Original Research Article

Comparative Neuroprotective Effects of Atorvastatin and Ethanolic Fruit Extract of *Fragaria Ananassa* on the Cerebellum of Methamphetamine- Intoxicated Adult Male Wistar Rats

Abstract

Methamphetamine (METH) is a powerful, highly addictive stimulant that affects the central nervous system. It takes the form of a white, odourless, bitter-tasting crystalline powder that easily dissolves in water or alcohol. Oxidative stress and elevated levels of reactive oxygen species (ROS) is one of the main cause of cellular damage as a result of METH exposure. Numerous plants are known to be rich in potent phytochemicals which have been used in folkloric and orthodox medicine in the prevention and treatment of different ailments. Strawberry (*Fragaria ananassa*) as a plant contains several polyphenols with strong antioxidant and anti-inflammatory activities as well as flavonoids, anthocyanins, and ellagic acid with strong neuroprotective efficacy. This research was designed to compare the neuroprotective effects of atorvastatin and ethanolic extract of *Fragaria ananassa* on the cerebellum of methamphetamine-intoxicated Wistar rats. Forty adult male Wistar rats were divided into 8 groups of 5 rats each. Group A was the negative control and had food and water only. Group B was the positive control and was exposed to 10mg/kg/body weight of METH without treatment. Groups C and D were administered 200mg/kg/body weight of extract and 10 mg/kg/bw of atorvastatin (ATVS) (a standard drug) without exposure to METH respectively. Groups E was exposed to 10mg/kg of METH and treated with 10mg/kg/bw of ATVS while F and G were exposed to 10mg/kg of METH and were treated 50mg/kg/bw and 100mg/kg/bw of the ethanolic extracts respectively. Group H was exposed to 10mg/kg of METH and treated with 200mg/kg/bw of the extract plus 10mg/kg/bw of ATVS.The results of antioxidant studies show that the atorvastatin conferred more protection against oxidative stress than the ethanolic extract of strawberry. However, its histological results show that both have equal neuroprotective effects as depicted with normal cerebellar architecture whereas its combined treatment conferred more neuroprotection on the cerebellum of Wistar rats.

Keywords: **Methamphetamine, Atorvastatin, Ethanol, Neuroprotective, Cerebellum, *Fragaria ananassa.***

Introduction

"Methamphetamine (METH; also called crystal, chalk or ice) is a powerful central nervous system stimulant which elevates mood, alertness, energy levels and concentration in the short-term. In addition, it is also a psychomotor drug highly addictive and toxic to the brain. METH is an addictive stimulant that can be administered orally, smoked, snorted or injected resulting in immediate and intense euphoria" (Hauer, 2010). "Its addictive nature has led to METH abuse becoming a global problem. At a cellular level, METH exerts a myriad of effects on the central and peripheral nervous systems, immune system and the gastrointestinal system" (Rommel *et al*., 2015). Several studies "with human subjects have shown that METH chronic users demonstrate structural abnormalities in the brain, namely loss of grey matter, white matter hypertrophy and altered glucose metabolism in specific regions like hippocampus, prefrontal cortex, cingulated gyrus and amygdale" (Thompson *et al*., 2004).

"Atorvastatin (a family member of statins) is a class of anti-cholesterolemic drugs widely used for the prevention and treatment of cardiovascular, cerebrovascular and metabolic disorders" (Sizar *et al.,* 2021). It is generally accepted that atorvastatin exhibit a number of biological activities that are unrelated to their cholesterol-lowering effect. These include antioxidant properties, effects on inflammation and amyloid processing, modulation of apolipoprotein E expression (Naidu *et al.,* 2002) and possible activation of neuroprotective pathways in the brain (Zacco *et al*., 2003) but these are not without side effects.

"With the medical and technological advancement today, plants are now screened for the presence of bioactive compounds which are known to possess antioxidant and other therapeutic properties" (Brookie *et al.,* 2018). "Parts of plants like the leaves, bark and roots are very rich in phytochemicals such as phenols, alkaloids, flavonoids, terpenes, glycogens with the inherent potential to mitigate diseases and reduce toxicities" (Ekaluo *et al*., 2015). Several medicinal plants are of much interest due to their antioxidant and free radical scavenging properties.

Fragaria ananassa (Strawberry fruit) has been a functional food plant. Reports show that it is rich source of minerals such as manganese, potassium**,** magnesium, copper, iron and phosphorus (Giampieri *et al.,* 2015), ascorbic acid (Singh *et al.,* 2010), thiamine, riboflavin, niacin, vitamin B6, vitamin K, vitamin A and vitamin E (Tulipani *et al.,* 2010), folate (Tõnutare *et al.,* 2014), flavonols, catechins, hydroxycinnamic acids, ellagitannins, and ellagic acid have also been associated with the beneficial effect of strawberries on human health (Tulipani *et al.,* 2009). "The most significant contributors of the antioxidant capacity of strawberries are considered to be ascorbic acid, ellagitannins, and anthocyanins whereas fisetin is the major phytochemical implicated for neuroprotection effects" (Aaby *et al.,* 2007). "In fact, there is multiple evidence supporting the role of strawberries against inflammatory- and oxidative-based diseases, like cardiovascular diseases and several types of cancer as well as neuroprotection during oxidative stress" (Battino *et al.,* 2021; Cianciosi *et al.,* 2019; Giampieri *et al.,* 2018). Different parts of the

herb have been used over the years all over world including the leaves, the fruit and the seed. This study was carried out on the fruits. Hassan *et al.* (2021) reported that "it is a good source of minerals such as manganese, potassium**,** magnesium, copper, iron and phosphorus, ascorbic acid, thiamine, riboflavin, niacin, vitamin B6, vitamin K, vitamin A and vitamin E, folate, flavonols, catechins, hydroxycinnamic acids, ellagitannins, and ellagic acid, hence its wide acceptability".

"Oxidative stress has been associated with the aetiology of several ailments across the globe. Oxidative stress itself is induced by free radicals released into the body in the course of metabolic activities. Antioxidants on the other hand are electron sufficient compounds with the readiness to donate electron to electron-deficient free radicals to stabilize their actions. Free radicals are electron-deficient compounds because they possess unpaired valence electrons and in their quest to finding electrons, they can easily attack cells and biomolecules in the body resulting in generation of diseases" (Kadam, 2010). "Several studies has linked the generation of reactive oxygen species (free radicals) such as hydroxyl (OH^-) radical, superoxide (O^{2-}), nitric oxide (NO^-), nitrogen dioxide (NO^{2-}), peroxyl (ROO^-), hydrogen peroxide (H_2O_2) to the development of pathological conditions including protein oxidation, lipid peroxidation, DNA damage and cellular degeneration and these conditions have been implicated in the aetiology of diseases such as diabetes, cancer, Alzheimer and Parkinson disease, cardiovascular disease, aging process, arthritis and inflammation" (Hassan and Barde, 2019; Sahlin *et al.*, 2004).
"Notwithstanding, our body generates natural antioxidants to deal with the free radical load but there is always need to boost and augment the efforts of these natural antioxidants with natural and synthetic products so as to support the body's defence, especially when they are overwhelmed by the load of free radicals generated. However, the continuous use of synthetic antioxidants from pharmaceutical stores has over the years create panic following scientific assertions on their side effects" (Emmanuel-Ikpeme *et al.*, 2014).

Methodology

Collection and authentication of plant material

The strawberry fruit used for this study were obtained from Shoprite in Enugu State, Nigeria and identified by Dr. Okoh Augustine Okoh of the Department of Plant and Biotechnology, Faculty of Biological sciences, Ebonyi State University Abakaliki, Nigeria with Herbarium number EBSU/P/065.

Preparation of plant materials

The Strawberry fruits were cut into fine slices and wereoven dried for 5.5 hours at 50°C, the oven dried strawberries were grinded in electrical grinder and fine powder was prepared.

Extraction Method

"During its extraction, 400g of the macerated plant powder was soaked in 1000ml of absolute alcohol and kept in a container for 24 hours. The mixture was also shaken vigorously at intervals for another 2 hours, to allow complete extraction. The resulting mixture was rapidly filtered through Whatman no 1 filter Paper to obtain a homogenous filtrate. This filtrate was then concentrated in vacuum at low temperature (37-40°c) to about one tenth the original volume using a rotary evaporator. The concentrates were allowed open in water bath (40°c) for complete dryness yielding about 25g of a dark reddish gummy substance. The extract was later reconstituted in distilled water at a concentration of 1g/ml before administration. The extract was then refrigerated until use" [Aguwa et al. 2020].

The reference doses of *Fragaria ananassa* used in this experiment were 50mg/kg, 100mg/kg and 200mg/kg for low and high doses respectively. 1g of the extract (*Fragaria ananassa*) was dissolved in 10ml of water to get the stock solution of 10mg/ml using this method.

Phytochemical analysis

Qualitative and quantitative phytochemical screeninghighlighted by standards phytochemical methods. Phytochemical analysis of alkaloids, anthocyanins, flavonoids, polyphenols, saponins and tannins were performed according to the methods described by Phillipson (2000). Phytochemical explored includes alkaloids, tannins, flavonoids, saponins, phenols and anthocyanin.

Preparation of stock solutions

Methamphetamine used for this study was of analytical grade. 1g of Methamphetamine was dissolved in 100ml of distilled water yielding 10mg/ml (stock solution). Atorvastatin used for this study was of analytical grade. 10g of atorvastatin was dissolved in 100ml of distilled water yielding 100mg/ml (stock solution).

Experimental design

Forty Wistar rats weighing between 120-180g were used for this study. The animals were purchased from the animal house of Nnamdi Azikiwe University, Nnewi Campus. They were housed in standard cages and left to acclimatize for 14 days under natural conditions in the animal house before the commencement of the experiment. The animals were fed with standard pellet and water *ad libitum*.

The rats were divided into 8 groups - A, B, C, D, E, F, G and H of 5 rats each.

Rats in group A received only food and water. Rats in group B received 10mg/kg of methamphetamine only, C received 200mg/kg of the extract only, D received 10mg/kg of atorvastatin only, E received 10mg/kg of methamphetamine and 10mg/kg of atorvastatin, F received 10mg/kg of methamphetamine and 50mg/kg of extract, G received 10mg/kg and

100mg/kg and H received 10mg/kg of methamphetamine and 200mg/kg of extract and 10mg/kg of atorvastatin for 28days. All the administrations were done orally using oral gavage.

After 28days of administration, the rats were subjected to the neurobehavioural test (hanging wire test), after which the animals were sacrificed by cervical dislocation. Blood was collected via ocular puncture for antioxidant studies formalondialdehyde (MDA), superoxide dismutase (SOD), and catalase (CAT). The brains were fixed in 10% formal saline for histological studies using H&E procedure according to the method described by Drury and Wallington (1980).

Statistical Analysis

Data obtained were analyzed using students T test with Microsoft excel and expressed as Mean ± SD. Differences between means were regarded significant at $P < 0.05$

Results and Discussion

Results

Qualitative Phytochemical Screening

For this investigation, different phytochemicals from *Fragaria ananassa* fruits were extracted and highlighted by different methods; their presence (+) is shown in Table 1. The results indicated that *Fragaria ananassa* fruits contained alkaloids, anthocyanins, flavonoids, polyphenol, saponins and tannins which are the main phytochemical groups.

Table 1: Result of Qualitative Phytochemical Analysis of *Fragaria ananassa* fruits.

S/N	Chemical Test (parameters)	Extract
1.	Alkaloids	+
2.	Polyphenols	+
3.	Flavonoids	+
4.	Anthocyanins	+
5.	Tannins	+
6.	Saponins	+

(+) Present

Quantitative Phytochemical Screening

The results of this study are presented in **table 2** shows the content of anthocyanin, flavonoids, other flavonoids and phenolic acids of fruit extracts of *Fragaria ananassa* determined by dosage or calculation. We noted that flavonoids are the majority of the compounds with 20.70 mg/g representing 89.20% of the extract components. This is followed by anthocyanin compound with

12.34 mg/g and represents 53.19 % of the phenolic compounds (polyphenols), followed by the other flavonoids with 8.36 mg/g (36.03 %) and finally phenolic acids with 2.50 mg/g (10.77 %).

Table 2: Quantitative Data of Various Phytochemicals in the fruit extracts of *Fragaria ananassa*

S/N	Compounds	Contents (mg/g)	Ratio (%)
1.	Anthocyanin	12.34 ± 2.30[c]	53.19[c]
2.	Flavonoids	20.70 ± 1.50[b]	89.20[b]
3.	Other Flavonoids*	8.36 ± 0.72[d]	36.03[d]
4.	Polyphenols	23.21 ± 2.70[a]	99.98[a]
5.	Phenolic Acids*	2.50 ± 0.12[e]	10.77[e]

Values are expressed as Mean ± Standard deviation (SD), n = 3.
Means followed by the same letter were not significantly different at 5 % (test of Newman Keuls).
Ratio: content of a compound relative to the total content of compound.
*: values determined by calculation.

Table 3: Result of Antioxidant Studies.

Groups	**Malondialdehyde (MDA) level (um/L)**	**Superoxide Dismutase (SOD) (U/ml)**	**Catalase (CAT) (kw/l)**
	MEAN±SEM	**MEAN±SEM**	**MEAN±SEM**
Group A	5.8±0.62	17.18±2.06	23.32±1.25
Group B	5.40±0.32[a] **p-value = 0.4304**	15.78±1.22[a] **p-value =0.4948**	19.51±1.37[a] **p-value =0.1013**
Group C	5.49±0.45[a] **p-value =0.5526**	17.76±6.36[a] **p-value =0.9128**	20.38±5.31[a] **p-value =0.5269**
Group D	5.58±0.42[a] **p-value =0.6365**	17.95±2.63[a] **p-value =0.7756**	15.98±0.91[*] **p-value =0.0216**

Group E	3.28±0.12[*] **p-value =0.0281**	15.30±1.11[a] **p-value =0.3754**	23.53±1.40[a] **p-value =0.8863**
Group F	4.36±0.23[a] **p-value =0.0822**	24.16±3.97[a] **p-value =0.1583**	22.48±2.89[a] **p-value =0.7424**
Group G	3.96±0.56[a] **p-value =0.0830**	18.54±8.87[a] **p-value =0.8529**	24.68±2.48[a] **p-value =0.5605**
Group H	2.95±0.28[*] **p-value =0.0258**	23.68±1.79[a] **p-value =0.0780**	25.04±1.81[a] **p-value =0.3831**

Data was analyzed using ANOVA followed by post Hoc multiple comparison, and values considered significant at $p<0.05$. SEM: Standard error of mean, significant ([*]) and not significant ([a]). Results were presented as Mean ± SD of five (5) rats in each group.

The result of antioxidant studies is presented in table 4 below. Malondialdehyde (MDA), Superoxide dismutase (SOD) and Catalase (CAT) was studied. Our results show that MDA levels were highly significantly increased in all the experimental groups such as B, C, D, F and G compared to the control group A. Malondialdehyde is an indicator of lipid peroxidation which itself is a pointer to cell damage. MDA levels were raised in all the experimental groups, but it was highest and statistically significantly higher in group B that was exposed to methamphetamine without treatment compared to the control. It was however observed that with administration of extract of *Fragaria ananassa* and atorvastatin, MDA levels began to reduce groups E and H towards the normal control value in a dose dependent manner. So we see that the H groups were more reduced than the E groups. It may therefore imply that extract of *Fragaria ananassa* and atorvastatin administration may be interfering with lipid peroxidation and hence, the rate of generation of malondialdehyde (MDA).

It was further observed that the atorvastatin has more interfering potential against lipid peroxidation than the ethanolic extract of *Fragaria ananassa.*

Superoxide dismutase (SOD) and catalase (CAT) are body's antioxidant enzymes that fight to neutralize the free radicals generated due to oxidative stress. Their mechanism of action is reviewed in chapter two. Therefore during oxidative stress, the tissue levels of these enzymes are depleted, typical of what happens with leucocytes in fighting germs. Therefore, it was observed that there were reductions in levels of SOD and CAT in all the experimental groups compared to the control. But only that of group D showed significantly reduced tissue CAT levels compared to group A attained statistical significance.

Further observation revealed that for SOD and CAT showed the highest rise for all treated groups most especially in groups C, E, F and G respectively. These observations further lend

credence to the assertion that the ethanolic extract of *Fragaria ananassa* is conferring more neuroprotection than the atorvastatin.

Our result showed that the fruit extracts of *Fragaria ananassa* used for this research showed antioxidant properties in a dose dependent fashion. This is inferred following our results which reveals that for all the tests carried out, the G and H groups which received 100mg/kg/b.w and 200mg/kg/b.w of *Fragaria ananassa* performed better than the F groups which received 50mg/kg/bw.

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Another study by Mohammed*et al.* (2017) "on Cadmium-Induced Lipid Peroxidation in the testes of rats showed that strawberry crude extracts decreased the levels of serum malondialdehyde.

However, our result reveals that atorvastatin showed better antioxidant properties than the ethanolic fruit extract of *F. ananassa* in a dose-dependent fashion. This was evident in the decreasing levels of malondialdehyde (MDA) in the treatment groups E to H when compared to group B which received neither atorvastatin nor extracts. Furthermore, the levels of the antioxidant enzymes which were significantly depleted in the experimental group B showed a rise with *F. ananassa* administration in a dose-dependent manner. We are therefore convinced by these results in line with the finding of other researchers that *F. ananassa* and atorvastatin are potent antioxidants. But we observed from our result that the combined dose of both atorvastatin and ethanolic extract conferred better resistance to oxidative stress than individual doses. This is as the levels of MDA were the least in the group H compared to their individual counterparts (C to G)".

Reports also show that "plant phenolics are major group of compounds acting as primary antioxidants or free radical scavengers (Deepak *et al*., 2019). Oxidative stress occurs when the production of reactive oxygen species (ROS) exceeds the level the body's natural antioxidant defence mechanisms can cope with, causing damage to macromolecules such as DNA, proteins and lipids" (Dimuthu, 2019). "Although many synthetic drugs exist which are used as

antioxidants, reports have shown that the users are also exposed to other dangers. Synthetic antioxidants like butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) are suspected to be tumorigenic. This is the reason for preference of natural alternatives, and this research goes ahead to confirm that *F. ananassa* is one of such natural alternatives" (Dimuthu, 2019).

The results of our research as presented in table 3 shows that both ethanolic extracts and atorvastatin used for this study showed antioxidant quality. A decline in cellular level has been considered to be indicative of oxidative stress. The decrease in this endogenous antioxidant in our experimental groups B, E, F to H supports the presence of oxidative stress in our experimental animals. Also reduced were levels of catalase (CAT) and super oxide dismutase (SOD). This trend (increase in MDA levels with corresponding decrease in SOD and CAT) leave us with no doubt that methamphetamine (METH) administered once a day orally at 10mg/kg body weight of rat induced oxidative stress in the rats in the experimental group when placed in the light of the results of the rats in the control group. Also the result of the biochemical analysis presented in table 3 shows that the rats in the experimental group B which received methamphetamine only were under oxidative stress. We therefore put forth that the result of our study adds to the existing literature that exposure to methamphetamine is toxic to the brain of wistar rats. These evidences lend support to our results which shows that methamphetamine not only elicited oxidative stress in the rats, but also led to cell deaths and cytoarchitectural distortion of the tissues. These toxicities were expressed as the rats performed poorly in the neurobehavioural tests where they actually performed relatively better before exposure methamphetamine. Reports from several studies showed "long-term exposure to METH causes molecular changes in the dopamine system, contributing to nerve terminal damage in the brain and leading to impaired motor skills, rapid cognitive decline, increased anxiety, psychotic disorders, violent behaviour, hallucination, delusions and depression. These brain changes persist for many years after METH use has ceased" (Rusyniak, 2011). "METH exposure still remain a public health issue across different countries in the world especially the developing nations and as such its regulation must be strictly adhered to ensure the proper control among the users. Thus, exposure to METH is detrimental to the good health and well-being of living organisms and as a result, its toxicity might predispose to chronic ailments over time and eventually lead to reduced life expectancy. However, the toxicity of METH is no longer in doubt as several researchers have reported the toxic effects of METH on different organs and systems of the body, including the nervous system" [Aguwa et al. 2020]. Park *et al.* (2013) reported "its toxic effect on the blood-brain barrier inducing damage by altering the structure of proteins that are involved in BBB stability in mice". Rusyniak (2011) reported that "METH causes molecular changes in the dopamine system, contributing to nerve terminal damage in the brain and leading to impaired motor skills, rapid cognitive decline, increased anxiety, psychotic disorders, violent behaviour, hallucination, delusions and depression". Talloczy *et al.* (2008) reported that "METH is a weak base and alkalizes the acidic organelles within macrophages, leading to impaired

phagocytosis, antigen processing and presentation resulting to a reduction of pathogen uptake and processing, increasing infections in immune system".

Table 4 Result of Hanging Wire Test

Groups	INITIAL (secs)	FINAL (secs)
	MEAN±SD	**MEAN±SD**
Group A	107.00 ± 4.58 **p-value = 0.00079**	39.00 ± 14.73 **p-value = 0.00079**
Group B	53.63 ± 10.41* **p-value = 0.00102**	10.00 ± 2.00* **p-value = 0.00102**
Group C	53.67 ± 23.69* **p-value = 0.018755**	11.67 ± 1.53* **p-value = 0.018755**
Group D	78.33 ± 32.25* **p-value = 0.003809**	11.00 ± 2.00* **p-value = 0.003809**
Group E	47.67 ± 22.03* **p-value = 0.032802**	13.00 ± 9.17* **p-value = 0.032802**
Group F	66.67 ± 45.54[a] **p-value = 0.065775**	16.67± 4.73[a] **p-value = 0.065775**
Group G	44.33 ± 34.44[a] **p-value = 0.121207**	17.00 ± 2.65[a] **p-value = 0.121207**
Group H	22.00 ± 19.16[a] **p-value = 0.203231**	11.33 ± 5.51[a] **p-value = 0.203231**

Data was analyzed using ANOVA followed by post Hoc multiple comparison and values considered significant at $p<0.05$, significant (*) and not significant ([a]).

Table 4 shows the results of the hanging wire test carried out on 5 rats in each of the 8 groups used for the experiment. INITIAL represents the data collected prior to experimentation (after acclimatization) while FINAL represents data collected at the end of the experimental period of 28 days.

The result for the control group shows that rats spent significantly shorter time at the final test compared to the initial test. This could be due to acquaintance with the technique to fall without injury, coupled with increase in body weight.

The same trend was observed in rats from the experimental groups B, C, D, E, G and H.

However, only those of groups B, C, D and E were statistically significant. The remaining, although high but did not attain statistical significance. It is therefore difficult to ascertain the

comparative impacts of the extract and atorvastatin on improving suspension time in the rats and hence neuroprotection.

The poor result of the hanging wire test presented in Table 4 reveal that the rats had muscle weakness and so could not hold up long enough on the hanging wire. The hanging wire test was carried out to test for muscular strength and by extension cerebellar function. In this case the animals were suspended on a hanging wire using their forelimbs. The rationale is to determine how long they can suspend there which by extension is an indicator of muscle strength and motor activity. The results were compared with those taken before METH and ethanolic extracts of *Fragaria ananassa* as well as atorvastatin administration. So, the initial records are here referred to as INITIAL while the final data collected at the end of the experiment were referred to as FINAL. Our result as presented in table 4 shows statistically significant reduction for FINAL when compared to INITIAL in all the experimental groups (B, E, F, G, & H). In the control group however, there was no statistically significant difference between INITIAL and FINAL. The reduction in the FINAL for the experimental groups is an indication of weakness as well as reduced muscular strength. This result is supported by the histology results which show varying degrees and stages of necrosis following METH intoxication. It has been shown that "at a cellular level, METH exerts a myriad of effects on the central and peripheral nervous systems, immune system and the gastrointestinal system" (Rommel *et al*., 2015). Several studies with human subjects have shown that METH chronic users demonstrate structural abnormalities in the brain, namely loss of grey matter, white matter hypertrophy and purkinje fibres atrophy (Thompson *et al*., 2004). However, we observe that the groups treated with the atorvastatin had longer time of suspension compared to those that received ethanolic extracts of *Fragaria ananassa*. This was evident in both the E and H groups when compared to F and G groups. Based on the result of the neurobehavioural studies, we could infer that the atorvastatin conferred more protection on the cerebellum leading to a better retention of the functions sub served by it.

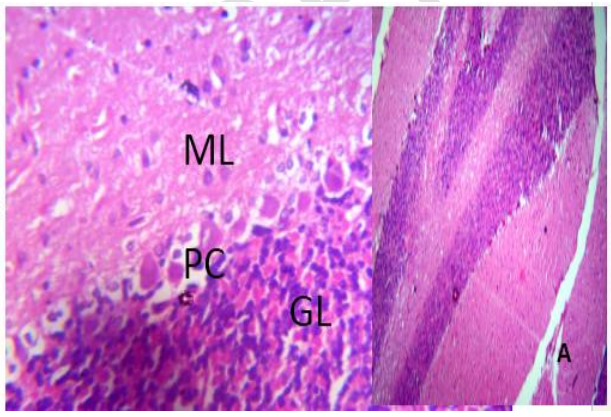

Plate 1: Photomicrograph of group A control section of rat cerebellum showing normal cerebellar cytoarchitecture with normal molecular layer (ML), granular layer (GL) and well outlined active

pyramidal cell (PC) layer (x100 X400/(H/E).

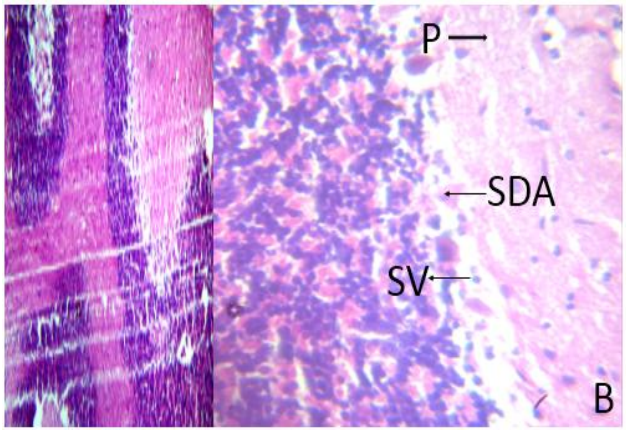

Plate 2: Photomicrograph of group B section of rat cerebellum induced with methamphetamine only shows severe degeneration and atrophy (SDA) of pyramidal cells with severe vacoulation (SV) and pyknotic (P) of pyramidal cells (x100 X400/(H/E)

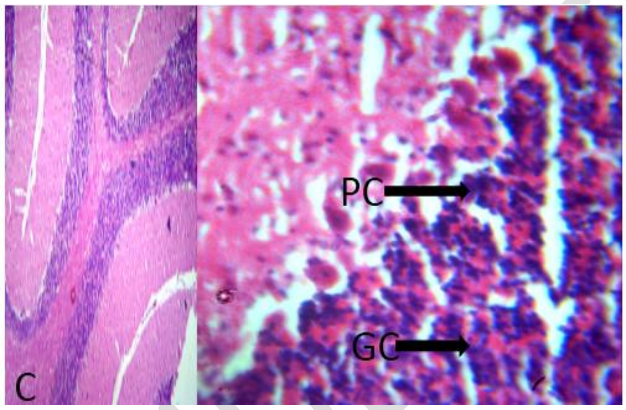

Plate 3: Photomicrograph of group C section of cerebellum administered with extract only shows cerebellum with normal pyramidal cells (PC) and granular cell (GC) (x100 X400/(H/E).

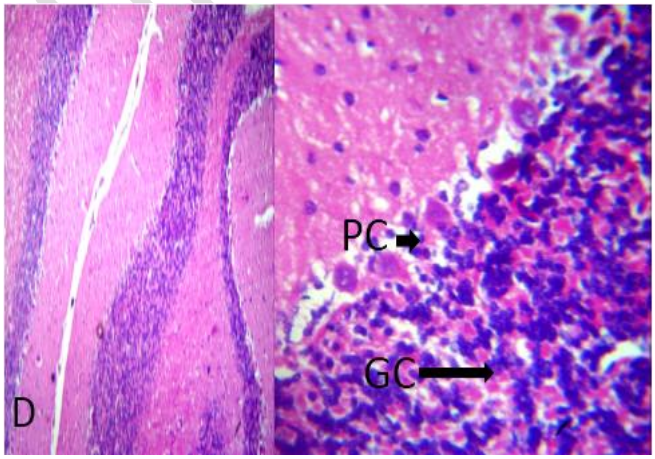

Plate 4: Photomicrograph of group D section of cerebellum administered with atorvastatin shows cerebellum with active pyramidal cells (PC) and granular cell (GC) (x100 X400/(H/E).

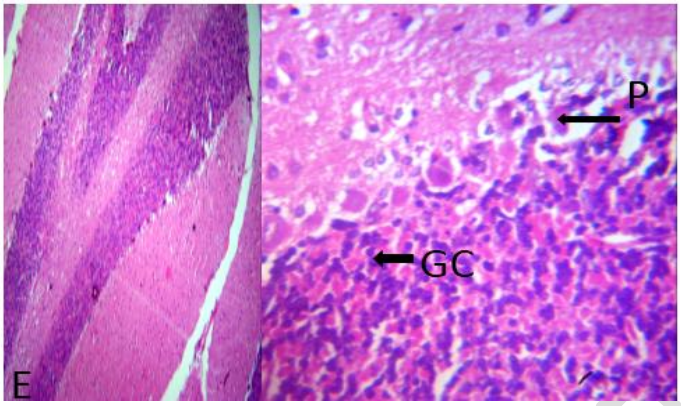

Plate 5: Photomicrograph of group E section of rat cerebellum induced with methamphetamine and atorvastatin shows mild area of pyknotic (P) pyramidal cells (dying purkinje cell) and active granular cells (GC) (x100 X400/(H/E) .

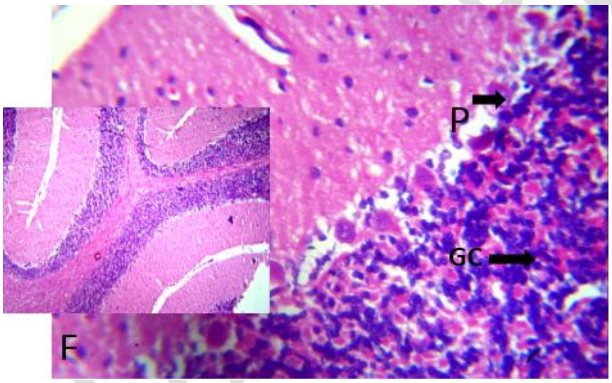

Plate 6: Photomicrograph of group F section of rat cerebellum induced with methamphetamine treated with low dose extract shows mild area of pyknotic (P) pyramidal cells (dying purkinje cells) with active granular cells (GC) (x100 X400/(H/E).

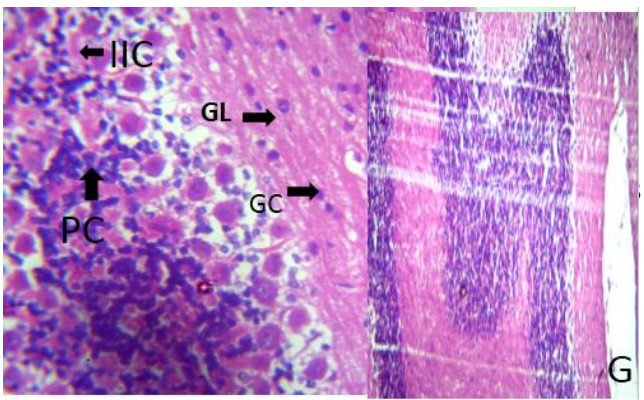

Plate 7: Photomicrograph of group G section of rat cerebellum induced with methamphetamine treated with medium dose extract shows mild infiltration of inflammatory cell (IIC), active granular cells (GC), active granular layer (GL) as well as active pyramidal cells (PC)(x100 X400/(H/E).

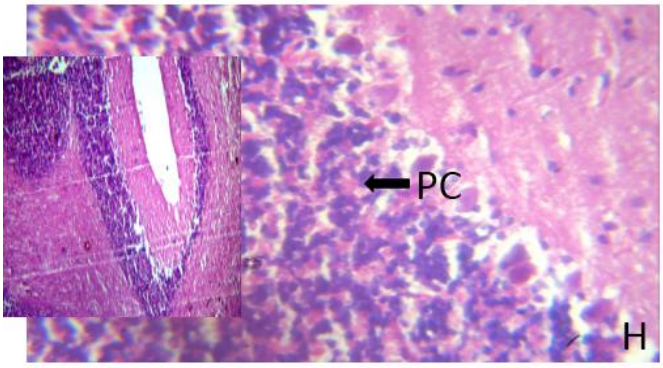

Plate 8: Photomicrograph of group H section of rat cerebellum induced with methamphetamine treated with high dose extract and atorvastatin shows moderately active Purkinje cells (PC) indicating normal cerebellar architecture(x100 X400/(H/E).

"Histopathological assessment of the cerebellum showed various degrees and stages of cell death, distortion of the Purkinje cell layer, infiltration of cells in the granular cell layer and general disorientation of the architecture of the Purkinje cell layers. There was scanty distribution of Purkinje cells as well as vacoulation accompanied by pykonitc of the Purkinje cell layer especially in group B which received METH without treatment. Notwithstanding, there is a slightly normal cerebellar architectures in the treated groups with atorvastatin and ethanolic extract of *F. ananassa*, thus conferring neuroprotection. The Purkinje cell is the sole motor output of the cerebellar cortex. Reduction in its number, size or efficiency could lead to interferences with the motor functions such as loss of fine movement, loss of grasping, maintenance of equilibrium and loss of regulation of muscle tone. Moreover, it has been shown that neuronal degeneration in the cerebellum can affect the level of copper concentration in the cerebellum and this can affect the action of the neurotransmitter, dopamine which is very crucial in motor activity. This is because copper serve as a modulator for the neurotransmitter, dopamine and a decreased in copper level may reduce dopamine activity" [Aguwa et al. 2020]. Rusyniak (2011) reported that "long-term use of METH causes molecular changes in the dopamine system, contributing to nerve terminal damage in the brain and leading to impaired motor skills, rapid

cognitive decline, increased anxiety, psychotic disorders, violent behaviour, hallucination, delusions and depression".

Northrop *et al.* (2016) reported that "METH induces peripheral kidney and liver damage that leads to toxic ammonia levels in the blood and subsequently, the brain. Ammonia that is not cleared by the liver as normal accumulates and causes oxidative damage of endothelial cells, activation of matrix metalloproteases (MMPs) and neuro-inflammation via microglia and astrocyte activation, leading to BBB disruption as observed in our present study where there is infiltration of inflammatory cells which an evidence of oxidative stress". This could explain the poor performance recorded in the hanging wire test for cerebellar function carried out after exposure to METH when compared to the results gotten prior to exposure to METH.

Conclusion

From our results, it could be concluded that strawberry ethanolic fruit extract (*Fragaria ananassa)* confers protection on the human brain against oxidative stress induced by methamphetamine exposure in rats as comparable to that conferred by atorvastatin, a synthetic drug. Strawberry extract exhibited better antioxidant properties, and as such can be used to substitute for atorvastatin as neuroprotective agents. Also, the administration of strawberry and atorvastatin did not have significant effect on the performance of the rats in the hanging wire neurobehavioural tests. Both atorvastatin and extract conferred protection on the histomorphology of the cerebellum in Wistar rats.

Recommendation

Conscious consumption of raw strawberry and in form of juice as a natural source of antioxidants is therefore highly recommended. We also recommend that further researches be carried out with several solvent combinations to know the combination that can give the best antioxidant yield.

Declarations

Ethical Approval

Due ethical approval was gotten from the research ethics committee of the Faculty of Basic Medical Sciences, Nnamdi Azikiwe University, Nnewi

REFERENCES

Aaby K, Ekeberg D and Skrede G: Characterization of phenolic compounds in strawberry (*Fragaria ananassa*) fruits by different HPLC detectors and contribution of individual compounds to total antioxidant capacity. Journal of Agricultural and Food Chemistry 2007; 55: 4395-4406.

Abubakar, M. B., Abdullah, W. Z., Sulaiman, S. A., and Suen A. B. (2012) *Int. J. Mol. Sci.* 13, 15054-15073

Battino, M., Giampieri, F., Cianciosi, D., Ansary, J., Chen, X., Zhang, D.i., Forbes- Hern´andez, T. (2021). The roles of strawberry and honey phytochemicals on human health: A possible clue on the molecular mechanisms involved in the prevention of oxidative stress and inflammation. *Phytomedicine: International Journal of Phytotherapy and Phytopharmacology, 86*, 153170. https://doi.org/10.1016/j. phymed.2020.153170

Brookie, K. L., Best, G. I. and Conner, T. S. (2018). "Intake of raw fruits and vegetables is associated with better mental health than intake of processed fruits and vegetables," *Front. Psychol*., vol. 9, pp. 487,.

Carlson, C.G., Rutter, J., Bledsoe, C., Singh, R., Hoff, H., Bruemmer, K. Sesti, J.,.Gatti, F., Berge, J. and McCarthy, L. (2010). "A simple protocol for assessing inter-trial and inter-examiner reliability for two noninvasive measures of limb muscle strength." *Journal of neuroscience methods* 186, no. 2: 226-230. https://doi.org/10.1016/j.jneumeth.2009.11.006

Cianciosi, D., Simal-G´andara, J., & Forbes-Hern´andez, T. Y. (2019). The importance of berries in the human diet. *Mediterranean Journal of Nutrition and Metabolism, 12*(4), 335–340. https://doi.org/10.3233/MNM-190366

Deepak, S. K. , Mahabaleshwar, V. H. and Hanhong. B (2015). Significance of Antioxidant Potential of Plants and its Relevance to Therapeutic Applications. doi: 10.7150/ijbs.12096

Dimuthu, D (2019). "Basic Assessment of Community Based Water Projects in Sri Lanka to Analyze Impacts of it for Health and Social Development and Sustainable Community Development Approaches." *GSJ* 7, no. 1

Ekaluo, U.B., Ikpeme, E. V., Ekerette, E. E. and Chukwu, C. I. (2015).. "In vitro antioxidant and free radical activity of some Nigerian medicinal plants: bitter leaf (Vernonia amygdalina L.) and guava (Psidium guajava Del.)." *Research Journal of Medicinal Plant* 9, no. 5: 215-226. DOI: 10.3923/rjmp.2015.215.226

Ekaluo, U.B., Ikpeme, E. V., Ibiang, Y. B. and Omordia, F. O. (2013). "Effect of soursop (Annona muricata L.) fruit extract on sperm toxicity induced by caffeine in albino rats." *J. Med. Sci* 13, no. 1: 67-71. DOI:10.3923/jms.2013.67.71

Emmanuel-Ikpeme, C., Peters, H. and Orim, A.O. (2014). "Comparative evaluation of the nutritional, phytochemical and microbiological quality of three pepper varieties." *Journal of food and nutrition sciences* 2, no. 3: 74-80. doi: 10.11648/j.jfns.20140203.15

Fouillaud, M., Caro, Y., Venkatachalam, M., Grondin, I. and Dufossé, L. (2018). "Anthraquinones. Leo M. L. Nollet; Janet Alejandra Gutiérrez-Uribe. Phenolic Compounds in Food Characterization and Analysis", CRC Press, pp.130-170, 978-1-4987-2296-4.

Giampieri F, Tulipani S, Alvarez JM, Quiles JL, Mezzetti B and Battino M (2015). The strawberry: Composition, nutritional quality, and impact on human health. Nutrition; 28: 9-19.

Giampieri, F., Gasparrini, M., Forbes-Hernandez, T. Y., Mazzoni, L., Capocasa, F., Sabbadini, S., Battino, M. (2018). Overexpression of the Anthocyanidin Synthase Gene in Strawberry Enhances Antioxidant Capacity and Cytotoxic Effects on Human Hepatic Cancer Cells. *Journal of Agricultural and Food Chemistry*, *66*(3), 581–592. https://doi.org/10.1021/acs.jafc.7b0417710.1021/acs.jafc.7b04177.s001

Gornall, A.G., Bardawill, C. J. and David, M. M. (1949) "Determination of serum proteins by means of the biuret reaction." *Journal of biological chemistry* 177, no. 2: 751-766.

Hassan, S. A., Abo-Elwafa, S. M., El-Baz, H. A., Hussein, M. A., and Gobba, N. A (2021). The potential protective effect of strawberry extract against indomethacin-induced liver toxicity andgastric ulceration in rats: biochemical, histopathological and genetic studies. *IJPSR*, 2021; Vol. 12(6): 3120-3133.

Hassan, Y. and Barde, M (2019). "Phytochemical Screening and Antioxidant Potential of Selected Nigerian Vegetables", *Int. Ann. Sci.*, vol. 8, no. 1, pp. 12-16, doi: 10.21467/ias.8.1.12-16.

Hauer, P (2010). Systemic effects of methamphetamine use. *S D Med.* 63(8):285-287.

Misra, H.P. and Fridovich, I (1972). "The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase." *Journal of Biological chemistry* 247, no. 10: 3170-3175. *The Journal of Biological Chemistry 247, 3170-3175.*

Mohammed, I. Y. E., Manal, F. E., Ebtesam, M. A., and Ahmed, E. A. M (2017). Protective Effect of *Fragaria ananassa* Crude Extract on Cadmium-Induced Lipid Peroxidation, Antioxidant Enzymes Suppression, and Apoptosis in Rat Testes. *International Journal of Molecular Sciences*

Northrop NA, Halpin LE, Yamamoto BK (2016). Peripheral ammonia and blood brain barrier structure and function after methamphetamine. *Neuropharmacology.* 107:18-26.

Park, M., Kim, H.J., Lim, B., Wylegala, A. and Toborek, M. (2013). Methamphetamine-induced occludin endocytosis is mediated by the Arp2/3 complex-regulated actin rearrangement. *J Biol Chem.* 288(46):33324-33334.

Phillipson, J.D (2000). Phytochemistry of medicinal plants. Phytochem; 56(3): 237-248.

Rommel, N., Rohleder, N.H., Wagenpfeil, S., Haertel-Petri, R. and Kesting, M.R (2015). Evaluation of methamphetamine-associated socioeconomic status and addictive behaviors, and their impact on oral health. Addict Behav. 50:182-187.

Rusyniak, D.E. (2011). Neurologic manifestations of chronic methamphetamine abuse. *Neurologic clinics.* 29(3):641-655.

Sahlin, E., Savage, G. P. and Lister, C. E. (2004). "Investigation of the antioxidant properties of tomatoes after processing." *Journal of Food composition and Analysis* 17, no. 5: 635-647. https://doi.org/10.1016/j.jfca.2003.10.003

Singh R, Gupta RK, Patil RT, Sharma RR, Asrey R, Kumar A and Jangra KK (2010). Sequential foliar application of vermicompost leachates improves marketable fruit yield and quality of strawberry (*Fragaria ananassa* Duch.). Scientia Horticulurae; 124: 34–39.

Sizar O, Khare S, Jamil RT, (2021). Statin Medications. Treasure Island, FL: StatPearls Publishing. Available at: https://www.ncbi.nlm.nih.gov/books/NBK430940/

Talloczy, Z., Martinez, J., Joset, D., Ray, Y., Gacser, A., Toussi, S., Mizushima, N., Nosanchuk, J.D., Goldstein, H., Loike, J., Sulzer, D. and Santambrogio, L (2008). Methamphetamine inhibits antigen processing, presentation, and phagocytosis. PLoS Pathog. 4(2):e28.

Thompson, P.M., Hayashi, K.M., Simon, S.L., Geaga, J.A., Hong, M.S., Sui, Y., Lee, J.Y., Toga, A.W., Ling, W. and London, E.D. (2004). Structural abnormalities in the brains of human subjects who use methamphetamine. *J. Neurosci*. 24, 6028–6036.

Tõnutare T, Keert K, Szajdak L and Moor U (2014). Composition of commercially produced organic and conventional strawberries. Nutrition and Food Science; 44: 562-75.

Tulipani S, Alvarez JM, Busco F, Bompadre S, Quiles JL, Mezzetti B and Battino M (2011). Strawberry consumption improves plasma antioxidant status and erythrocyte resistance to oxidative haemolysis in humans. Food Chemistry; 128: 180-86.

Tulipani S, Romandini S, Bompadre S, Capocasa F and Mezzetti B (2009). Variation in strawberry micronutrients, phytochemical and antioxidant profiles: the combined effect of genotype and storage. Acta Horticulturae; 842: 867-71.

Valerino, D.M. and. McCormack, J. J (1971) "Xanthine oxidase-mediated oxidation of epinephrine." *Biochemical pharmacology* 20, no. 1: 47-55. https://doi.org/10.1016/0006-2952(71)90470-9

Zacco A., Togo J., Spence K., Ellis A., Lloyd D., Furlong S. and Piser T. (2003.) 3-Hydroxy-3-methylglutaryl coenzyme A reductase inhibitors protect cortical neurons from excitotoxicity. *J. Neurosci. 23, 11 104–11 111.*

Aguwa US, Okeke SM, Okeke CM, Eze CE, Obinwa BN, Izuogu M, Ovie FO, Onyejike DN, Ogbuokiri DK, Ezejindu DN. Comparing the Neuroprotective Effects of Aqueous and Methanolic Extracts of Vernonia Amygdalina on the Cerebellum of Adult Male Wistar Rats. International Annals of Science. 2020 Jul 1;9(1):145-59.