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Original Article

Carica papaya Fruit Extract Protects the Cerebellum of Wistar Rats Against Acrylamide-Induced Oxidative Stress

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ABSTRACT

Acrylamide is a water-soluble vinyl monomer produced industrially as an agent for the production of plastics and cosmetics, in waste water management as a flocculation agent, and naturally in fried, baked, and roasted foods, especially when heated beyond 120 °C. Acrylamide (AC) is associated with severe neurotoxic complications through the initiation of oxidative stress. Hence the use of *Carica papaya* (CP), a natural antioxidant, in protecting the cerebellum of Wistar rats against acrylamide-induced oxidative stress. Forty adult female Wistar rats (180-200 g) were divided into four groups (n=10): Control (1 mL distilled water), AC (25 mg/kg), CP (300 mg/kg), and CP+AC (300 mg/kg+25 mg/kg). All treatments were administered orally for 21 days. Thereafter, the rats were weighed, and neurobehavioural tests were done. The rats were sacrificed, while their cerebella were dissected out and preserved for oxidative stress, antioxidant markers, and histological and immunohistochemical studies. Data were analysed using analysis of variance at $p<0.05$. There was decreased body weight, locomotor activities, and forelimb grip strength in the AC-treated group compared with the control and other treated groups. Increased lipid peroxidation and nitric oxide levels, decreased glutathione (GSH) levels, and catalase, superoxide dismutase, and glutathione peroxidase activities in the AC-treated groups compared with the control and other treated groups. Histologically, degeneration, pyknosis, shrinking and loss of Purkinje neurons, reactive astrogliosis, and apoptosis occurred in the AC-treated group compared with the control and other treated groups. In conclusion, acrylamide induced oxidative stress in the rat cerebellum, and administration of CP fruit extract offered protection from its neurotoxic effects.

Keywords
Acrylamide neurotoxicity, Cerebellar cortex, Neurobehaviour, Astrogliosis, Apoptosis

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INTRODUCTION

Acrylamide is a white, solid, odourless chemical that is produced industrially and naturally. Industrially, it is produced in the plastic or cosmetic industries and used as water-soluble thickeners and flocculation agents in waste water management. It is highly toxic and likely to be carcinogenic (America Cancer Centre 2019). Acrylamide, discovered naturally in foods in April 2002 by Eritrean scientist Eden Tareke in Sweden, is widely present in fried, baked, and roasted foods, such as French fries, breakfast cereals, and roasted coffee (Tareke et al. 2002; Abbes et al. 2016; Markovic et al. 2018), above the temperature of 120 °C (248 °F). Due to its water solubility, it easily crosses the blood-brain barrier (BBB) (Yildizbayrak and Erkan 2018), generates reactive oxygen species (ROS) (Abdel-Daim et al. 2015), and has been implicated in oxidative stress in the cerebrum, cerebellum, and hippocampus (Sameh et al. 2021). The major change in lifestyle, feeding pattern, and use of some industrial chemicals resulted in the synthesis of acrylamide, which is a neurotoxin and causes the disassembly or rearrangement of intermediate neurofilaments and leads to a neurotoxic syndrome characterized by ataxia, skeletal muscle weakness, and weight loss (Riboldi et al. 2014), hence, the cerebellum is implicated.

The cerebellum is the largest part of the hindbrain and weighs about 150 g in humans. It is separated from the

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brainstem by the fourth ventricle. The cerebellum functions in maintaining equilibrium, tone, and posture of trunk muscles as well as being involved in the smooth performance of skilled voluntary movements (Kulkami 2012). However, the cerebellum has been implicated in the acrylamide toxicity that compromised the cerebellar functions outlined above in Wistar rats (Wesam et al. 2019). It becomes necessary to introduce strategies that can protect or reduce acrylamide-induced oxidative stress in the cerebellum of Wistar rats. Hence, the use of *Carica papaya* fruit extracts with strong antioxidant properties in this study

Carica papaya is one of the natural remedies that have been used in traditional medicine for constipation, wound repair, skin infection, reproductive organ stimulation, and diabetes (Andrews et al. 2018). Previous studies provide proof for therapeutic effects on wound healing in diabetic rats (Nayak et al. 2007) and antibacterial effects on common wound microorganisms. Fermented papaya restores antioxidant enzymes and protects the liver from oxidative damage during N-methyl-N-nitrosourea-induced hepatocellular carcinoma in Balb/c mice. In Mauritian neo-diabetic subjects, short-term supplementation with fermented papaya reduced cardiovascular disease risk via decreasing inflammation and oxidative stress (Somanah et al. 2012). Moreover, fermented papaya also decreased the marker of oxidative damage to DNA, 8-hydroxy-2'-deoxyguanosine (8-OHdG), in patients with Alzheimer's disease (Barbagallo et al. 2015). Although the antioxidant and therapeutic effects of papaya on wound healing and some diseases have been reported, the benefit of papaya on endothelial cells (ECs) has not been mechanistically determined thus far. Unripe *Carica papaya* fruit appeared to diminish H_2O_2-induced cell death by two main strategies: elimination of intracellular stress (ROS reduction and NF-kB inactivation) (Daiber et al. 2016) and equipping the cells with antioxidant defence (enhanced CAT activity and Nrf2 modification). Therefore, unripe *Carica papaya* fruit can be a specimen for the development of nutraceuticals for the prevention of oxidative-related conditions such as cardiovascular disease and aging.

However, various factors have been attributed to the increased use of plant-based remedies. These include economic considerations such as the high cost of conventional medicines and the perceived lower toxicity and fewer side effects of plant-based medicines, as these plants have been used before. The increased cases of drug resistance that are being encountered with the use of conventional medicines have favourably contributed to the use of plant-based remedies (Pan et al. 2014). With the abundant phytochemical components of *Carica papaya* fruit, including alkaloids, flavonoids, tannins, and phenolic compounds (Sofowora et al. 2013; Choudhury et al. 2015), and the reported antioxidant properties, this study elucidated the neuroprotective potentials of *Carica papaya* fruit extract against acrylamide-induced oxidative stress in the cerebellum.

MATERIALS AND METHODS

Chemicals

Acrylamide (Batch No: 62021, Mol. Gew. 7108, Hannover, Germany) and all reagents were purchased from a Reliable Chemical Shop in Enugu State, Nigeria. In this study, 25mg/kg body weight of acrylamide solution was freshly prepared by dissolving the powder in a saline solution.

Plant authentication and extract preparation

Matured, fresh, ripe *Carica papaya* fruits were obtained from Bodija market, Ibadan, Oyo State, Nigeria, and authenticated at the Department of Botany, University of Ibadan, with UIH-23127**.** The fruits were peeled and the cream coloured seeds were discarded. Five hundred grams (500 g) of the fruit mesocarp was weighed and blended into a beaker, and 1.5 L of clean water was used to soak *Carica papaya* fruit Extract. The extract was sieved into a clean container and kept in the refrigerator until use.

Animals

Forty adult female Wistar rats (180-200g) used for the study were kept in rat cages in the well-ventilated Central animal house of the College of Medicine, University of Ibadan, Nigeria, with free access to tap water and dry rat pellets. The rats were divided into four groups of ten rats per group. All animals received humane care and handling after the protocol was reviewed and approved by the University of Ibadan Ethical Review Committee with approval number UI-ACUREC/062-0621/30.

Experimental Design

The rats were assigned to four groups: Control group (1 mL distilled water); acrylamide group (AC, 25 mg/kg body weight of acrylamide); *Carica papaya* group (CP, 300 mg/kg body weight of fruit extract of *Carica papaya)*; *Carica papaya*-Acrylamide group (CP+AC, 300 mg/kg + 25 mg/kg body weight of fruit extract of *Carica papaya* and of acrylamide, respectively).

All treatments were given orally for 21 days. Thereafter, the rats were weighed, and neurobehavioural tests (open-field and forelimb grip strength) were done. The rats were sacrificed, and the cerebella were dissected out and preserved for oxidative stress and antioxidant markers (lipid peroxidation, nitric oxide, reduced glutathione, catalase, superoxide dismutase, and glutathione peroxidase), as well as histological (haematoxylin and eosin, and Cresyl fast violet stains) and immunohistochemical studies (glial fibrillary acidic protein, GFAP, for the astrocyte, and B-cell lymphoma 2, Bcl2, for apoptosis).

Open Field Test

In this test, rats were taken to the test room in their home cages and handled by the base of their tails at all times. Rats were placed in the centre of the open field and allowed to explore the apparatus for 5 minutes, after which they were returned to their home cages, and the open field was cleaned with 70% ethyl alcohol and allowed to dry between tests (Carrey et al 2000). This test evaluated exploratory movement, exploratory behaviour and anxiety

in the rats. The following parameters were measured: Horizontal movements (measured by the number of transitions or lines crossed): Vertical movements or rearing (number of times the rat balances only on its hind feet); Centre square duration (length of time spent in the centre square before exploring the platform); Freezing (duration of times the rat stops without moving); Grooming (duration of times the rat stops in order to use its forelimbs for either face or body cleaning, genital licking, or scratching). All these parameters were assessed and manually recorded by the same set of observers.

Forelimb Grip Strength Test
This test involved the forepaws of the rats being placed on a horizontally suspended metal wire (measuring 2 mm in diameter and 1 m in length) placed one meter above a landing area filled with soft bedding. The length of time each rat was able to stay suspended before falling off the wire was recorded. A maximum of 2 min was given to each rat, after which it was removed. This test reflects muscular strength in the animal (Pichon et al. 2010).

Oxidative Stress and Antioxidant Markers
The cerebella of rats in each group were taken for biochemical assays to determine the level of oxidative stress and antioxidants. The assays include protein concentration, lipid peroxidation (LPO), nitric oxide (NO), reduced glutathione (GSH), catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx).

Histological preparations
Cerebellar tissues from the rats of all groups were fixed in 10% formal-saline, processed employing routine paraffin embedding techniques, and stained with haematoxylin and eosin for histological and histomorphometric evaluation (Ahmed 2016; Mahmoud 2017). They were examined and then evaluated under a light microscope.

Immunohistochemistry
Cerebellar tissues were immunostained with GFAP for astrocyte using the avidin-biotin immunoperoxidase method (Mahmoud 2017) and avidin-biotin peroxidase system for localization of apoptotic cells for Bcl2 (Atiba et al. 2021).

Evaluation of Slides
The slides were first viewed at lower magnification (×40 and ×100), and an area of interest was selected. The selected area was then viewed under higher magnification (×400) using a 500-pixel Leica binocular microscope. Five high-power fields were selected for different cell counts using the imageJ software.

Statistical Analysis
Data obtained and expressed as mean ± S.D. were further analysed employing one-way analysis of variance, followed by Dunnett's post-test for multiple comparisons using GraphPad Prism California, USA, version 9.0 for Windows, with the level of statistical significance set at $p<0.05$.

RESULTS

General Observation
During the experiment, the rats in the control and *Carica papaya* groups were very active, while some of the rats in the acrylamide group were morbidly inactive, with displays of general body weakness, gait abnormalities, an unsteady walking pattern with abduction and external rotation of hind limbs, and reduced food and water intake compared with their consumption rate during the period of acclimatization, hence loss of weight.

Body Weight Changes
There was significant body weight loss in the acrylamide-treated group compared with the control and *Carica papaya*-treated groups and relative improvement in the Carica papaya-acrylamide group compared with the acrylamide group-only at $p<0.05$, as shown in Table 1.

Table 1: Body Weight of Rats

Group	Initial weight (g)	Final weight (g)	Weight gain/loss (g)	Weight gain/loss (%)
CNTL	197.70 ±8.20	223.30 ±17.80	25.67 ±17.50	6.00 ±3.90
AC	195.50 ±5.20	138.30 ±7.30^{+}	-57.17 ±5.50^{+}	-17.16 ±2.00^{+}
CP	188.70 ±6.10	219.20 ±7.40^{a}	30.50 ±6.00^{a}	7.47 ±1.40^{a}
CP+AC	187.30 ±7.40	204.80 ±10.30*	17.50 ±12.90*	4.44 ±3.20*

Values (n=10) are expressed as mean±SD (g) at $p<0.05$. +Acrylamide vs control, ^{a}*Carica papaya* vs acrylamide group and **Carica papaya*+acrylamide vs acrylamide group. CNTL-Control, AC-Acrylamide, CP-*Carica papaya*, CP+AC -*Carica papaya*+acrylamide

Neurobehaviour
There was a significant decrease in the drop-off time of the acrylamide-treated group in the forelimb grip test compared with the increased drop-off time in the control and *Carica papaya*-treated groups at $p<0.05$. There were improvements in the *Carica papaya*-acrylamide group at $p<0.05$. In the open field platform, there was a decreased number of lines crossed, an increased amount of time spent at the centre square, and an increased amount of time spent by the rats immobile (freezing) in the acrylamide group compared with the control and *Carica papaya* groups, with an increased number of lines crossed, decreased time spent at the centre square, and decreased freezing time at $p<0.05$. There were mild improvements in the above parameters in the *Carica papaya*-acrylamide group. There were decreased grooming, and rearing frequencies in the acrylamide-treated group compared with the control and *Carica papaya* groups, with increased grooming and rearing at $p<0.05$. However, there were relative improvements in the above parameters in the *Carica papaya*+acrylamide group compared with the acrylamide group only at $p<0.05$ (Fig. 1).

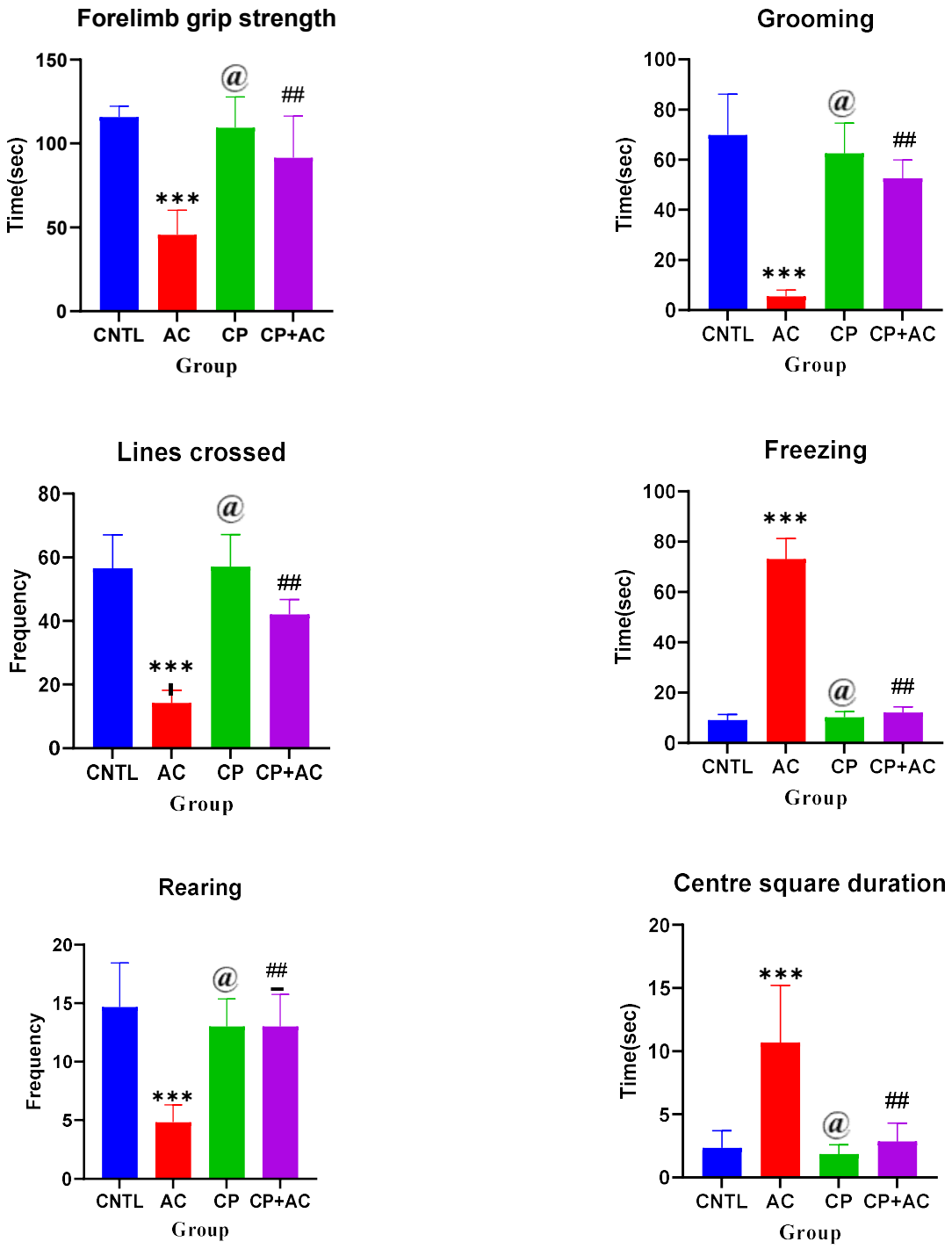
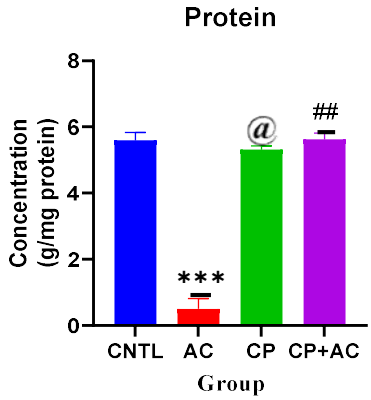
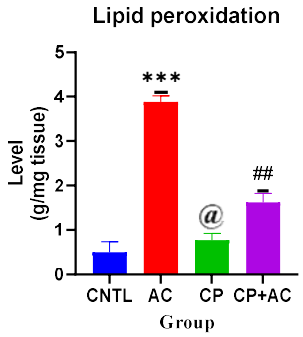


Fig. 1: Neurobehavioural assessment. Values (n=6) are expressed as mean±SD at P<0.05. ***Acrylamide vs control, [@]*Carica papaya* vs acrylamide group and [##]*Carica papaya*+acrylamide vs acrylamide group. CNTL-Control, AC-acrylamide, CP-*Carica papaya*, CP+AC -*Carica papaya*+acrylamide

Oxidative Stress Markers

There were increased oxidative stress markers (lipid peroxidation and nitric oxide) and decreased protein concentrations in the acrylamide-treated group compared with the control and other treated groups. There was a relative amelioration of these parameters in the *Carica papaya*-acrylamide group compared with the acrylamide group only at p<0.05 (Fig. 2).





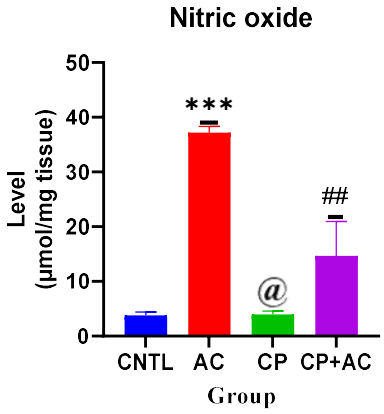
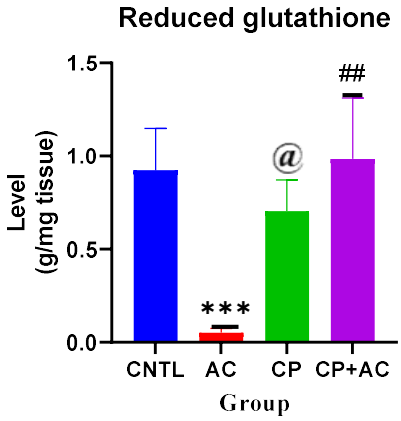
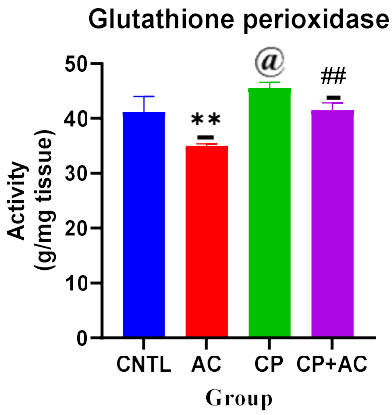


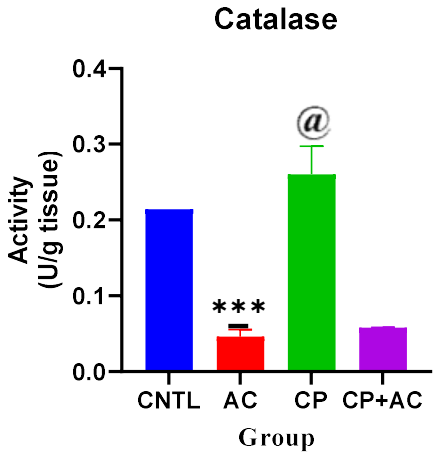
Fig. 2: Oxidative stress markers. Values (n=5) are presented as mean±SD at P<0.05. ***Acrylamide vs control, @*Carica papaya* vs acrylamide group and #*Carica papaya*+acrylamide vs acrylamide group. CNTL- Control, AC-acrylamide, CP-*Carica papaya*, CP+AC -*Carica papaya*+acrylamide

Antioxidants Biomarkers

There were decreased antioxidant biomarkers (GSH, CAT, SOD, and GPx) in the acrylamide-treated group compared with the control and other treated groups. There was relative protection offered in ameliorating the levels of these parameters in the *Carica papaya*-acrylamide group compared with the acrylamide group at p<0.05 (Fig. 3).







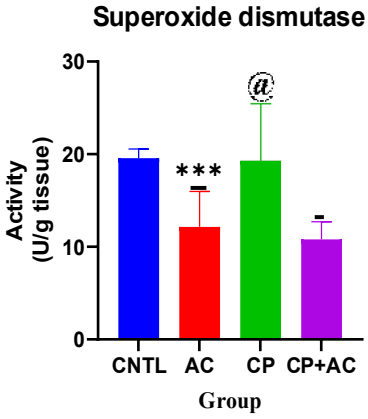


Fig. 3: Antioxidant biomarkers. Values (n=5) are presented as mean±SD at P<0.05. ***Acrylamide vs control, @*Carica papaya* vs acrylamide group and #*Carica papaya*+acrylamide vs acrylamide group. CNTL-control, AC-acrylamide, CP-*Carica papaya*, CP+AC -*Carica papaya*+acrylamide

Histomorphology

There was drastic loss of Purkinje cells (blue arrow) and neuronal cell vacoulations (red arrow) in the cerebellar cortex of the acrylamide group compared with the control and the *Carica papaya* treated groups which showed normal architecture. There was improvement in the *Carica papaya*-acrylamide group when compared with the acrylamide group (Fig. 4).

Nissl Substance Expression

There were drastic neuronal loss (Purkinje cells) and severe condensation of neuronal cells in the acrylamide group compared with the control and *Carica papaya* treated groups with normal neuronal densities. There were mild neuronal densities in the *Carica papaya*-acrylamide group (Fig. 5).

Immunohistochemistry

GFAP expression

GFAP was remarkably and highly expressed in the acrylamide-treated group, hence the increase in astrocyte population therein compared with the reduced astrocyte population in the control and the *Carica papaya* group due to least expression of GFAP and mild expression of GFAP with mild astrocyte population in the *Carica papaya*-acrylamide treated group (Fig. 6).

Apoptotic cell count (Bcl2)

There was intense positive immunoreaction for Bcl-2 protein expression observed in the acrylamide-treated rats, hence increased number of cells susceptible to die by apoptosis compared with the decreased expression of Bcl2 protein in the control and *Carica papaya* group but mild expression of bcl2 in the *Carica papaya*-acrylamide group (Fig. 7).

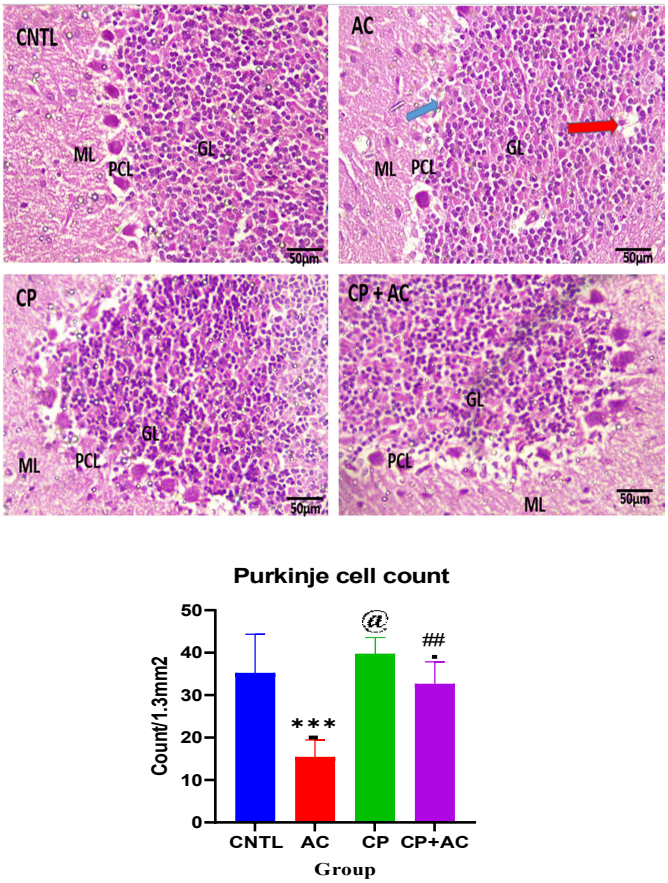
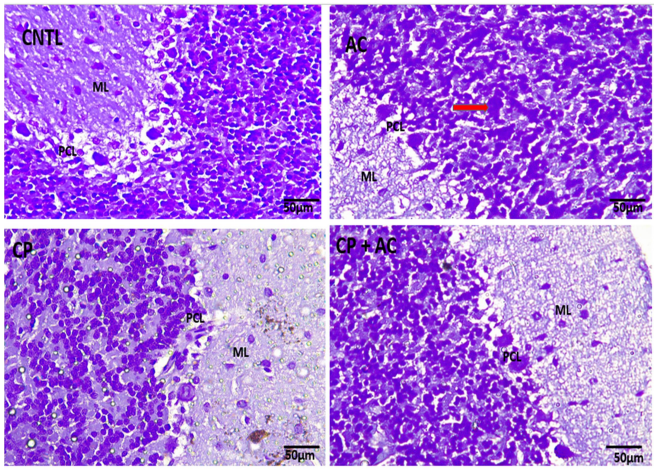


Fig. 4: Histomorphology (Purkinje cell count). Control group (CNTL) showing the normal architecture of the cerebellar layers (ML, PCL, GL) with intact cells therein. Acrylamide-treated group (AC) showed drastic loss of Purkinje cells in PCL- Blue arrow and vacuolation of cells in the GL- Red arrow. *Carica papaya* group (CP) only showed normal architecture of the cerebellar cortex. *Carica papaya*-acrylamide group (CP+AC) showed some restoration of some damaged Purkinje cells. H&E, scale bar: 50µm. Values (n=5) are presented as mean ± SD at P<0.05. ***Acrylamide vs control, $^{@}$*Carica papaya* vs acrylamide group and $^{\#\#}$*Carica papaya*+acrylamide vs acrylamide group



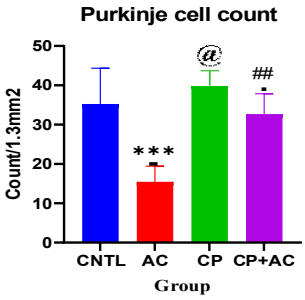
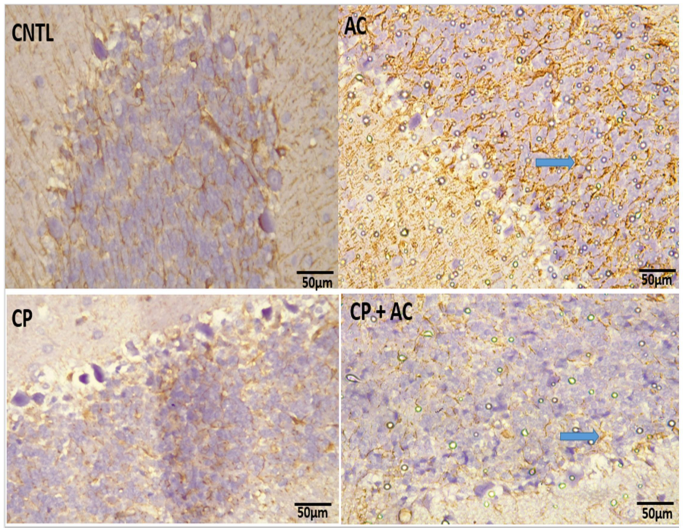


Fig. 5: Nissl substance expression. Control group (CNTL) showing the normal architecture of the cerebellar layers (Molecular layer (ML), Purkinje cell layer (PCL), Granule layer (GL) with intact cells therein. Acrylamide-treated group (AC) showed drastic Purkinje cell loss in the PCL, vacuolations and condensation of neurons signifying neuronal death in the GL- Red arrow. *Carica papaya* group only (CP) showed normal architecture of the cerebellar cortex. *Carica papaya*-acrylamide group (CP+AC) showed some restoration of some damaged Purkinje cells. Cresyl violet stain, scale bar: 50µm



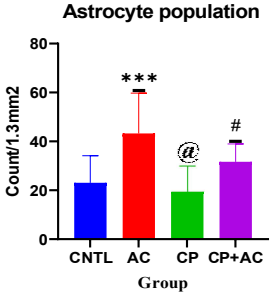
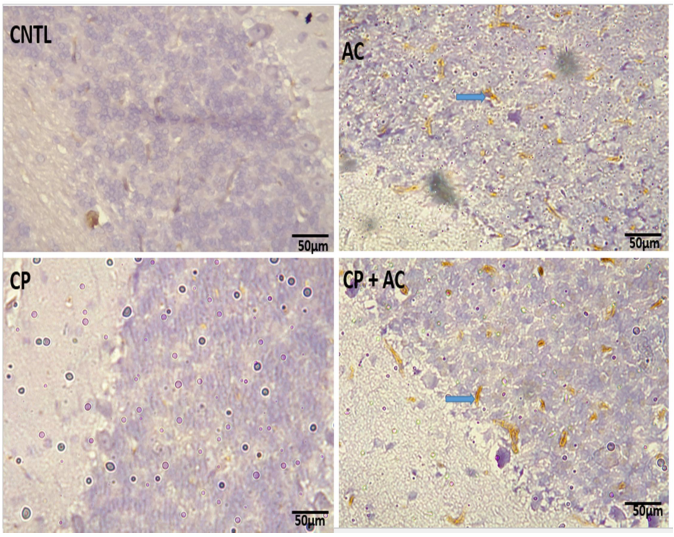


Fig. 6: Astrocyte population. Values (n=5) are presented as mean±SD at P<0.05. ***Acrylamide vs control, @*Carica papaya* vs acrylamide group and #*Carica papaya*+acrylamide vs acrylamide group. Control (CNTL) with normal astrocyte density. Acrylamide group (AC) with high level of astrogliosis. *Carica papaya* group (CP) with normal astrocytes density. *Carica papaya*-acrylamide group (CP+AC) with less dense astrocytes. The arrow(s) in each photomicrograph point at each astrocyte. GFAP, scale bar: 50µm



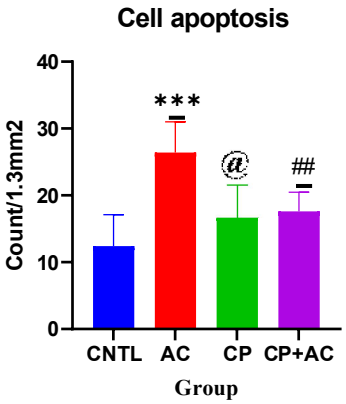


Fig. 7: Apoptotic cell count. Values (n=5) are presented as mean±SD at P<0.05. ***Acrylamide vs control, @*Carica papaya* vs acrylamide group and ##*Carica papaya*+acrylamide vs acrylamide group. Control (CNTL) with normal cell density. Acrylamide group (AC) with abnormally increased cell density susceptible to die. *Carica papaya* group (CP), with normal cell density. *Carica papaya*-acrylamide group (CP+AC) with less dense death susceptible cells. The arrows show the susceptible apoptotic cell. Bcl_2, scale bar: 50µm

DISCUSSION

This study investigated the protective effects of *Carica papaya* fruit extract against acrylamide-induced oxidative stress in the cerebellum of Wistar rats. There was a percentage weight loss in the acrylamide-treated group when compared to the control and other groups. The decreased body weight may be due to the drastic reduction in food and water intake observed following the administration of acrylamide, which was very significant when compared with their consumption rate during the periods of acclimatization. The weight loss may also be attributed to the direct effect of acrylamide on growth, through excessive breakdown of tissue proteins, decreased plasma proteins (Arihan et al. 2011), and other haematological parameters

(Lebda et al. 2015). Acrylamide is not only neurotoxic and carcinogenic but also damages the erythrocyte membrane and alters blood viscosity parameters (Lebda et al. 2015).

The cerebellum maintains balance and equilibrium, fine-tunes voluntary movements, enhances motor learning and the acquisition of skilled movements, and regulates non-motor processes including linguistic, cognitive, and affective functions (Charles and Guy 2017).

Animals from all the groups were evaluated for their performance in the open field arena for 5 minutes using various behavioural, locomotor, neurological, and sensory-autonomic measurements. The open-field test was performed to evaluate the exploratory movement and anxiety-related behaviour of the rats. The increased number of lines crossed and decreased duration of time spent in the centre zone by the rats in the control group are indications of an increase in anxiety-related behaviour.

However, on exposure of rats in the acrylamide group to the same platform, there were signs of peripheral neuropathy, such as the progressive development of gait abnormality, an unsteady walking pattern with abduction, and an external rotation of the hind limbs (Tian et al. 2015). Hence, the rats' exploratory abilities were seriously distorted, suggesting a severe degree of neurotoxicity. The rearing count is a measure of an animal's spontaneous motor activity. Acrylamide treatment showed a significant decrease in rearing counts.

Other activities and alertness in the open field were also severely affected, where animals showed minimal exploratory movements with longer periods of immobility in the acrylamide-treated rats. There were increased exploratory abilities in the control and the *Carica papaya* groups, while the *Carica papaya*-acrylamide group showed some improvement when compared to the acrylamide group only. These findings were in accordance with observations by Patil et al. (2015), where a statistically significant, severe gait abnormality was found in acrylamide treated rats. This also corresponded with Mehri et al. *(*2014).

The forelimb grip strength procedure is the commonly used technique to measure chemically-induced weakness in rodents and measures the force required to cause an animal to release its grip from a bar or wire screen (Pichon et al. 2010). This test supports the observation in the open field. The forelimb grip strength was significantly reduced in the rats treated with acrylamide when compared to the control, and the *Carica papaya* groups, which increased significantly, while the *Carica papaya*-acrylamide group only showed improvement when compared with the acrylamide group only. The rats in the acrylamide group have reduced muscular strength, which might be due to damage in the layers of cerebellar cortex as a result of oxidative stress, affecting the motor coordination of the cerebellum.

The mechanism by which acrylamide induces neurotoxicity has been proposed as an interference with kinesin-related motor proteins in neurofilaments that are involved in fast anterograde transport of nerve signals between axons (Patil et al. 2015). Acrylamide induces synaptic dysfunction through the adduction of presynaptic thiol groups and a reduction in neurotransmitter release. Consequent upon that, nerve dysfunction and muscle weakness set in (Lopachin et al. 2004). That could be the reason for the reduced grip strength observed in the present study in the acrylamide-induced group.

The mammalian bodies produce reactive oxygen species (ROS), which are the natural metabolism of oxygen when neutralized by the endogenous enzymatic and non-enzymatic antioxidant defence system that also includes GST, SOD, and GSH (Al-Khalaf and Ramadan 2013). Nevertheless, humans and animals are exposed to ecological contamination through chemicals and xenobiotic that increase ROS production, thereby creating a disproportion between their generation and neutralization (Al-Khalaf and Ramadan 2013; Al-Khalaf 2014). Cell membranes are attacked by ROS, which deteriorates the biomolecules, proteins, lipids, and DNA. The intracellular generation of ROS induced by acrylamide has a damaging role owing to their toxicity (Zhang et al. 2013).

The consequences of acrylamide-induced oxidative stress on the tissues are characterized by increased levels of MDA and NO and a decrease in the functions of the antioxidant enzymes glutathione peroxidase, superoxide dismutase, and catalase. The enhancement of lipid peroxide is a consequence of GSH depletion to certain critical levels.

GSH is a crucial component of natural antioxidant cellular defence and is necessary to protect tissues by eliminating different types of ROS and acting as a non-enzymatic oxygen radical scavenger and coenzyme. Glutathione content is significantly reduced under oxidative stress due to its consumption in rapidly generated ROS scavenging. The decrease in GSH content reduces the activity of enzymes that depend on its concentration, such as GST and GPx, which also play a very important role in free radical scavenging (Bano et al. 2020).

In the course of this study, there was a decrease in the level of GSH in the acrylamide-treated group and a significant improvement in the group administered *Carica papaya* fruit extract and acrylamide compared to the control group and the group given only *Carica papaya* fruit extract. Notably, acrylamide disrupts cellular redox homeostasis and then leads to an excessive increase in ROS (Orta-Yilmaz 2020). *In vitro* and *in vivo* studies have also shown that acrylamide causes oxidative stress via increasing ROS levels, such as peroxides, superoxide, and hydroxyl radicals, and by lowering GSH levels (Elhelaly et al. 2019).

Oxidative stress causes cellular injuries accompanied by the generation of ROS that exceeds antioxidant defences. The results of this study on acrylamide toxicity correlate with those of Sameh et al. (2021), who observed a significant decline in cerebral, cerebellar, and hippocampal content of GSH and GPx activities along with a significant increase in MDA and NO in the same tissues. This is also consistent with Zamani et al. *(*2018), who recorded that oxidative stress induced by acrylamide was demonstrated by an increase in MDA content and a decrease in GSH levels.

Considering that glutathione is a crucial intracellular non-enzymatic antioxidant demonstrating defensive action

against varied oxidative stress-induced stimuli, GST makes up a fundamental portion of brain toxicity by combining it with glutathione, while superoxide dismutase detoxifies superoxide anions.

Carica papaya treatment hindered the peroxidation of lipids and oxidative stress in the tissues of the brain through the elevation of the accumulation of antioxidant functions and enzyme systems. Hence, the treatment of *Carica papaya* produced a shielding effect against acrylamide-induced oxidative stress, possibly elaborated by the potential restrictive consequences on peroxidation of lipids and scavenging of free radicals due to *Carica papaya's* inhibition of peroxidation of lipids and elevation of the capacity of the total antioxidant status in the rat cerebellum. This is because *Carica papaya* has a biologically active polyphenol component that possesses anti-inflammatory (Tahir et al. 2013) and antioxidant (Srinivasan and Pari 2012) properties. Addai et al. (2013) reported that *Carica papaya* extract has high antioxidant activity because it is an important dietary source of vitamin C, vitamin E, mineral salts, amino acids, flavonoids, phenolic compounds, and tannins. Flavonoids present in *Carica papaya* fruit exhibit strong antioxidant activity, which helps protect cells against the damaging effects of reactive oxygen species such as singlet oxygen, superoxide, and peroxyl radicals. This flavonoid has been able to prevent an imbalance between antioxidants and reactive oxygen species, which may result in oxidative stress and lead to cellular damage. The antioxidant action of *Carica papaya* has been attributed to the scavenging of superoxide anions and hydroxyl radicals. Therefore, by counteracting oxidative stress and rejuvenating antioxidant defences, *Carica papaya* fruit extract protects against acrylamide-induced brain injury: The elevated consequences on the molecules of the antioxidants CAT, SOD, and GPx. This is in consonance with the studies of Arab et al. (2015) and Germoush (2016), who reported that *Carica papaya* can exert its antioxidant effect directly via free radical scavenging or indirectly by up-regulating cellular antioxidant enzymes such as GPx, CAT, and SOD.

With regard to detailed histological examinations, the current study further indicated that acrylamide can induce obvious histological changes ranging from pronounced loss of Purkinje neurons to degenerated and shrunken neurons in the acrylamide group. The degree of distortion in Purkinje cells, ranging from deep staining of their cytoplasm to loss of their dendrites and axons, establishes the pathological and neurotoxic consequences of acrylamide. These suggest that the shrinkage of the cells and deep staining of the cytoplasm are signs of neuronal cell death. Also, the shrinkage and irregularity in shape of the Purkinje cells referred to disorganization of the cytoskeletal elements in the cell body and processes of the cell. The induction of free radicals and oxidative stress by acrylamide causes membrane necrosis and mitochondrial dysfunction in Purkinje cells. Tissue damage produced by acrylamide administration might be attributed to an increase in acrylamide-induced free radicals. These were improved over the *Carica papaya*-acrylamide group. This finding is in harmony with the study by Mansour et al. (2017).

Immunohistochemically, GFAP, as a member of the cytoskeletal protein family, modulates astrocyte motility and shape by providing structural stability to astrocyte processes. Incidences of neurotoxicity stimulate astrocytes to become reactive in response to the trigger, a term called astrogliosis. Astrogliosis is characterized by rapid recruitment of GFAP, as demonstrated by an increase in protein content or by immunostaining with GFAP antibodies (O'Callaghan and Sriram, 2005). In this study, GFAP levels were remarkably high in the acrylamide group, as shown by the highest astrocyte density in the photomicrograph. This could be a result of the oxidative stress exposed to this group by the acrylamide toxicity, hence the stimulation of reactive hyperplasia of GFAP in the rat cerebellum. The *Carica papaya*-acrylamide group's expression of astrocyte density was highly ameliorated; therefore, *Carica papaya* fruit extract offered some degree of protection against neurotoxicity by reducing GFAP expression. The increment in the activity of GFAP in brain tissue of treated rats is compatible with Saini et al. (2020), so a decline in GFAP activity might reflect a decrease in the existence of astrocytosis as well as reactive gliosis in the cerebellum of the rats.

The present study also showed a mild positive immunoreaction for Bcl-2 in the cerebellar cortex of the control and *Carica papaya* groups with a reduced number of cells targeted to die by apoptosis, a moderate immunoreaction in the *Carica papaya*-acrylamide group with mild apoptotic cells, and intense immunoreactions in the acrylamide group only with the highest density of apoptotic cells. This finding is in agreement with Li et al. (2006), who postulated that expression of bcl-2 in the nervous system significantly changed after acrylamide administration and that overexpression of the anti-apoptotic Bcl-2 gene, or Bax gene, disruption provides significant neuroprotection in several *in vivo* and *in vitro* disease degenerative models. In the whole CNS, the expression of Bcl-2 followed the same change. In the current study, a higher expression of Bcl-2 in the acrylamide-treated group was part of the normal protective defence mechanism against neuronal injury and damage by acrylamide.

Conclusion

This study revealed that acrylamide induced oxidative stress, caused neurobehavioural deficits, loss of Purkinje cells, astrogliosis and apoptosis, and that *Carica papaya* fruit extract provided relative protection against acrylamide-induced oxidative stress in rats' cerebellum.

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Conflict of Interest

None declared.

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Authors' Contribution

Conceptualized, designed and supervised – IOI: Data collection and analysis - IOI, LSO: Animal handling, experimentation and sacrifice - LSO: Writing - LSO, IOI.

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