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Carica papaya ameliorates Nissl Bodies, Grey Matter distortion in Streptozotocin Induced Cerebral Hyperglycemia.

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Abstract

Carica papaya have been reported for treatment of numerous diseases such as glandular tumors, blood pressure, dyspepsia, constipation, amenorrhea, stomach ulcer, malaria, Dengue fever, cancer and Diabetes Mellitus. Diabetes is characterized by hyperglycemia. This study evaluated the ameliorating effect of Carica Papaya leave extract on distorted histoarchitecture of cerebrum in hyperglycemic states. The experimental animals (20 Wistar rats) weighed 100-130g were divided randomly into Group 1 which consisted five (5) normoglycemic rats administered with intraperitoneal (i.p.) 2m/ls of distilled water, Group 2 which consisted five (5) hyperglycemic rats administered i.p. streptozotocin (STZ) at 100mg/kg with 2mls of i.p. distilled water, Group 3 made up of five (5) hyperglycemic rats treated i.p. STZ at 100mg/kg with 800mg/kg of oral *Carica papaya* leave extract and Group 4 were five (5) normoglycemic rats treated with 800mg/kg of oral *Carica papaya* leaves extract. Treatment was carried out for 21 days. The cerebrum of hyperglycemic group under microscopic examination showed disorganized and distorted nissle bodies, grey and white matters. Studied hyperglycemic group treated with 800mg/kg of *Carica papaya* leaves showed marked amelioration of the examined parameters. Conclusively, the study showed *Carica papaya* leaves are of possible ameliorative importance in managing integrity of cerebral cell structures caused by diabetes- induced cell disruptions.

Keywords: Hyperglycemia; Diabetes Mellitus; *Carica papaya*; Histoarchitecture; Nissl bodies

1.0 Introduction

Diabetes mellitus termed as a series of metabolic disorder that are characterized by chronic carbohydrate intolerance (hyperglycemia) and the defect in Insulin production which lead to serious medical complications (Banday et al., 2020; Atilade et al., 2020). There are four main types of diabetes mellitus: type 1, type 2, gestational Diabetes and other specific types of Diabetes mellitus (Rahman et al., 2019). Medical complications caused by Chronic diabetes were long-term damage, dysregulation, and failure of various organs, especially the eyes (retinopathy), kidneys(nephropathy),pancreas (Pancreatopathy), nerves (neuropathy), heart, and blood vessels

(Caro-Ordieres et al., 2020; Stefanou et al., 2022).

Manifestation of diabetes are due to autoimmune destruction of B- cell of pancreas which lead to insulin deficiency to abnormalities that result in high blood glucose and resistance to insulin action (Balaji et al., 2019). According to the American Diabetes Association, an estimated 1.3% of the population was affected from diabetes around the world in 2017 (Einarson et al., 2018). India is considered as the diabetic Centre of the world due to increasing number of diabetic patients (Fralick et al., 2022). The International Diabetes Federation reported in the IDF Diabetes Atlas 2019 that 463 million adult possess diabetes mellitus (DM), and by 2045, approximately 700 million will develop diabetes mellitus, and 374 million people are risky for developing type II diabetes (Ferrannini et al., 2020).

Different people around the world use medicinal plants as an alternative treatment of their diseases; due to the fact that orthodox medical treatments were costly, or may be as a consequence of the worldwide opt for natural, rather than manmade products. Hence, products with antioxidant properties, such as *Carica papaya* have in recent times been given special attention as potential medicinal and therapeutical agents (Singh et al., 2020). *Carica papaya* possesses excellent antioxidant activity which play role in the neutralization of free radical generation and prevent the pathogenesis (Singh et al., 2020). *Carica papaya* leaf extract possesses remedial role in the treatment of various human ailments due to the presence of crucial phytoconstituents (alkaloids, glucosides, Tannins, Saponins and flavonoids) minerals and vitamins (Sharma et al., 2020). Study has been documented about antibacterial, anti-cancer, Immunomodulatory, anti-dengue, gastro-protective and anti-hyperglycemic effects of *Carica papaya* leaf in recent time(Singh et al., 2020).
According to study by Banday et al. (2020), hyperglycemia in Wistar rats causes neuronal distress usually evidenced by the increased lipoperoxidation and nitration along with the lowering of antioxidant molecules. Neuroinflammation and cell death are inevitable in the brain tissue of diabetic rats.

This research work aimed at evaluating degree of histological distortion of Nissle bodies, grey and white matters in cerebral hyperglycemia in Wistar rats and the Neurorecuperative effects of *Carica papaya* leaf extract in same.

2.0 Methods

2.1 Plant Material Collection and Preparation of Plant Extraction

Fresh *Carica papaya* leaves (pawpaw leaves) collected at UnderG, Ogbomoso North Local Government was authenticated at Department of Pure and Applied Biology, Ladoke Akintola University (LAUTECH), Ogbomoso, Nigeria. The leaves were then air-dried at room temperature for a week and were grinded using grinded machine at WAZO market and weighed to be 500g. It was taken to the Department of Food Science and Engineering (FSE), LAUTECH, Ogbomoso, where the extraction process was carried out. It was soaked in 2000mls of distilled water for 48hours and thoroughly stirred for proper mixing. The mixture was filtered with muslin cloth for the purpose of separating the residue from the filtrate. The filtrate was condensed and subjected to evaporation through water bath at temperature between 45-57^{o}C for one (1) week. Temperature higher than this can damage the phytoconstituents in the botanical.

2.2 Animal Care and Management

Twenty Wistar rats of both sexes weighing 100-130g were purchased from CCTABREEDER in Ogbomoso for this study. The twenty Wistar rats were divided randomly into 4 groups of five rats per each group. Male and female in each group were separated within the cage. The animals were kept in animal house of the Department of Anatomy for a period of two weeks, for

acclimatization purpose before the commencement of the experiment. Standard feed (Pelletized grower mash) and water were administered ad libitum. The animals' room was well ventilated and maintained under standard temperature condition range from 25 to 27^{o}C under day/night in a 12-12 hour photoperiodicity. The experimental procedures were carried out in guidelines of the Institutional Animal Ethics Committee (IAEC) of Ladoke Akintola University of Technology, Ogbomoso, Oyo State.

2.3 Determination of LD_{50}

LD_{50} of the extract was carried out in Pharmacology Department, Ladoke Akintola University of Technology, Ogbomoso. The LD_{50} was found to be 800mg/kg.

2.4 Induction of Diabetes Mellitus

Hyperglycemia was induced in forty Wistar rats fasted overnight and randomly selected by a single intraperitoneal administration of streptozotocin (STZ) at 100mg/kg (Khan et al., 2016). The forty Wistar rats selected for hyperglycemia induction were in excess of the only twenty needed for this study to be able to select best twenty suitable following successful inductions. Usually, there might be failed induction. STZ was dissolved in citrate buffer (0.1M, pH 4.5) just before the injection. Hyperglycemia was allowed to develop for 72hrs (Atilade, 2020). A glucometer was used to determine the blood glucose level, using tail bleeding. A glucometer reading of above 100mg/dl is considered hyperglycemic. The twenty selected Wistar rats were divided randomly into 4 groups of five rats per each group. Group 1– Comprised of five Normoglycemic Wistar rats and serves as the control group. They were fed with standard diet and water and treated with intraperitoneal (i.p.) 2mls of distilled water. Group 2- Comprised of five diabetic (hyperglycemic) Wistar rats. They were fed with standard diet and water and also treated with i. p. 2mls of distilled water. Group 3- Comprised of five diabetic (hyperglycaemic) Wistar rats. They were administered with oral 800mg/kg *Carica papaya* leaves extract and were fed with standard diet and water. Group 4 - Comprised of Normoglycemic Wistar rats. They were administered with oral 800mg/kg of *Carica papaya* leaves extract and were fed with standard diet and water. . Standard feed (Pelletized grower mash) and water were administered ad libitum.. The animals were treated daily at around 8am daily for 21days.The animals were weighed alternate days using weighing scale. Blood glucose of the Wistar rats was checked weekly.

2.5 Animal Sacrifice and Collection of Organs

All the Wistar rats were weighed and sacrificed 24 hours after the last administration by cervical dislocation method. The cranium of each animal was opened up using brain forceps and the whole brain was carefully removed and weighed, the cerebrum was then excised. Tissues were then fixed in formol calcium, and processed for histological observation using routine Haematoxylin and Eosin staining techniques and Nissl stain (Nissl).

2.6 Histological Techniques

Histological examination was done on the cerebral tissue fixed in formol calcium. Tissue blocks were sectioned for routine Hematoxylin and Eosin (H& E) and Nissl Stain. The fixed cerebral were cut in slabs of about 0.5cm thick transversely and moved to 70% alcohol for dehydration. The tissues were passed subsequently through 90% and absolute alcohol and then to xylene for clearing before they were transferred into two changes of molten paraffin wax for 1 hour each in an oven at 65 degree centigrade for infiltration. They were embedded and serially sectioned using a rotary microtome at six microns. The tissues then moved onto albumenized slides and allowed to dry on hot plate for 2 minutes. The Slides were dewaxed with xylene and passed through absolute alcohol (2 changes); 70% alcohol, 50% alcohol and then to water for 5 minutes. The slides were then stained with hematoxylin and eosin and Nissl stain were carried out too on the tissue.

2.7 Data Analysis

The results were subjected to statistical analysis using the ANOVA Graph-pad prism software package 6 for data analysis. They were expressed as mean ± standard error of the mean (SEM).

3.0 Result

Figure 1 showed that Group 1 indicated an increase in body weight (g) of the animals from the 1st week to the 3rd week. Group 2 indicated a decrease in body weight (g) of the animals from the 1st week to the 3rd week. Group 3 indicated a rapid increase in body weight (g) of the animals throughout the weeks. Group 4 indicated a rapid increase in body weight (g) of the animals throughout the weeks.

Table 2 provides information on descriptive nature of weight of animals for every weeks

Group 1 indicated an increase in body weight of the animals from the 1st week to the 3rd week.

Group 2 indicated a decrease in body weight of the animals from the 1st week to the 3rd week.

Group 3 indicated a rapid increase in body weight of the animals throughout the weeks.

Group 4 indicated a rapid increase in body weight of the animals throughout the weeks.

Table 1: Descriptive statistics on animals grouping

GROUPS	
Group 1	Normal + distilled water
Group 2	Diabetic + distilled water
Group 3	Diabetic + *Carica papaya* leaf
Group 4	Normal + *Carica papaya* leaf

Table 2: Weight of animals (g)

Groups	Week -2	Week -1	Week 0	Week 1	Week 2	Week 3
Group 1	100	120	125	130	140	150
Group 2	105	115	125	120	110	100
Group 3	130	130	135	130	140	160
Group 4	100	115	125	130	135	140

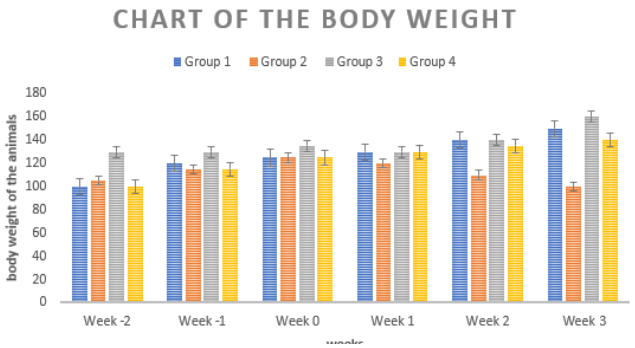

Figure 1: The body weight of the animals (g).

	N	Mean	SD	±SEM	p-value
Group 1	5	127.5	17.2482	7.0415	0.9574
Group 2	5	112.5	9.3541	3.8188	0.9606
Group 3	5	137.5	11.7260	4.7870	0.0155*
Group 4	5	124.2	14.637	5.9740	0.6820

*p-value<0.05 indicates significance

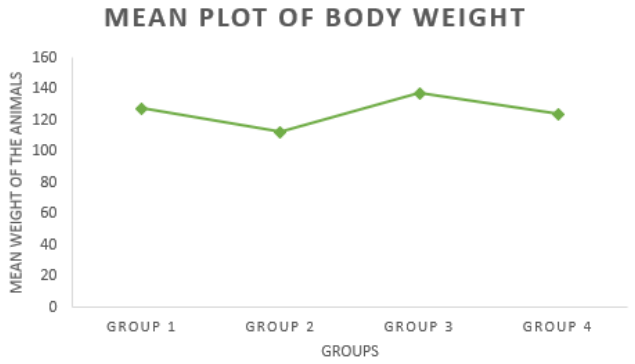

Table.4: Compares the p-values between groups

Groups	p-value
Group 1 and group 2	0.0906
Group 2 and group 3	0.0022*
Group 1 and group 4	0.7256
Group 2 and group4	0.1398
Group 3 and group 4	0.1122

*p-value <0.05 indicates statistical significance. One-way ANOVA Statistical significance: true. P = 0.0357. Calculated F Value = 3.4615

Critical F Value = 3.0984

Post Hoc table: (adjusted P values)

Table 5: '*'means statistically significant difference)

	Group 1	Group 2	Group 3	Group 4
Group 1	---	0.2537	0.5878	0.9734
Group 2	0.2537	---	0.0219*	0.4622
Group 3	0.5878	0.0219*	---	0.3488
Group 4	0.9734	0.4622	0.3488	---

3.2: Blood Glucose Statistics

Table 6: Provides data for descriptive nature of the data on blood glucose level (mg/dl).

Groups	Week -2	Week -1	Week 0	Week 1	Week 2	Week 3

Group 1	89	90	90	92	95	96
Group 2	80	85	490	495	505	560
Group 3	100	105	429	395	265	182
Group 4	75	78	85	92	99	102

Group 1 indicated an increase in blood glucose of the animals from the 1st week to the 3rd week. Group 2 indicated an increase in blood glucose of the animals from the 1st week to the 3rd week. Group 3 indicated a rapid decrease in blood glucose of the animals throughout the weeks. Group 4 indicated a rapid increase in blood glucose of the animals throughout the weeks.

Table 7: Descriptive statistics on Blood glucose of the animals (mg/dl)

Groups	N	Mean	SD	±SEM	p-value
Group 1	6	92.0	2.898	1.183	0.2709
Group 2	6	369.2	223.460	91.227	0.0127*
Group 3	6	246.0	142.369	58.122	0.3164
Group 4	6	85.5	11.041	4.507	0.6197

*p-value<0.05 indicates significance

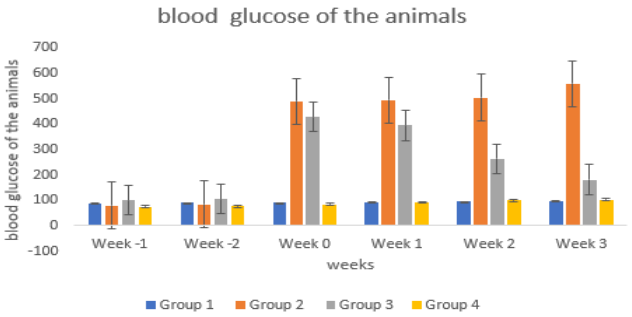

Fig 2: Graphical representation and Error bar of blood glucose of the animals (mg/dl)

Fig 2: Group 1 indicated an increase in blood glucose of the animals from the 1st week to the 3rd week. Group 2 indicated an increase in blood glucose of the animals from the 1st week to the 3rd week. Group 3 indicated a rapid decrease in blood glucose of the animals throughout the weeks. Group 4 indicated a rapid increase in blood glucose of the animals throughout the weeks.

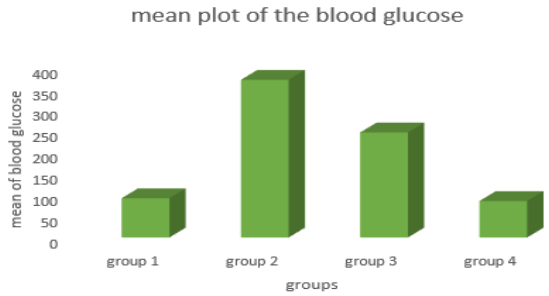

Table 8: Compares the p-values between groups

Groups	p-value
Group1 and group 2	0.0125*
Group 2 and group 3	0.2814
Group 1 and group 4	0.4699
Group 2 and group 4	0.4321
Group 3 and group 4	0.0222*

One-way ANOVA Statistical significance: true
P = 0.0037
Calculated F Value = 6.236
Critical F Value = 3.0984
Post Hoc table: (adjusted P values)

Table 9: ('*' means statistically significant difference)

	Group 1	Group 2	Group 3	Group 4
Group 1	---	0.0085*	0.217	1.0
Group 2	0.0085*	---	0.3964	0.0077*
Group 3	0.217	0.3964	---	0.2012
Group 4	1.0	0.0077*	0.2012	---

basophilia is evident with lack of prominent nucleoli and presence of necrotic debris (NB), a few reactive astrocytes are evident GRP 3 Shows degenerating shrinking neurons (N) with hyperchromatic staining nuclei and no evident nucleoli, evident necrotized neurons (NC) with reactive astrocytes are seen GRP 4 Shows a the grey matter consisting of the cell body of neurons (CB), few necrotized neuronare present, some neurons appear shrunken with abnormal basophilia, the astrocytes appears reactive (N).

Plate 1.0 showing; GRP1 shows the grey matter (G) consisting of the cell body (CB) of neurons appearing normal with evident nucleoli without any pathological lesion (NC), the astrocytes appear normal and non-reactive GRP 2 Shows a cerebrum with poor morphology with evident degeneration of the neurons (N) with abnormal shrinkage with a reduced number of neurons in relation to the unexposed control group, abnormal

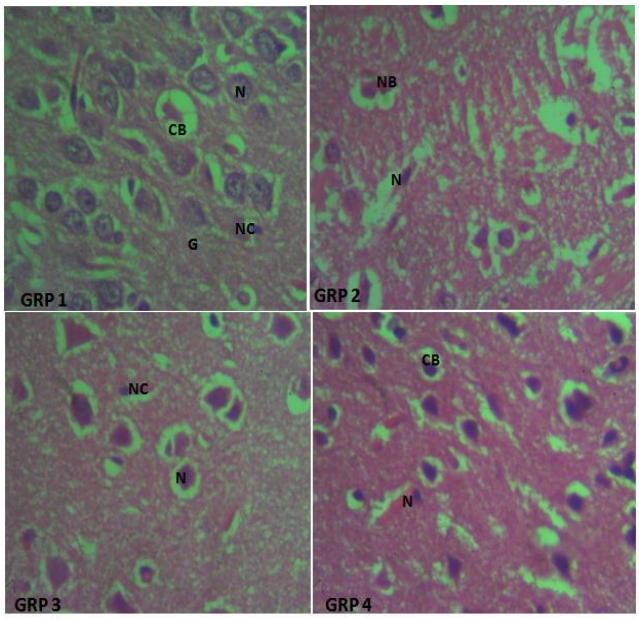

Plate 1.0 Photomicrograph of Cerebrum section X400

Plate 2.0 reveals: GRP 1 showing a prominent staining nucleus with nucleoli with a prominent cytoplasmic staining Nissl substance (CB). GRP 2 Prominent staining nucleus with an absence of cytoplasmic Nissl substance, some of the neurons appear necrotized (N). GRP 3 Pale staining neurons with prominent nucleoli and cytoplasmic staining of Nissl substance, there is evident nuclear shrinkage of some neurons without evidence of necrosis (N). GRP 4 Cell body (CB) of neurons with abnormal basophilia without evident nucleoli, some of the neurons appear shrunk with the absence of Nissl bodies in their cytoplasm (N).

4. 0. Discussion

The brain was once thought to be an insulin-insensitive organ. However, it is now widely recognized that insulin plays an important role in neuronal survival and brain function. Insulin action is, in fact, needed for neuronal synaptic plasticity and promotes brain functional roles such as learning and memory (Sharma et al., 2014; Capucho et al., 2022). It has also been shown that insulin promotes dendritic spine and synapse development, neuronal stem cell activation, neurite integrity and neuroprotection (Tumminia et al., 2018). Therefore, alterations in insulin metabolism due to hyperglycemia and response in the Central Nervous System (CNS) can generate many brain disorders (Tumminia et al., 2018; Belsham and Dalvi, 2021).

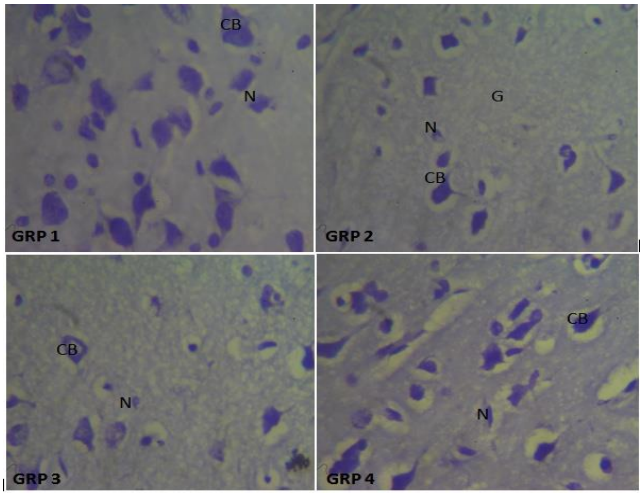

Plate 2.0 A High-power view of a section of cerebrum stained with CresylEcht Violet Stain X 400.

The brain is vulnerable to oxidative damage as a result of its high oxygen utilization rate, rich lipid content, and relative sparseness of antioxidant enzymes as compared to other tissues. Neuronal cells are particularly responsive to oxidative abnormalities, and therefore reactive oxygen species (ROS) are involved in many neurodegenerative processes such as diabetes mellitus (Lee et al., 2020). Additionally, recent studies have demonstrated that peripheral Insulin Resistance results in loss of the brain function, which indicates concrete evidence between metabolic disturbances and cerebral degeneration, cognitive impairment, depression, as well as Alzheimer's disease (Cui et al., 2022). It is suggested that one possible common denominator of all these conditions could be chronic oxidative stress which can be diabetic induced factor (Maciejczyk et al., 2019). Due to the fact that *Carica papaya* has extra ordinary medicinal and therapeutic properties we have selected *Carica papaya* leaves, and studied its effect on diabetic disrupted cerebral histostructure. Notable is the poor cerebrum morphology with evident degeneration of the neurons, abnormal shrinkage with a reduced number of neurons in group 2 compared to control group, abnormal presence of necrotic debris. The decrease in distribution of Nissl bodies is caused by loss of functional protein

synthesis and packaging in cells, thus it was caused by oxidative insults through increment in ROS leading to failure of essential enzymes, distorted membrane integrity and genomic damage (Khan, 2016). Administration of *Carica papaya* leaves extract revamped the hyperglycemic effects on rats, served to maintain the tissue integrity. Pale staining neurons with prominent nucleoli and cytoplasmic staining of Nissl substance, evident nuclear shrinkage of some neurons without evidence of necrosis seen in group 3. There is also evidence of reactive astrocytes, indicating repair and reduced degeneration, in extensive as the group induced with diabetes and treated with water only. This suggested that *Carica papaya* help to ameliorate tissue distortion by restoring its integrity after being affected by oxidative insults (Kong et al., 2021). It has vital antioxidants which help to reduce oxidative stress by regulating protein biosynthesis (Mansour et al., 2022).

5.0. Conclusion

Streptozotocin induced hyperglycemia in rats possibly through disrupting glucose and insulin metabolism, and initiating oxidative stress. The brain is essentially prone to oxidative damage as a result of its high oxygen utilization rate and overly dependence on glucose and lipids as its major fuel. *Carica papaya* ameliorates nissl bodies, grey matter distortion in streptozotocin induced cerebral hyperglycemia most likely via antioxidation and neutralization of free radicals. Immunohistochemical study is recommended to further strengthen our findings.

6.0 Ethical Approval

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee".

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

7.0. Competing Interests

Authors have declared that no competing interests exist.

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