Prepubertal continuous dietary folate fortification enhances brain function of adult mice by modulating antioxidant status, inflammation and brain neurotransmitter levels

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Research Article

Keywords: Metabolism, Neurobehaviour, Nutrients, Neurodevelopment, Puberty

Posted Date: January 9th, 2023

DOI: https://doi.org/10.21203/rs.3.rs-2433962/v1

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Abstract

Background

There is a growing body of knowledge in support of the beneficial effects of folic acid supplementation. However, while ample evidence exists concerning beneficial effects on growth and haematologic parameters, possible effects of continuous folic acid supplementation on the brain are less examined.

Objectives

To investigate possible effect of dietary folic acid supplementation (beginning in the prepubertal period) on neurobehaviour, oxidative stress, inflammatory parameters and neurotransmitter levels in adult mice.

Methods

Forty-eight prepubertal male mice (postnatal day 21) were randomly assigned into four groups of 12 (n = 12) animals each. Mice were grouped into normal control (fed standard diet) and three groups fed folic acid supplemented diet at 2.5, 5 and 10 mg/kg of feed. Daily food intake and weekly body weight were assessed. Animals were fed standard diet, or folic acid supplemented diet for a period of eight weeks. On postnatal day 78, animals were exposed to behavioural paradigms (Open-field, Y maze, radial arm maze, elevated plus maze, bar test and models of behavioural despair). Twenty-four hours after the last behavioural test, animals were fasted overnight following which they were sacrificed, and blood taken for assessment of blood glucose, leptin, and insulin levels. The brain of the animals were also homogenised for the assessment of biochemical parameters (lipid peroxidation, total antioxidant capacity, inflammatory markers, dopamine, brain derived neurotropic factor, acetylcholine and acetylcholinesterase activity).

Results

Results showed a concentration dependent increase/improvement in body weight, antioxidant status, memory scores (in the radial arm and Y maze) and acetylcholine levels; and a decrease in food intake, blood glucose, insulin, and leptin level. A reduction in open field behaviours, anxiety-related behaviours, and proinflammatory markers were also observed.

Conclusion

The beneficial effects of prepubertal continuous dietary folate fortification in specific contexts relating to behaviour, cognition, oxidative status, metabolic hormones and brain neurochemistry (as the animal ages) are shown in the study.

1.0 Introduction

In the last few decades, there have been significant advances in our understanding of the crucial roles played by nutrition, nutritional supplements and dietary choices in brain development, maintenance of brain health, and the development of age-related neurodegenerative disorders [1–7]. Evidence from several studies have demonstrated the benefits of adequate nutrition and dietary supplementation (with vitamins and micronutrients) in normal brain development, prevention of brain disorders, and the mitigation of psychiatric and neurodegenerative disorders [3–6, 8–18]. In humans, the periods during pregnancy and infancy are crucial for the formation of the brain as well as the laying down of the foundation for the development of motor, cognitive, and socio-emotional skills. There are also strong indications that nutritional deficiencies (in addition to causing abnormal brain development in the immediate postnatal period) can also impair brain function and increase susceptibility to diseases later in life [12, 15].

Folate is an essential vitamin that is crucial for nucleic acid synthesis and a methyl donor in one-carbon metabolism [11, 12, 19, 20]. Folate deficiency is associated with impaired growth, low energy, and the development of megaloblastic anaemia [21]. There is also overwhelming evidence supporting the crucial role of folate in normal brain development, necessitating an increase in the advocacy for prenatal and antenatal folic acid supplementation.
Periconceptional folate deficiency has been associated with increased risk of the development of congenital malformations, particularly neural tube defects and anencephaly. Particularly, several reports demonstrate the importance of folate supplementation in preventing the development of neural tube defects [22–25]. In addition, there is evidence that periconceptional folic acid supplementation improves maternal health (reducing incidence of miscarriages), ensures neonatal wellbeing, and reduces the risk of the development of other congenital malformations, including cleft lip and palate [26–28]. There are also renewed interests in folic acid supplementation following suggestions that folic acid fortification (in addition to protecting against the development of congenital anomalies) also supports healthy brain development throughout the pre and peri-adolescent years.

While there is scientific evidence supporting the beneficial effects of folic acid supplementation on growth, haematologic parameters and in aging related brain memory dysfunction [29–32], there isn’t enough evidence of its effects on the periadolescent brain as it matures to adulthood. This study examined the potential benefits (in adulthood) of prepubertal dietary folic acid fortification on brain function, oxidative stress markers, inflammatory markers, and neurotransmitter levels in Swiss mice.

2.0 Materials And Methods

2.1 Chemicals and drugs

High Potency Folic acid tablets 800 mcg (Organic Health) was procured from the Pharmacy. Kits for inflammatory markers were from ENZO Life Sciences, U.S.A. Acetylcholine and dopamine (ABCAM U.S.A), while Brain derived Neurotropic factor, acetylcholinesterase, lipid peroxidation and antioxidant tests were purchased from BioVision Inc. USA.

2.2 Diet

At the beginning of the experimental period, animals were either fed standard chow (29% protein, 11% fat, 58% carbohydrate) or folic acid supplemented chow (at 2.5, 5 and 10 mg/kg of feed), which were administered ad libitum for a period of eight weeks. Concentrations of folic acid were guided by results of previous studies using folate in diet [11, 33, 34].

2.3 Animals and Experimental design

Healthy male prepubertal (postnatal day 21) Swiss mice used in this study were obtained from the animal house of the Empire Farms and Research Laboratory Ogbomoso, Oyo State, Nigeria. All experiments and animal handling were carried out in accordance with the institutional guidelines for animal experimentation of the Ladoke Akintola University of Technology, and within the provisions for animal care and use prescribed in the scientific procedures on living animals, European Council Directive (EU2010/63).

Pregnant mouse dams were housed singly in plastic cages measuring 12 x 9 x 6 inches and maintained in controlled conditions of 23 ± 2°C, with 12 h light-dark cycle, and a humidity of 50 ± 5% until delivery of pups (day 0). Mouse dams and their pups remained in the same cage were the dams continued to have access to normal rodent diets and water ad libitum. On day 21, male pups from several dams were weaned into a single large box. On postnatal day 22, forty-eight prepubertal male mice were randomly assigned into four groups of 12 (n = 12) animals each. Mice were grouped as follows, normal control (standard diet) and three groups of folic acid incorporated into standard diet at 2.5, 5 and 10 mg/kg of feed. Daily food intake and weekly body weight were assessed using a weighing balance (Mettler Toledo Type BD6000, Greifensee, Switzerland). Animals were fed standard diet or folic acid supplemented diet daily for a period of ten weeks. At the end of the experimental period (postnatal day 92–94), adult animals were exposed to the open-field (for assessment of locomotor, rearing and grooming behaviours), Y maze and radial arm maze (for assessment of spatial working memory) and elevated plus maze (for evaluation of anxiety related behaviours). Other behavioural tests were catalepsy and behavioural despair tests which were carried out 24 hours after the initial tests (to allow animals recover from stress of the initial tests).

Twenty-four hours after the last behavioural test, animals in all groups were fasted overnight following which blood was taken for the assessment of blood glucose, leptin and insulin levels. Whole brain homogenates were also prepared and used for the assessment of lipid peroxidation (using malondialdehyde (MDA) levels); superoxide dismutase, glutathione peroxidase, reduced glutathione, total antioxidant capacity, tumour necrosis factor (TNF)-α, interleukin (IL)-10, IL6 acetylcholine levels and acetylcholinesterase activity.

2.4 Determination of body weight and food intake
Weekly body weight and daily food intake measurements were carried out using an electronic weighing balance as described previously [35–37]. Relative change in body weight or food intake was calculated for each animal using the equation below, following which results for all animals were computed to find the statistical mean.

\[
\frac{\text{Final body weight (or food intake)} - \text{Initial body weight (or food intake)}}{\text{Initial body weight (or food intake)}} (g)
\]

### 2.5 Behavioural tests

Behavioural tests were conducted in the following sequence: 1) elevated plus maze, 2) open field and memory tests. The catalepsy and behavioural despair tests were carried out 24 hours after the initial tests to allow animals recover from the stress of the initial behavioural tests.

#### 2.5.1 Anxiety Model: Elevated plus-maze

The elevated plus-maze which is a plus-shaped apparatus with four arms arranged at right angles to one another was used to measure anxiety-related behaviours. Anxiety behaviours were scored as previously described [38, 39].

#### 2.5.2 Open field behaviours

Open-field responses in rodents are representative of dersive and inspective exploration, arousal, inhibition and anxiety behaviours. Stereotypic behaviours such as grooming are also observable using this box. Behaviours observed in this paradigm are considered central behaviours and are indicative of the animal's exploratory ability, which is modulated by interaction of cortical and subcortical structures. Ten minutes of locomotion, rearing and grooming behaviours were observed and scored in the open field apparatus. The open-field box used was a rectangular arena composed of a hard floor measuring 36 x 36 x 26 cm. It is made of white (painted wood), with its floor divided by permanent red markings into 16 equal squares. Animal placement, movement and scoring system used are as previously described [40, 41].

#### 2.5.3 Memory tests (Y- and Radial arm- maze)

The Y-maze and the radial-arm maze are used to measure general activity and spatial working-memory. Spontaneous alternation behaviour is generally used to measure spatial working-memory. Spontaneous alternation behaviour measures the tendency for rodents to alternate conventionally non-reinforced choices of Y-maze or radial maze arms on successive opportunities. The Y-maze spontaneous alternation is validated in rodents as a measure of working-memory, general locomotor activity and stereotypic behaviour. Spontaneous alternation was assessed using a Y-maze made of white painted wood with three equally spaced arms (120°, 41 cm long, 15 cm high and 5 cm wide). The floor was also made of hard wood and painted white. Each mouse was placed in one of the arm compartments and allowed to move freely until its tail completely entered another arm. The sequence of arm entries was recorded as previously described [42, 43].

The radial arm maze apparatus consists of eight equidistantly-spaced arms, each measuring 33 cm long, all radiating from a small circular central platform. This maze tests the ability of the mouse to recall which maze arm last visited, as it tries to enter as many different arms as possible during a test session. Each mouse is placed on the central platform of the maze, allowed to move freely through each arm and its behaviours recorded for 5 min. Working memory is scored when the mouse enters each arm a single time as previously described [41, 44].

#### 2.5.4 Catalepsy measurements

Catalepsy was assessed using the bar test previously described [45, 46]. Behaviours of the mice on the bar test was measured at intervals of 30 and 60 minutes. This was done by lifting the mouse and placing it with its front paws on a steel bar (15 cm long, 0.5 mm diameter, and 5.5 cm above the horizontal surface) with the hind legs on the plane surface.

#### 2.5.5 Tail suspension test

The tail suspension test used to assess behavioural despair was carried out as previously described [11]. Briefly, animals fastened securely to a flat platform by the tip of their tail were suspended for 6 minutes about 30 cm below the platform. The total time the animal remained immobile was measured during the 6-minute period of the testing session.
2.5.6 Forced swim test

The forced swim test, which is also an animal measure of behavioural despair, was also used in this study. The test protocol and scoring system was as previously described [47].

2.6 Brain Homogenisation

Within 24 hours of the completion of the behavioural tests, animals in all groups were euthanised by cervical dislocation, post-anaesthesia with diethyl ether. Whole brain homogenates were prepared in ice-cold phosphate buffered saline, using a Teflon-glass homogeniser. The homogenate was centrifuged at 5000 rev/min, 4°C, for 15 minutes. The supernatant obtained was used for estimation of biochemical assays.

2.7 Biochemical tests

2.7.1 Insulin, Leptin, Adiponectin and Lipid profile

Plasma insulin, leptin, adiponectin levels; and lipid profile were assessed as described in an earlier study [42, 48].

2.7.2 Lipid peroxidation (Malondialdehyde)

The lipid peroxidation kit was used to determine malondialdehyde (MDA) levels as previously described [42, 48].

2.7.3 Antioxidant activity

Superoxide dismutase activity was assayed using the method previously described [48]. Superoxide dismutase activity was expressed as u/ml (serum) or units/mg protein (brain tissue). Total antioxidant capacity measures the quantity of free radicals scavenged by the test solution in any biological sample [41, 44]. Glutathione and reduced glutathione levels were assayed according to the manufacturers directives.

2.7.4 Tumour necrosis factor-α and Interleukin (IL) -6 and 10

Tumour necrosis factor-α and interleukin (IL)-6 and 10 were measured using enzyme-linked immunosorbent assay (ELISA) techniques with commercially available kits (Enzo Life Sciences Inc. NY, USA) designed to measure the ‘total’ (bound and unbound) amount of the respective cytokines.

2.7.5 Dopamine, Acetylcholine levels, Brain derived neurotropic factor and Acetylcholinesterase activity

Brain acetylcholinesterase activity (Biovision, USA), and levels of dopamine and acetylcholine (ABCAM, USA) were determined using commercially-available Enzyme linked immunosorbent assay kits, following the protocols of the manufacturer.

2.8 Statistical analysis

Statistical analysis was carried out using Chris Rorden's ezANOVA for windows. Data obtained were subjected to analysis of variance (ANOVA) and post-hoc tests (Tukey HSD). Results were expressed as Mean ± S.E.M. while p < 0.05 was taken as the accepted level of significant difference from control.

3.0 Results

3.1 Effect of prepubertal folate supplementation on body weight, food intake and metabolic markers (blood glucose, insulin, leptin and adiponectin levels)

Figure 1 (upper panel) represents the effect of dietary folate supplementation on percentage relative change in body weight. There was a significant (p < 0.001) increase in body weight in the groups fed folic acid (FA) supplemented diet at 5 and 10 mg/kg compared to control. Figure 1 (lower panel) represents the effect of dietary FA supplementation on percentage relative change in food intake. There was a significant (p < 0.001) decrease in food intake in the groups fed FA supplemented diet at 2.5, 5 and 10 mg/kg compared to control.
Table 1 shows the effect of folic acid supplementation on metabolic parameters including blood glucose levels, insulin level, leptin, and adiponectin levels. There was a significant decrease in fasting blood glucose, fasting insulin levels and leptin levels in the groups fed FA supplemented diet at 2.5, 5 and 10 mg/kg compared to control. There was however no significant difference in adiponectin levels in any of the groups fed folic acid diet compared to control.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Glucose mg/dl</th>
<th>Insulin (µU/ml)</th>
<th>Leptin (µg/ml)</th>
<th>Adiponectin (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>116.2 ± 0.22</td>
<td>7.56 ± 0.21</td>
<td>6.24 ± 0.02</td>
<td>5.01 ± 0.10</td>
</tr>
<tr>
<td>FA 2.5</td>
<td>95.80 ± 0.24*</td>
<td>6.26 ± 0.23*</td>
<td>4.14 ± 0.04*</td>
<td>5.11 ± 0.13</td>
</tr>
<tr>
<td>FA 5.0</td>
<td>89.21 ± 0.21*</td>
<td>5.15 ± 0.13*</td>
<td>4.12 ± 0.05*</td>
<td>5.13 ± 0.11</td>
</tr>
<tr>
<td>FA 10</td>
<td>86.32 ± 0.22*</td>
<td>5.04 ± 0.14*</td>
<td>4.10 ± 0.03*</td>
<td>5.14 ± 0.14</td>
</tr>
</tbody>
</table>

Values are presented as Mean ± S.E.M, *p < 0.05 significantly different from control. FA: Folic acid, number of animals/group-12.

### 3.2 Neurobehavioural and neurochemical parameters

#### 3.2.1 Effect of prepubertal folate supplementation on open-field behaviours (locomotor activity, rearing and self-grooming) and catalepsy score

Figure 2(upper panel) represents the effect of dietary folate supplementation on horizontal locomotion in the open field, measured as number of lines crossed. There was a significant (p < 0.001) decrease line crossing in groups fed FA supplemented diet at 2.5, 5 and 10 mg/kg compared to control. Figure 2 (lower panel) represents the effect of dietary folate supplementation on rearing in the open field. There was a significant (p < 0.001) decrease in rearing behaviour in groups fed FA supplemented diet at 5 and 10 mg/kg compared to control. Figure 3 represents the effect of dietary folate supplementation on self-grooming behaviour in the open field. There was a significant (p < 0.001) decrease in self-grooming in groups fed FA supplemented diet at 5 and 10 mg/kg compared to control.

Figure 4 represents the effect of dietary folate supplementation on catalepsy score in the bar test. There was no significant difference in catalepsy scores in any of the groups fed FA supplemented diet compared to control.

#### 3.2.2 Effect of prepubertal folate supplementation on spatial working memory (Y-maze, and radial arm maze), anxiety related behaviours and behavioural despair

Figure 5 (upper panel) represents the effect of dietary folate supplementation on Y-maze spatial working memory measured as percentage alternation in 5 minutes. There was a significant (p < 0.001) increase in spatial working memory score in the groups fed folic acid (FA) supplemented diet at 2.5, 5 and 10 mg/kg compared to control. Figure 5 (lower panel) represents the effect of dietary folate supplementation on radial arm-maze spatial working memory measured as alternation index. There was a significant (p < 0.001) increase in working memory score in the groups fed folic acid (FA) supplemented diet at 2.5, 5 and 10 mg/kg compared to control.

Figure 6 (upper panel) represents the effect of dietary folate supplementation on time spent in the open arm of the elevated plus maze. There was a significant (p < 0.001) increase in open arm time in the groups fed folic acid (FA) supplemented diet at 2.5, 5 and 10 mg/kg compared to control. Figure 6 (lower panel) represents the effect of dietary folate supplementation on time spent in the closed arm of the elevated plus maze. There was no significant difference in closed arm time in any of the groups fed folic acid (FA) supplemented diet compared to control.

Figure 7 (upper panel) represents the effect of dietary folate supplementation on immobility time in the tail suspension test. There was a significant (p < 0.001) decrease in time spent immobile in the groups fed folic acid (FA) supplemented diet at 2.5, 5 and 10 mg/kg compared to control. Figure 7 (lower panel) represents the effect of dietary folate supplementation on immobility time in the forced swim test. There was a significant (p < 0.001) decrease in time spent immobile in the groups fed folic acid (FA) supplemented diet at 2.5, 5 and 10 mg/kg compared to control.
3.3 Effect of prepubertal folate supplementation on Lipid peroxidation, antioxidant status, acetylcholinesterase activity and inflammatory markers

Table 2 represents the effect of dietary folate supplementation on antioxidant status and lipid peroxidation while Table 3 represents the effect of dietary folate supplementation on acetylcholinesterase activity and brain levels of dopamine, brain derived neurotropic factor, acetylcholine, TNF-α, IL-6 and 10. Lipid peroxidation measured as malondialdehyde levels (MDA) in serum decreased significantly with FA at 2.5, 5 and 10 mg/kg compared to control. Total antioxidant capacity, glutathione peroxidase (GPX) and reduced glutathione (GSH) increased significantly with FA at 5 and 10 mg/kg compared to control. Superoxide dismutase (SOD) and Catalase activity decreased significantly with FA at 5 and 10 mg/kg compared to control.

Dopamine levels, acetylcholinesterase activity and TNF-α levels decreased significantly with FA at 5 and 10 mg/kg compared to control while acetylcholine, IL-10 and IL-6 levels increased significantly with FA at 5 and 10 mg/kg compared to control. Brain derived neurotropic factor increased significantly with FA at 2.5, 5 and 10 mg/kg compared to control.

Table 2

<table>
<thead>
<tr>
<th>Groups</th>
<th>MDA µmol/g</th>
<th>TAC (TE mM)</th>
<th>GSH nmol/mL</th>
<th>GPX IU/L</th>
<th>SOD (u/mg)</th>
<th>CAT (u/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.39 ± 0.10</td>
<td>11.20 ± 0.10</td>
<td>0.80 ± 0.11</td>
<td>10.55 ± 1.01</td>
<td>1.20 ± 0.12</td>
<td>28.22 ± 0.50</td>
</tr>
<tr>
<td>FA 2.5</td>
<td>0.29 ± 0.10*</td>
<td>10.89 ± 0.10</td>
<td>1.12 ± 0.04</td>
<td>11.22 ± 1.03</td>
<td>1.12 ± 0.11</td>
<td>27.17 ± 0.23</td>
</tr>
<tr>
<td>FA 5.0</td>
<td>0.22 ± 0.10*</td>
<td>22.23 ± 0.10*</td>
<td>2.32 ± 0.03*</td>
<td>23.11 ± 1.44*</td>
<td>0.65 ± 0.12</td>
<td>18.20 ± 0.11*</td>
</tr>
<tr>
<td>FA 10</td>
<td>0.16 ± 0.10*</td>
<td>27.10 ± 0.10*</td>
<td>2.76 ± 0.04*</td>
<td>29.12 ± 1.12*</td>
<td>0.30 ± 0.12*</td>
<td>12.20 ± 0.10*</td>
</tr>
</tbody>
</table>

Values are expressed as Mean ± S.E.M, *p < 0.05 significantly different from control. FA: Folic acid, number of animals/group-12.

Table 3

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dopamine (pg/mg)</th>
<th>Acetylcholine (µM)</th>
<th>Acetylcholinesterase (nmol/mg)</th>
<th>BNDF (pmol/mg tissue)</th>
<th>TNF-α (ng/g/protein)</th>
<th>IL-10 (pg/mg/protein)</th>
<th>IL-6 (ng/g/protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>88.12 ± 0.10</td>
<td>66.37 ± 0.42</td>
<td>1.43 ± 0.02</td>
<td>18.01 ± 0.11</td>
<td>70.30 ± 0.18</td>
<td>29.22 ± 0.18</td>
<td>90.17 ± 1.28</td>
</tr>
<tr>
<td>FA 2.5</td>
<td>87.10 ± 0.22</td>
<td>67.14 ± 0.20</td>
<td>1.40 ± 0.02</td>
<td>25.11 ± 0.10</td>
<td>69.41 ± 1.11</td>
<td>30.13 ± 0.15</td>
<td>89.42 ± 1.10</td>
</tr>
<tr>
<td>FA 5.0</td>
<td>74.20 ± 1.05*</td>
<td>77.30 ± 0.33*</td>
<td>1.15 ± 0.01*</td>
<td>27.20 ± 0.10*</td>
<td>50.10 ± 1.13*</td>
<td>64.01 ± 0.11*</td>
<td>121.30 ± 1.09*</td>
</tr>
<tr>
<td>FA 10</td>
<td>73.24 ± 1.16*</td>
<td>84.25 ± 0.45*</td>
<td>1.05 ± 0.03*</td>
<td>27.60 ± 0.10*</td>
<td>35.11 ± 1.22*</td>
<td>78.1-±0.17*</td>
<td>130.43-±1.10*</td>
</tr>
</tbody>
</table>

Values are expressed as Mean ± S.E.M, *p < 0.05 significantly different from control. FA: Folic acid, number of animals/group-12.

4.0 Discussion

This study examined the possible effects of continuous dietary folic acid supplementation (beginning on day 22) on body weight, food intake, markers of metabolism, brain function, oxidative stress markers, antioxidant status, inflammatory parameters and neurotransmitter levels in adult mice. The results of the study revealed an increase in body weight, and improvement in antioxidant status; memory scores (in the radial arm and Y-maze), acetylcholine levels, brain derived neurotropic factor and interleukin levels; while a reduction in food intake, acetylcholinesterase activity, and levels of glucose, insulin and leptin were also observed.

It also showed open field behavioural changes that were suggestive of central inhibition (decreased horizontal locomotion, rearing and self grooming behaviours); while in the elevated plus maze, an anxiolytic behaviour was observed.
The continuous supplementation of folic acid from day 22 till adulthood (day 92) was associated with an increase in body weight (5 and 10 mg) and a reduction in food intake, blood glucose, insulin and leptin levels at the three concentrations of folic acid. This would suggest that folic acid supplementation beginning in the prepubertal period possibly increases feed efficiency, while also reducing the risk of developing dysmetabolism in adulthood. Effects of folic acid on metabolic parameters such as weight, food intake, blood glucose levels, lipid levels and metabolic hormones have been reported severally [11, 49–53]; and there have been reports suggesting that a possible relationship exists between folic acid metabolism and the maintenance of metabolism in the body. While the results of this study showed that folic acid supplementation beginning in the prepubertal period had the ability to increase body weight in rats, corroborating an earlier study using adult mice from our laboratory [11], it also demonstrated that this increase in body weight was not accompanied by derangement of metabolism markers such as glucose, insulin and leptin. This also corroborates the result of the study that evaluated the effect of folic acid supplementation in broiler chickens and reported that supplementation of folic acid at 5 mg.kg was associated with increased average body weight, and reduced adiposity [54]. The absence of metabolic derangement (as observed in this study) or adiposity following folate supplementations differentiates the body weight increase observed in this study from that reported with folate deficiency in humans, or with the administration folate deficient diet in animals [52, 53, 55]. Results of this study also suggest that folic acid has the ability to increase feed conversion efficiency, supporting a number of studies that had also reported improvement in body weight and feed conversion efficiency following folic supplemented diet [54, 56].

In the last few decades, folic acid has been shown to be an essential micronutrient necessary for optimal brain functioning. It is important in the synthesis of nucleotides, myelin and neurotransmitters. It is also crucial in methylation reactions, and ensuring that homocysteine is kept at non toxic levels [57]. More important is the association of low maternal folic acid intake during pregnancy with an increased risk of neural tube defects and other neurodevelopmental disorders including schizophrenia and autism spectrum disorders. In this study, folic acid supplementation was associated with a decrease in horizontal locomotion, rearing and self-grooming; with no significant effect on catalepsy. The results of this study contrast the results of an earlier study from our laboratory that examined the effect of folic acid supplemented diet fed at 25, and 50 mg/kg of feed in adult rats [11], and reported an increase in locomotor activity, rearing and self grooming behaviours in healthy adult rats. In this study, folic acid supplementation beginning in the prepubertal period was associated with an overall central inhibitory effect with a concentration dependent decrease in locomotor activity, rearing, and grooming behaviours. Locomotor activity, rearing and self grooming are brain behaviours that are modulated or regulated at multiple brain regions under the influence of several neurotransmitters such as glutamate, dopamine, gamma amino butyric acid and serotonin [58–66]. Also, in this study, we observed a decrease in brain levels of dopamine (a crucial neurotransmitter in the modulation of locomotor and self grooming behaviours) with increasing concentrations of folic acid (Table 3), suggesting that commencing dietary fortification of folic acid supplementation (at the higher concentrations) in the prepubertal period was associated with a decrease in dopamine levels in adulthood. While there is a dearth of scientific literature assessing the effects of folic acid on ligand/ receptor interactions in the brain, studies have shown that folic acid supplementation in adult rodents was associated with an increase in the level of dopamine and norepinephrine neurotransmitter in the brain [67]. Also, the relationship between brain neurotransmitter activity and neurobehavioural modulation has been studied severally. Reports have shown that dopaminergic transmission can modulate locomotor activity via ascending fibres that project to the basal ganglia and finally to locomotor networks in the brain. A decreased dopaminergic tone has been linked to a decrease in locomotor activity [68] while an inhibition of dopaminergic D1 receptors has been linked to a decrease in self-grooming. Also, the suppression of dopaminergic transmission in the nucleus accumbens results in hypolocomotion, while suppression of caudate-putamen dopaminergic transmission results in decreased rearing activity [64]. Therefore, the decrease in brain dopamine levels observed in this study could possible account for the central inhibitory response observed in the open field. However, other neurotransmitters could also be responsible for the central inhibitory effect observed in this study. Gamma amino butyric acid (GABA) is regarded as the most common inhibitory neurotransmitter in the brain, with its effect crucial to the regulation of central pattern generators and modulation of neuromuscular junction/ higher brain processing centres [66]. Another mechanism through which folic acid supplementation could result in central inhibitory effect would be through its effect on S-adenosylmethionine (SAM). Studies have shown that folic acid is important in one carbon metabolism as well as in the production of Sadenosyl methionine [69] which is a universal methyl donor required for various reactions and has been shown to result in hypolocomotion (horizontal and vertical) and decreased self-grooming. Also, SAM can be remethylated to methionine which is also associated with central inhibitory effects [60]. Motor coordination (measured as catalepsy score on the bar test) was however not altered by folic acid supplementation. Although, a 2012 study by Shooshstari et al [70] carried out in adult male rats had observed that oral administration of FA at 5 and 10 mg/kg but not at 15 mg/kg improved motor coordination in the rotarod.
Several clinical and preclinical studies have demonstrated the important roles played by folic acid and folate metabolism in memory processes [70–76], anxiety related behaviours, and depression [71, 77]. In this study dietary folic acid supplementation improved spatial working memory, decreased anxiety and depression-like behaviours. This supports the results of a number of studies that had reported the benefits of folic acid supplementation on memory processes, anxiety and ameliorating depression particularly in adults, the aged or following peripartum folic acid supplementation [67, 73, 78, 79]. There have been suggestions that these beneficial effects of FA supplementation could be attributed to its effects on brain derived neurotropic factor (an increase was observed in this study), its antioxidant effects, its ability to reduce lipid peroxidation (which was also observed in this study) and its ability to increase levels of serotonin, and the expression of glutamate receptor 1 [67, 77]. Folic acid's effects on memory have also been linked to its ability to decrease the activity of acetylcholinesterase and by extension increase acetylcholine levels in the brain [80] which was also observed in this study (Table 3).

The antioxidant, antiinflammatory and free radical scavenging properties of folic acid is not in dispute, decades of research continue to affirm this fact [81–83]. Also, the results of this study demonstrated that at the concentrations administered, the commencement of dietary folic acid supplementation in the prepubertal folic improved antioxidant status, IL-10 and IL-6 levels in adult mice while also decreasing levels of lipid peroxidation levels and TNF-α supporting the results of a number of in vitro and in vivo studies that had reported similar effects [67, 84]. There have been reports that folic acid's ability to increased total antioxidant capacity and decrease malondialdehyde levels could be linked to its effects on homocysteine concentration, although levels of homocysteine were not measured in this study. Also the antiinflammatory effects of folic acid have been linked to its ability to cause epigenetic regulation in cells [85]. These antiinflammatory effects have also been associated with a reduction in anxiety and improvement in memory and mood [85] which was also observed in this study. Supplementation with folic acid increased acetylcholine levels and decreased acetylcholinesterase activity corroborating the results of a few other studies [86]. The reduction in acetylcholinesterase activity with a corresponding increase in brain levels of acetylcholine could be responsible for the improvement in memory scores observed in this study.

**Conclusion**

Dietary folic acid supplementation beginning in the prepubertal period had significant benefits in mice. These beneficial effects were reflected in areas relating to behaviour, cognition, oxidative status, metabolic hormones and brain neurochemistry, in assessments conducted when the animals got older. Results obtained here shed more light on a wider beneficial central nervous system effect of folate supplements, and may serve to redirect our research focus towards understanding its further use in humans, especially in preserving the functional integrity of the brain possibly through its epigenomic influence.

**Declarations**

**Author Contribution Statement**

AYO and OJO conceived and designed research. ATO, FOO and JF conducted experiments and analyzed data. AYO and OJO wrote the manuscript. All authors read and approved the manuscript.

**Ethics Approval and Consent to Participate:**

All procedures performed on the animals were in accordance with approved protocols of the Faculty of Basic Medical Sciences, Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria and within the provisions for animal care and use as prescribed by the scientific procedures on living animals, European Council Directive (EU2010/63).

**Human and Animal Rights:**

No humans were used in this study. All procedures performed on the animals were in accordance with approved protocols of the Ladoke Akintola University of Technology

**Consent for Publication:**

Not Applicable.

**Availability of Data and Materials:**
The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Conflict of interest:

All authors of this paper declare that there is no conflict of interest related to the content of this manuscript.

Source of funding:

This research did not receive any specific grant from agencies in the public, commercial, or not-for-profit sectors.

References


10.1016/j.intimp.2016.05.007. Epub 2016 May 18. PMID: 27208432.


Figures

**Figure 1**

Effect of folic acid supplemented diet on change in body weight (upper panel) and change in food intake (lower panel). Each bar represents Mean ± S.E.M, *p<0.05 vs. control, number of mice/group=10, FA: Folic acid.
Figure 2

Effect of folic acid supplemented diet on Open-field horizontal locomotion (upper panel) and rearing (lower panel). Each bar represents Mean ± S.E.M, *p<0.05 vs. control, number of mice/group=10, FA: Folic acid.
Figure 3

Effect of folic acid supplemented diet on Open-field horizontal self-grooming behaviour. Each bar represents Mean ± S.E.M, *p<0.05 vs. control, number of mice/group=10, FA: Folic acid.
Figure 4

Effect of folic acid supplemented diet on catalepsy score. Each bar represents Mean ± S.E.M, number of mice/group=10, FA: Folic acid.
Figure 5

Effect of folic acid supplemented diet on spatial working memory in the Y-maze (upper panel) and radial arm maze (lower panel). Each bar represents Mean ± S.E.M, *p<0.05 vs. control, number of mice/group=10, FA: Folic acid.
Figure 6

Effect of folic acid supplemented diet on time spent in the open arm (upper panel) and closed arm (lower panel) of the elevated plus maze. Each bar represents Mean ± S.E.M, *p<0.05 vs. control, number of mice/group=10, FA: Folic acid.
Figure 7
Effect of folic acid supplemented diet on immobility time in tail suspension test (upper panel) and forced swim test (lower panel). Each bar represents Mean ± S.E.M, *p<0.05 vs. control, number of mice/group=10, FA: Folic acid.