effects of haloperidol and HFD on glucose levels. With regards to blood glucose level, on day 1, the group administered with Baicalein alone significantly reduced the blood glucose levels when compared with haloperidol- and HFD-treated groups, while HFD increased glucose levels when compared with control, though not significantly, which implies that it probably has no immediate significant effect with single dose administration. Though BAC alone decreased glucose level, in combination with Haloperidol, it may not have an immediate effect or impact on glucose levels. On day 8, the HFD- and Haloperidol-treated groups showed a significant increase in blood glucose levels when compared with the control, reinforcing the persistent impact on blood glucose levels. BAC alone plus BAC in combination with Haloperidol significantly reduced the glucose levels when compared with HFD and Haloperidol-treated groups which implies that Baicalein may help mitigate adverse effects of haloperidol and HFD on glucose levels. On day 14, HFD- and Haloperidol-treated groups showed similar elevation of glucose levels compared with control, and remained significantly higher, indicating a sustained hyperglycemia due to HFD and Haloperidol. Also, BAC alone group and BAC combined with HAL continued to show a significant reduction in glucose levels. This further suggests the lasting effect of BAC in reducing blood glucose levels over time, potentially normalizing them. One of the earliest pathological changes in many neurodegenerative disorders (NDDs), including AD, affects cell metabolism and, more specifically, glucose metabolism in neurons.[32] The results on blood glucose levels revealed the modulatory effects of baicalein as evidenced by the consistent reduction in blood glucose levels on all the days. Baicalein significantly reduced blood glucose levels over time which suggests that BAC may have protective effects against diet- and drug-induced hyperglycemia, making it a potential candidate for managing elevated blood glucose levels.

In the Hole Board test, the animals' exploration activity, known as head dipping, is thought to be a sign of anxiety. When both eyes vanished into the opening, it was considered a head dip.[33] It is thought that baicalein, along with other flavonoids, may underlie the anxiolytic effects of *S. baicalensis* and *S. lateriflora*.[34] The HFD-treated group showed a marked decline in head dips initially, but with fluctuations afterwards at a lower level when compared with control. This shows that it contributes to a reduction in exploratory behaviour, indicating increased anxiety levels, which could be due to dietary impact on brain function or overall energy levels. The haloperidol-treated group showed a decrease in head dips and was consistently lower than the control, which aligns with its pharmacological effects of sedation and reduction in exploratory activity. BAC- treated group showed an initial decline but significant recovery later, exceeding that of HFD and Haloperidol groups. Initial reduction of exploration may be a delayed anxiolytic or stimulating effect, leading to an increased number of head dips by later days, thus confirming its anxiolytic properties. The combined groups with BAC at different doses showed that the lower dose was not significant enough to counteract these effects thus the reduced exploratory behaviour. This suggests that in combination with Haloperidol, BAC may not be enough to suppress the effects of Haloperidol at that dose and that time frame.

HFD appeared to reduce exploratory behaviour thus increasing anxiety-like behaviour when compared with control. BAC alone shows a recovery of exploration over

time but in combination with HAL, not seemingly strong enough; so its ability to counteract Haloperidol effect is limited.

Catalepsy involves being insensitive to external stimuli or the limbs remain in whatever position they were placed in[35] and it is a major symptom of Parkinson's disease (PD). PD is characterized by motor symptoms like tremors, stiffness, and slow movement.[36] In the current investigation, haloperidol produced a significant amount of catalepsy in mice while high-fructose-diet did not induce a sustained catalepsy. This catalepsy was significantly reduced by the administration of baicalein. The various treatment groups of Baicalein appeared to mitigate the cataleptic effect of haloperidol suggesting possible protective effect against haloperidol-induced catalepsy.

Mitochondrial dysfunction and the formation of reactive oxygen species (ROS) are both common pathological mechanisms that trigger neurodegeneration in several dementia-associated diseases, including AD and PD.[37] Haloperidol has been found to impair the activity of enzymes such as catalase and superoxide dismutase in rat brain.[38] High fructose consumption has been implicated in the progression and severity of non-alcoholic fatty liver disease (NAFLD), increasing insulin resistance, oxidative stress, inflammation, and fibrosis.[39-40] This present study showed that baicalein significantly increased levels of superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH) while the HFD- and Haloperidol-treated groups significantly decreased their levels. This is in line with previous studies that have found Haloperidol and HFD to alter or negatively impact the levels of these antioxidants. This result also suggests that baicalein has a mitigatory effect on oxidative species by elevating the antioxidant biomarkers.

CONCLUSION

This study suggests and further confirms that high-fructose diet and haloperidol have negative impact on cognition, motor activity, anxiety, and blood glucose levels as well as on the antioxidant defense system. Thus, it can be concluded that Baicalein attenuates behavioural dysfunction and blood glucose levels in mice, as well as

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