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Research Article
Effects of Metformin and Aspirin on Spatial Working and Spatial Recognition Memory in an Aging Mouse Model: A Behavioural and Histological Study

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Abstract

Background and Objective: Memory loss and cognitive impairment are two of the most common age-related changes and the exploration of possible interventions has attracted significant attention. Metformin (MET), the most widely used antidiabetic drug and aspirin (AS), the most widely used anti-thrombotic drug, have both been shown to exert neuroprotective effects. This study explored the possible effects of MET and AS in attenuating memory loss in an aging model established by the administration of D-galactose (200 mg/kg). **Materials and Methods:** Forty adult mice were separated into 5 groups at random (n = 8 each): Control, aging, MET (aging model injected with 100 mg/kg MET), AS (aging model injected with 10 mg/kg AS) and MET+AS (aging model injected with 100 mg/kg MET and 10 mg/kg AS). Anxiety-like behaviour was evaluated using the elevated plus maze test; two memory tests spatial working and spatial recognition were evaluated using Y-maze. Haematoxylin and Eosin (H&E) staining was used for histological assessment of hippocampal tissue. **Results:** The MET attenuated anxiety-like behaviours and markedly ameliorated hippocampal damage in aging mice. Moreover, MET-mice group showed considerably improved spatial working memory in the Y-maze compared with the aging group. The AS did not significantly affect spatial working memory or spatial recognition memory performance and MET and AS co-treatment did not have any favourable additive effects on memory deterioration. **Conclusion:** These results suggested that MET treatment improved spatial working memory by reducing hippocampal tissue damage in this model of aging and that there was no significant effect of AS or combination treatment.

Key words: Aging, metformin, aspirin, spatial working memory, spatial recognition memory, anxiety-like behaviour

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The physiological process of aging is complex and unavoidable and is characterised by the progressive structural and molecular deterioration of various tissues and cells in the body[1]. Moreover, several pathological disorders can result from the gradual alterations in physiological functions that occur with aging[2]. Cognitive deterioration is a critical effect of aging, particularly when it affects learning and memory, as this can be a significant driver of various disabilities that can reduce quality of life[3,4]. Therefore, there has been an increasing interest in research on extending lifespan in animal models of aging using different nutritional and therapeutic interventions[5].

The Metformin (MET) has been the most widely used antidiabetic drug for more than 60 years. It controls blood glucose levels by improving glucose utilisation and by reducing gluconeogenesis[6]. Studies have established the high safety profile of long-term MET use and also its efficacy in attenuating the aging process via several physiological pathways[5,7]. Consequently, its anti-aging benefits are receiving increasing attention[8]. The MET administration was reported to ameliorate aging-related diseases, including cancer and cardiovascular diseases, as well as cognitive impairments[9,10]. Moreover, MET could increase life expectancy in the D-galactose-induced aging model in mice[11,12]. The MET administration can lower intracellular reactive oxygen species (ROS) levels by enhancing antioxidant enzyme activity and acting as an anti-inflammatory agent[13]. It can easily pass through the blood-brain barrier and disperse throughout several brain regions and has been demonstrated to have neuroprotective effects against several neurodegenerative conditions, such as Alzheimer's disease[14].

The aspirin (AS) acts as an effective anti-inflammatory agent in many tissues and has been shown to act on a variety of therapeutic targets[15]. Over the past three decades, AS at doses of 75-300 mg/day has been extensively used to reduce the risk of thrombotic cardiovascular and cerebrovascular disorders[15]. Significant biological effects, such as anti-inflammatory and anti-thrombotic actions have been reported in several neurological conditions as well[16]. The AS also acts as an antioxidant by reducing the generation of several ROS and by elevating the activity of antioxidant enzymes[17]. Long-term treatment with AS was demonstrated to reduce the learning and memory impairments associated with diabetes and these effects were shown to be related to the strength of its anti-inflammatory efficacy16. The low-dose AS treatment could improve learning and memory in an aging rat model, although a high dose aggravated the mental symptoms in aging animals[16,18,19].

The aging-related memory disorders have gained widespread research interest exploring possible therapeutic interventions. Numerous studies have investigated MET and AS as prophylactic and therapeutic agents for the treatment of cognitive disorders. The MET and AS have been found to share some underlying mechanisms of neuroprotective effects, including antioxidant and anti-inflammatory properties. However, the synergistic or additive effects of their combination on deteriorating learning and memory in D-galactose aging model have not been explored. Current work aimed to explore the effects of MET and AS alone and in combination on short-term spatial working memory and spatial recognition memory in aging mice as well as their impact on the hippocampus, which is the main memory-related area of the brain.

MATERIALS AND METHODS

Study area: This study was conducted in the Pharmacy College, King Abdulaziz, University, Saudi Arabia, from 2022 to 2023.

Animals: A total of 40 male SWR/J mice were used in this study (age: 6-8 weeks, corresponding to 14-20 years in humans, body weight, 18-22 g). The animals were purchased from the Laboratory Animal Center of King Abdul-Aziz University Pharmacy College, Jeddah, Saudi Arabia and housed in five plastic cages (8 mice/cage) at an average room temperature of 23±2°C and with *ad libitum* access to a standard diet and water and a standard feed.

Ethical consideration: The Pharmacy College Animal Unit Committee's Recommendations were followed when handling and caring for the mice. The King Abdul-Aziz University Biomedical Ethics Research Committee approved all experiments (Reference No. PH-1443-40).

Treatments: The D-galactose solution was prepared by dissolving extra pure D-galactose (Sigma-Aldrich, USA) in normal saline and given daily by subcutaneous injection at the dose of 200 mg/kg. Metformin (Glucophage™, 500 mg) was dissolved in normal saline and given daily by intraperitoneal (i.p.) injection at the dose of 100 mg/kg[20]. Aspirin (Bayer, Germany,100 mg) was dissolved in normal saline and given daily by i.p. at the dose of 10 mg/kg. Weekly dose adjustments were made based on mouse weight.

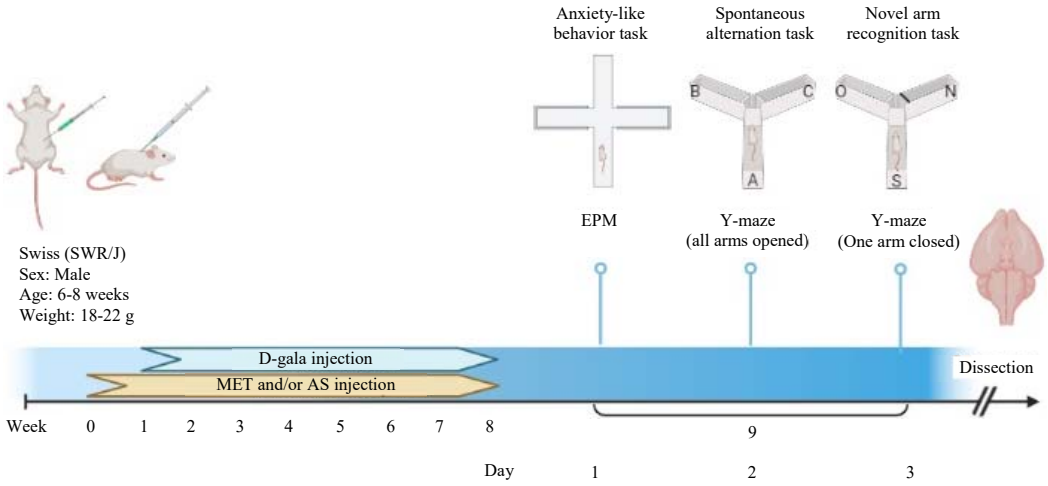

Fig. 1: Experimental timeline with an overview of the treatments and behavioural tasks

Total study duration was 8 weeks. The MET and AS were administered from week 0, whereas D-gala was administered from week 1. Behavioural assessments were conducted in week 9. On the 4th day of week 9, mice were sacrificed and the brains were removed for histological analysis. D-gala: D-galactose, MET: Metformin, AS: Aspirin, EPM: Elevated plus maze and created using BioRender.com

Experimental design: A schematic of the experimental schedule followed in this study was shown in Fig. 1. After 5 days of acclimatization, the 40 mice were separated into five groups at random (n = 8 each): The control group, aging group, aging group treated with MET, aging group treated with AS and aging group treated with a combination of both MET and AS. Mice in the control group were given 0.2 mL of normal saline intraperitoneally and 0.2 mL subcutaneously once daily. All treatments were administered for 8 weeks (six consecutive days per week). The MET and AS were administered from week 0, whereas D-galactose was administered from week 1. Behavioural tasks were initiated at week 9. On the 4th day of week 9, brain tissues of sacrificed animals were extracted for histopathological analysis.

Body weight assessment: During the experiment, the mice's weight was noted weekly and the percentage of weight gain was calculated using the following formula:

$$\text{Weight gain (\%)} = \frac{\text{Recent weight of animal} - \text{Initial weight of animal}}{\text{Initial weight of animal}} \times 100$$

Behavioural tests

Anxiety-like behaviour assessment: To measure anxiety-like behaviour in experimental mice, the elevated plus maze task (EPM) d maze made up of two closed arms (25×5×16 cm), two open arms (25×5×0.5 cm) and a central platform (5×5 cm). Each animal was placed on the maze's central platform, facing the closed arm and given 5 min to freely explore the maze which is known as a test period. The experiment was performed in a quiet room and the maze was cleaned with 70% ethanol to get rid of any lingering animal smells or residues before each session.

The following equation was used to calculate the anxiety index[21,22]:

$$\text{Anxiety index} = 1 - \frac{\left[\left(\frac{\text{Open arm time}}{\text{Total time in maze}}\right) + \left(\frac{\text{Open arm entries}}{\text{Total entries}}\right)\right]}{2}$$

The anxiety index has scores ranging from 0 to 1, the scores close to 1 indicating a high level of anxiety[23].

Spatial working memory: The spatial working memory of the mice has been assessed by several behavioural tasks including the assessment of spontaneous alterations in the Y-maze[24]. The Y-maze apparatus used in this experiment consisted of three arms of the same length (60 cm), width (10 cm) and height (15 cm) and labelled A, B and C. The mouse is allowed 5 min to explore the maze[25]. During the 5 min of the test, if the mouse visited the ABC, BAC or CBA arms consecutively without repetition, it was given an alternation score. An alteration score was assigned using the following equation:

$$\text{Spontaneous alterations (\%)} = \frac{\text{Number of alternations}}{\text{Total arm entries} - 2} \times 100$$

Additionally, the number of total arm entries was recorded to assess the locomotor activity of mice.

Spatial recognition memory assessment: Spatial recognition memory of mice is a type of memory that was measured by the novel arm recognition test in a previous by Alzahrani *et al.*[26]. The procedure included two trials: A sample and a test trial. In the sample trail, one arm of Y-maze was closed named as novel arm (N) and the mice freely explored the other maze arms for 3 min. A sample trial was implemented after 10 min, in which the N was unlocked and again the mouse was free to explore the maze arms for 3 min. Spatial recognition memory was measured by calculating of time spent in the N, as animal with normal recognition memory was spending more time investigating the N.

Histological assessment: The mice were sacrificed after being anaesthetised using isoflurane. The brain was extracted, fixed in 10% formalin for 24 hrs. The sagittal cut of the brain was embedded in paraffin wax and sectioned (section thickness, 4-5 µm). After fixing the brain section on the glass slide, Hematoxylin and Eosin (H&E) was used to stain it according to the manufacturer's protocol.

Statistical analysis: All values are presented as the Mean±Standard error of mean. GraphPad Prism (9.0.2) software was used for statistical analyses. One-way Analysis of Variance (ANOVA) followed by the appropriate *post-hoc* test was used for comparisons among groups. If the $p<0.05$, differences between groups were considered statistically significant.

RESULTS

Effect of MET and AS on body weight: The percentage weight change was measured over the entire 8-week period that MET and AS were administered. The weight change between the control group and the other treated groups did not differ significantly (two-way repeated-measures ANOVA ($F(28, 224) = 0.3367$, $p = 0.9994$) Fig. 2.

Effects of MET and AS on anxiety-like behaviour: The anxiety index in the EPM task was used to evaluate the effects of MET and AS on anxiety. The anxiety index was considerably higher in the aging group compared to control group ($p<0.0001$) (Fig. 3) and considerably lower in the MET group compared to aging group ($p<0.0001$). Anxiety was not significantly ameliorated in the AS and MET+AS groups compared to aging group ($p = 0.9582$ and 0.5921, respectively).

Effects of MET and AS on spatial working memory: The impacts of MET and AS on mouse spatial working memory

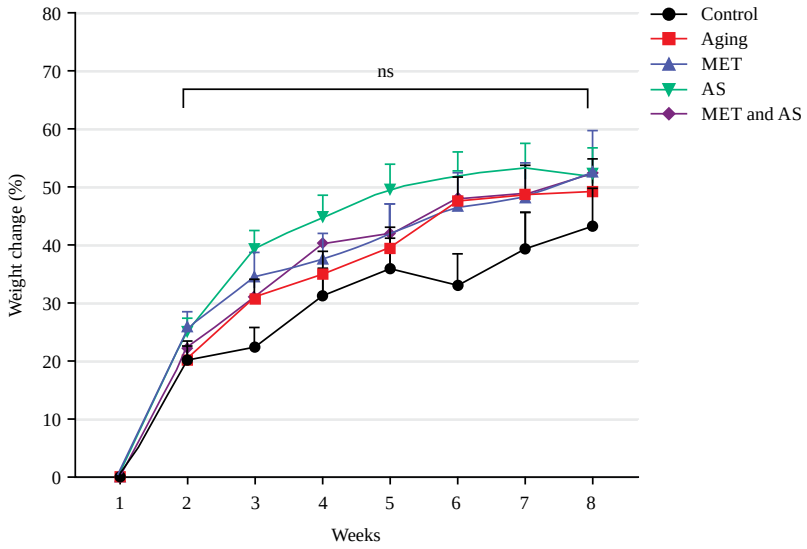

Fig. 2: Effects of MET and AS on percentage weight change

No statistically significant differences were seen in body weight between the groups, values represent as Means±SEM. Two-way repeated-measures ANOVA was used followed by the Bonferroni multiple-comparisons test. MET: Metformin, AS: Aspirin, SEM: Standard error of the mean, ANOVA: Analysis of Variance, ns: Non-significant, *$p<0.05$ and ****$p<0.0001$

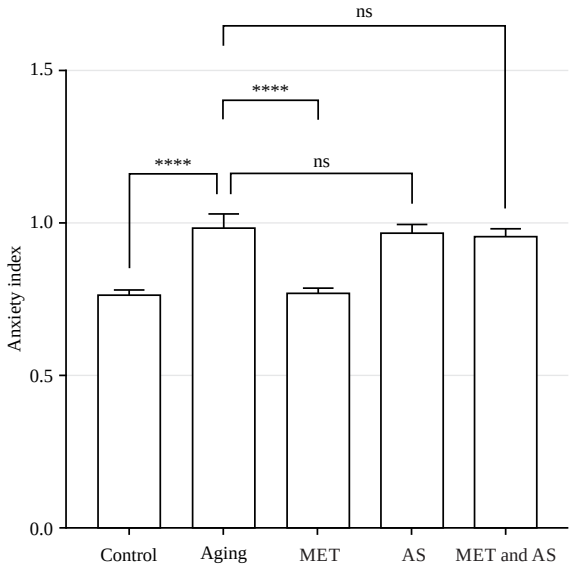

Fig. 3: Effects of MET and AS on anxiety index

A highly significant increase was showed in the anxiety index of the aging group compared to the control group. A highly significant decreases was showed in the anxiety index of the MET group compared to the again group. Values represent as Means±SEM and two-way repeated-measures ANOVA was used followed by Tukey's *post-hoc* test. MET: Metformin, AS: Aspirin, SEM: Standard error of the mean, ANOVA: Analysis of Variance, ns: Non-significant, *p<0.05 and ****p<0.0001

were assessed based on the SAP in the Y-maze test. Compared with the control, the SAP was considerably reduced in the aging group ($p = 0.0108$) (Fig. 4a). Compared with the aging group, the SAP was considerably improved in the MET group ($p = 0.0420$). Whereas, in the AS group and MET and AS group, the spatial working memory was not significantly ameliorated ($p = 0.2396$ and $p = 0.4631$). The locomotor activity of mice groups was determined by recording the number of arm entries in Y-maze. Among mouse groups, there was no considerable difference in the number of arm entries. The ($F (4, 35) = 0.557$ and $p = 0.6949$)). Between the aging group and the control, there was not a significant variance in the number of arm entries ($p = 0.7988$) (Fig. 4b). Moreover, no significant change was detected in the total number of arm entries in MET group ($p = 0.9843$), AS group ($p = 0.9994$) and MET+AS ($p = 0.9990$) compared to the control group.

Effects of MET and AS on spatial recognition memory: In the novel arm (N) recognition task, the aging group spent less time in the N comparing with the control group ($p = 0.0166$) (Fig. 5). No significant variance was observed in the time spent in the N between the aging and the MET ($p = 0.3446$), AS ($p = 0.2574$) and MET+AS ($p = 0.1830$) groups.

Histological examination of the effects of MET and AS on the hippocampus: The hippocampus was examined histologically to identify the histopathological alterations in the different groups. The dentate gyrus (DG), cornu ammonis 1 (CA1) and CA3 are the principal hippocampal regions involved in memory function. The H&E staining of the control group samples revealed that the shape and structure of the hippocampus, including the DG, CA1 and CA3 were normal.

In the control group, three distinct layers could be observed in both the CA1 and CA3 Fig. 6(a-b): The outer molecular layer (ML), middle pyramidal cells (PC) layer (PCL) and inner polymorphic layer (PML). The PCL was composed of several layers of PC, which appeared larger and loosely packed in the CA3 and smaller and densely packed in the CA1. The PC had a triangular shape, with pale basophilic cytoplasm and large, rounded vesicular nuclei. Both the ML and PML consisted of a pinkish neuropil that contained axons and dendrites, scattered neuroglial cells (NGs) and some blood capillaries.

Three layers could also be observed in the DG (Fig. 6c): Outermost PML, middle granular layer (GL) and innermost ML. The GL was composed of small, compact granular cells (GCs) with rounded nuclei. The ML and PML contained the axons of the GCs and a few NGs and capillaries. In the aging group, the PCL of the CA1 and CA3 had the most significant alterations and appeared disorganised compared to that in the control group. Many PC showed degenerative changes and appeared shrunken, with condensed nuclei and a dark cytoplasm with scattered vacuoles Fig. 6(d-e). Regarding the DG (Fig. 6f), the most significant alterations

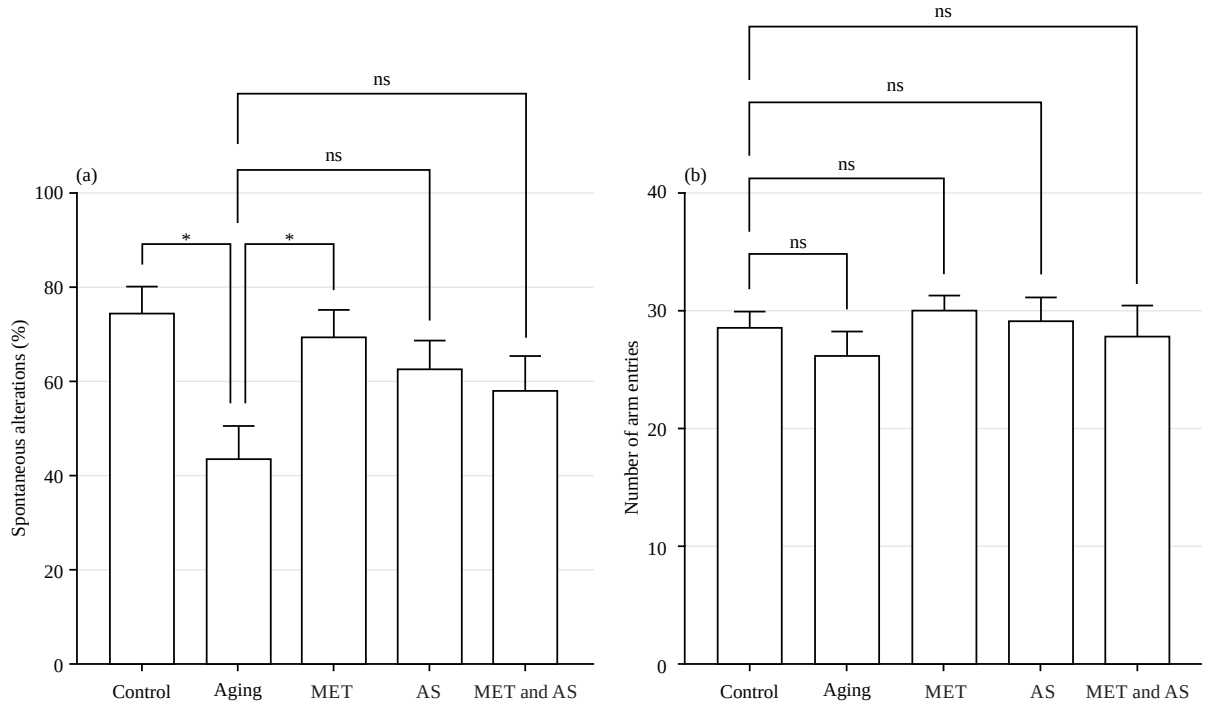

Fig. 4(a-b): (a) Effects of MET and AS on anxiety index and (b) Effects of MET and AS on the total number of arm entries

(a) SAP was significant reduced in the aging group compared to the control group. The SAP was significant improved in the MET group compared to the again group, (b) There was no significant variance in the number of arm entries among the group, Values are presented as Means±SEM and one-way ANOVA was used, followed by Tukey's *post-hoc* test. MET: Metformin, AS: Aspirin, SEM: Standard error of the mean, ANOVA: Analysis of Variance, ns: Non-significant, *p<0.05 and ****p<0.0001

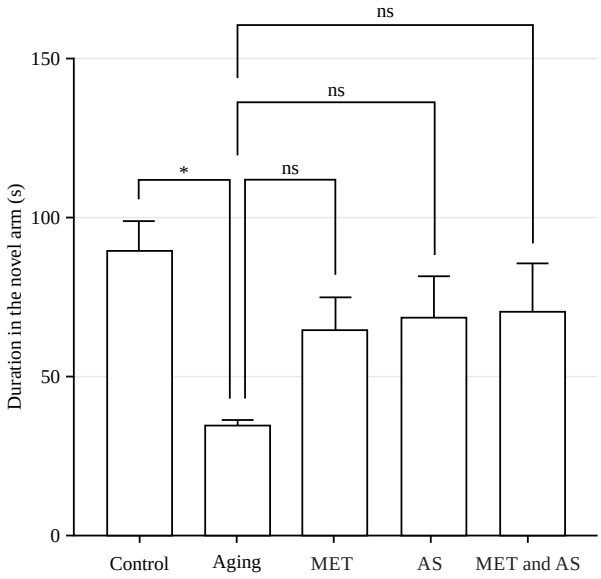

Fig. 5: Effects of MET and AS on time spent in the N

There was a significant reduction in the time spent in the N in the aging group compared to the control group. There was no significant elevation in the spent in the N arm in the other treatment group compared with the again group, Values are presented as the Means±SEM, one-way ANOVA was used, followed by Tukey's *post-hoc* test. MET: Metformin, AS: Aspirin, N arm: Novel arm, SEM: Standard error of the mean, ANOVA: Analysis of Variance, ns: Non-significant, *p<0.05 and ****p<0.0001

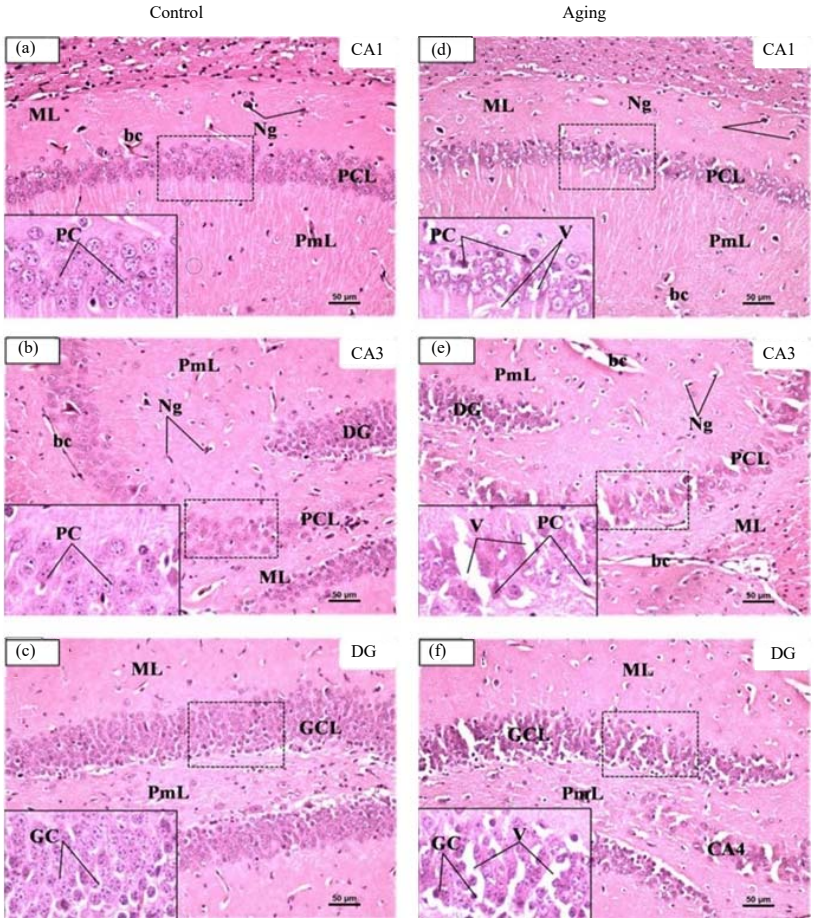

Fig. 6(a-f): Histological analysis of control and aging groups hippocampal tissue, (a) CA1 is composed of three layers, (b) CA3 was composed of three layers, (c) Three layers were observed in both the upper and lower limbs of the DG, (d) The CA1 had disorganised PCL cells compared to the control group, (e) CA3 also had a disorganised PCL compared to the control group and (f) DG had a dense GCL that contained many dark condensed GCs that separated by V (inset)

(a) Outer ML, middle PCL and inner PML. The PCL was composed of well-organized PCs with pale basophilic cytoplasm and large rounded vesicular nuclei. Both the ML and PML contained NGs and a few bc, (b) ML, PCL and PML. The NGs and a few bc were observed in the ML and PML, (c) ML, GCL and PML. GCL in the upper limb of the DG was composed of densely packed small, rounded to oval Gcs, (d) Several PCs appeared shrunken, with condensed nuclei and dark cytoplasm with scattered V (inset). There were more NGs and dilated bc in the ML and PML and (e) Several PCs appeared shrunken, with condensed nuclei and dark cytoplasm with scattered V. Both the ML and PML contained more NGs, V and dilated bc. Scale bar: 50 µm, CA1: Cornu ammonis 1, PCL: Pyramidal cell layer, PC: Pyramidal cell, V: Vacuoles, NG: Neuroglial cell, bc: Blood capillaries, ML: Molecular layer, PML: Polymorphic layer, CA3: Cornu ammonis 3, PCL: Pyramidal cell layer, DG: Dentate gyrus and GCL: Granular cell layer

were observed in the GCL, which contained several disorganised, condensed and shrunken GCs. Additionally, the ML and PML of the DG had increased numbers of dilated bc and NGs.

In the MET group, there was a marked improvement in the histopathological changes in the CA1 and CA3 in comparison with the aging group and the PCs were preserved in shape and showed vesicular, rounded nuclei Fig. 7(a-b). The architecture of the DG was also improved, with a few changes in the GCL (dark, condensed GCs) (Fig. 7c).

In the AS group, the hippocampus examination revealed relative improvements in histopathological changes compared to those in the aging group, most PCs in the CA1 and CA3 were preserved in shape, but a few cells appeared shrunken with a dark cytoplasm. In addition, the ML and PML had more dilated capillaries and NGs compared to those in the control group Fig. 7(d-e). In the DG (Fig. 7f), some GCs appeared shrunken and condensed.

In the MET and AS groups, some cells appeared shrunken with a dark cytoplasm in the CA1 and CA3 Fig. 7(g-h).

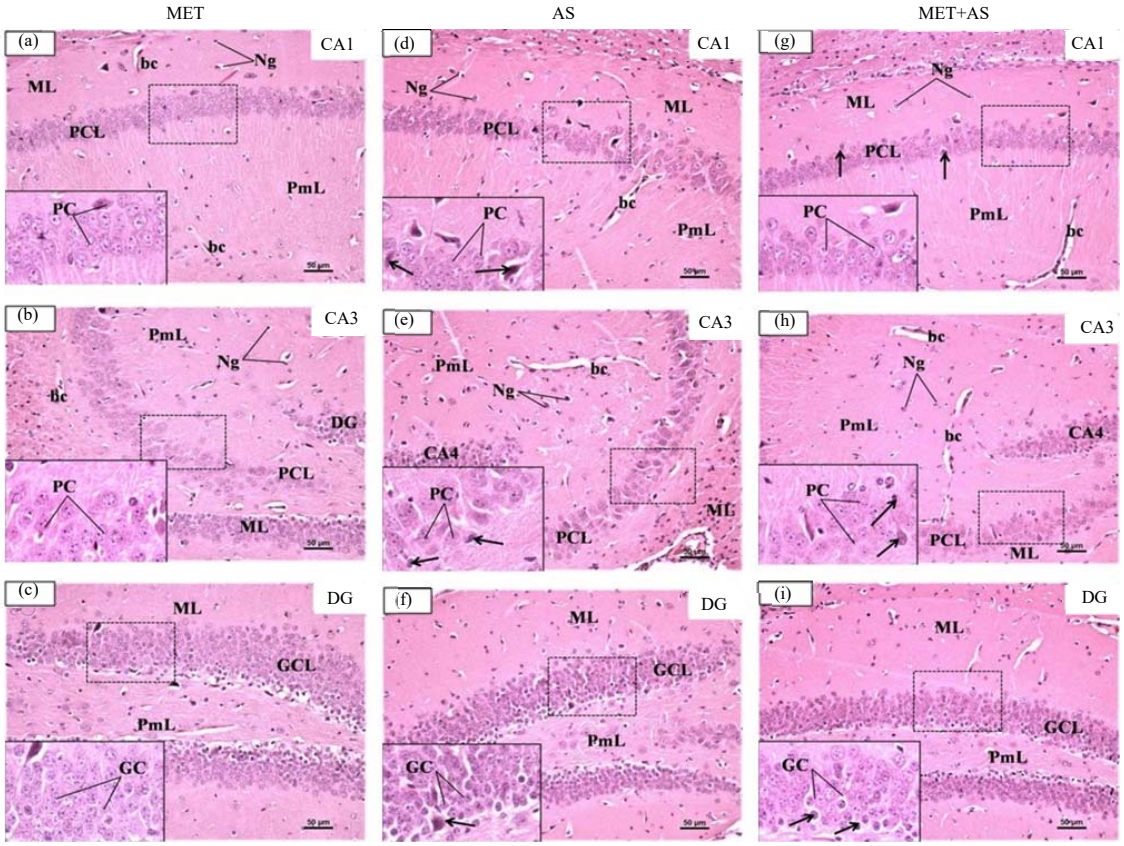

Fig. 7(a-i): Histological analysis of MET, AS and MET+AS groups hippocampal tissues, (a-b) In the CA1 and CA3 of MET, there was a marked improvement in the histopathological changes compared to those in the aging group. PCs were preserved in shape and showed vesicular, rounded nuclei, (c) There were few changes in the GCL of the DG (black arrow) in comparison with aging group, (d-e) In the CA1 and CA3 of the AS group, there were a few PCs in PCL that appeared shrunken and had a dark cytoplasm (black arrow). In the ML and PML, there were more NGs and some dilated bc than in the control group, (f) In the DG, GCL (inset) contained normal rounded GCs, except some cells that appeared condensed (black arrow), (g) In the CA1 PCL of the MET+AS group, some cells appeared shrunken and had dark condensed nuclei (black arrow). In the ML and PML, there were more NGs and bc, (h) In the CA3, the presence of some shrunken cells with dark condensed nuclei (black arrow) was observed in the PCL. There were some NGs and dilated bc in the ML and PML and (i) In the DG, GCL (inset) mainly had normal GCs, with some cells that appeared condensed (black arrow)

Scale bar: 50 µm, CA1: Cornu ammonis 1, PCL: Pyramidal cell layer, PC: Pyramidal cell, NG: Neuroglial cell, bc: Blood capillaries, ML: Molecular layer, PML: Polymorphic layer, CA3: Cornu ammonis 3, PCL: Pyramidal cell layer, DG: Dentate gyrus, GCL: Granular cell layer and GC: Granular cell

There was a moderate improvement in architecture, with the main changes occurring in the GCL of the DG in comparison with aging group (Fig. 7i).

DISCUSSION

The physiological process of aging is associated with the impairment of several memory-related abilities, which is known as age-associated memory impairment. The decline in both short and long-term memory associated with aging has been indicated in a number of human and animal studies[26,27]. Nevertheless, some of the intrinsic biological changes associated with aging can be targeted using therapeutic interventions[5]. The MET and AS have been in clinical use for many years, have been studied extensively, have good safety profiles and possess unique properties that can regulate several important pathways involved in aging and age-related conditions[5,15]. However, the neuroprotective potential of the

combination of the two against age-related memory impairment has not yet been investigated. Thus, this study, aimed to explore the potential neuroprotective impacts of MET and AS, alone and in combination, against memory decline in a D-galactose-induced senescence animal model, focusing on how these drugs can affect short-term memory.

Several studies investigating the impacts of MET and AS on aging have used natural aging animal models. However, we conducted our study on a D-galactose-induced aging model, which suitably mimics memory decline during aging[28,29]. The ability to analyze the progression of aging in isolation is one of the advantages of the senescence model induced by D-galactose. However, in natural aging animal models, comorbidities such as, malignancy, hypertension and diabetes are additional confounding factors[11].

Our results indicate that the administration of D-galactose (200 mg/kg for 6 weeks) leads to several age-related alterations, including short-term memory impairment, increased anxiety-like behaviour and histological alterations in the brain.

The percentage of weight gain to assess the general health of the mice was evaluated for the first time. Current findings indicated that there was no remarkable weight loss in the aging model and other treatment groups in comparison with the control. Thus, current data reported the good health of animals and the lack of possible toxicity. However, these results were inconsistent with those of a previous study, which demonstrated that the body weight of the D-gala-induced model was noticeably lower than the control[30]. The possible reason for this difference in results could be the differences in the models used and the duration of aging induction.

The locomotor activity of the mice was evaluated to ensure normal functioning and that there was no interference with memory. In this study, the result of Y-maze task indicated that the total number of arm entries, which is indicative of motor activity, did not differ among the groups. This result was consistent with the results of other investigations that revealed no considerable difference between the control and the aging group in terms of locomotor activity[11,30].

Working memory is commonly affected by aging owing to neuronal damage[31,32]. In our study, the Y-maze tests revealed a considerable decline in spatial working memory in aged mice compared to that of the control mice. Thus, the results found that the spatial working memory of aging mice was impacted as reported in previous studies[33,34].

Another type of spatial memory, spatial recognition memory, was also assessed in this study. The hippocampus-dependent "novel arm recognition task" is frequently used to assess spatial memory in rodents[35-37]. Current findings showed that the aging group significantly spent less time in the N than the control group, which was indicative of a decline in spatial recognition memory.

The aging process is commonly associated with memory impairment, but it is also associated with emotional dysfunctions such as anxiety. About 15-28% of older adults show anxiety symptom that affect their life quality[35,38,39]. Several previous studies reported that chronic administration of D-galactose elevates anxiety-like behaviour in rodents, mainly due to increasing ROS production[40,41]. Consistent with these investigations, current study EPM results demonstrated an increase in anxiety-like behavior in the D-galactose group.

Histological examination was performed using H&E staining to assess hippocampal neurodegeneration associated with cognitive decline. Examination of H&E-stained sections of the aging group showed the existence of degenerative alterations in CA1 and CA3 areas of the hippocampus compared to the control group. Similarly, several studies have also reported that D-galactose administration alters the typical hippocampal cells in several regions, indicating hippocampal degeneration[29,36,42]. As hippocampal damage often induces spatial memory impairments, these results link our behavioural observations with the histological findings[43-45].

Several studies have reported that MET improves the cognitive deterioration associated with aging[37-39]. In current study, the behavioural analyses showed that MET treatment considerably improved spatial working memory in the aging mice, similar to previous studies finding[4,11,46]. Results found that MET treatment, ameliorated anxiety-like behaviour in the aging mice which was consistent with several previous studies reported the anxiolytic effect of MET[47-49].

To our knowledge, no study has investigated the effects of MET on spatial recognition memory using novel arm-recognition. The novel arm recognition task results indicated that MET administration could not prevent the deterioration of spatial recognition memory in the aging group. However, according to the histological examination, MET treatment did result in a marked reduction in the histopathological changes in the hippocampus compared to those in the aging group, with a nearly normal appearance of the PCL in the CA1 and CA3 regions, which confirmed the neuroprotective effect of MET.

Neuroprotective effects of AS have been reported in several studies on aging, suggesting beneficial effects of AS on cognitive function[42,43]. However, few animal studies have

investigated the effects of AS on spatial working and spatial recognition memory in aging models. Current findings indicated no significant effect of low-dose AS treatment on spatial memory performance, which does not agree with a previous study that reported that chronic AS administration improved spatial learning in aged rats[47]. However, current findings agreed with those of several clinical studies indicating that low-dose AS does not improve cognitive function in older individuals[45,46]. Furthermore, histological examination of the hippocampus in the AS group revealed very mild improvements in histopathological changes.

The MET and AS have been found to share some underlying mechanisms of neuroprotective effects, including anti-oxidant and anti-inflammatory properties[47,48]. Several studies have investigated the possible synergistic effects of MET and AS on diabetes[48,49] and certain cancer types[48,50]. Interestingly, no previous study has investigated the combined effects of MET and AS on memory deterioration associated with aging. The present study indicated that MET does not potentiate the effects of AS on spatial working or spatial recognition memory, as indicated by the Y-maze test results. Furthermore, histological examination did not show a considerable difference between the histological improvements in the MET and combination groups. Consequently, no additive or synergistic effect was observed when MET and AS were combined, which may be attributable to several factors. The dose of MET (100 mg/kg) used in this study may have a maximal effect on enhancing spatial memory and alleviating anxiety in an aging model. Moreover, both MET and AS could have a competitor or drug interaction on the same receptor sites, which could have an impact on the potential synergistic or additive effects of administrating these drugs in combination.

In this work, AS alone and in combination did not considerably affect anxiety and spatial working or recognition memory. However, this could be due to the lower dose of AS. Further investigations, including biochemical and genetic analyses, are required to gain further insights into the underlying mechanisms of action of MET and AS.

CONCLUSION

The present study provides evidence indicating that chronic MET administration has a strong protective effect against the spatial working memory decline and anxiety-like behaviours associated with aging. However, administration of low dose AS alone had no effect on spatial memory impairment or anxiety and co-treatment with MET and AS did not exert additive or synergistic effects on cognitive impairment in the aging mouse model.

SIGNIFICANCE STATEMENT

Metformin (MET) and aspirin (AS) have been shown to exert neuroprotective effects. The present study was to evaluate the possible effects of MET and AS in attenuating memory loss in an aging model. The MET administration has a strong protective effect against the spatial memory impairment and anxiety-like behaviours associated with aging. However, administration of AS had no effect on spatial memory impairment or anxiety, this could be due to the lower dose of AS. The evaluation of the neuroprotective potential of these drugs and results will be helpful in future studies of optimal doses and the underlying mechanisms of these drugs action against memory dysfunctions.

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