

# Stroke vulnerability and soft diets: histological and biochemical analysis in a brain ischemic rat model

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## SUMMARY

Diet is an important contributor to human healthiness. The firmness of food can impact both the quantity of chews and the duration of chewing. The significance of mastication has been firmly established in recent years, with studies indicating that it has several favorable impacts on the entire body. Stroke constitutes a serious risk to human health because of its high morbidity, disability, recurrence, and fatality rates. As a result, the objective of this study was to assess the prolonged soft-diet impact on both body weight and lipid profiles with and without brain ischemia-reperfusion (IR). In addition, we estimated the brain oxidative and inflammatory states with the histological appearance, and an immunohistochemical investigation was accomplished on inducible nitric oxide synthase (iNOS) and caspase 3 (Casp-3). The current study found that rats fed an SD had higher body weight and dyslipidemia, as well as significantly higher levels of oxidative and inflammatory brain markers, as compared to rats on an ordinary laboratory diet. Feeding with soft diet (SD) also notably worsened the deficiencies in overall antioxidant capabilities. Moreover, chronic consumption

of SD up-regulated the brain IR-induced over-expression of iNOS and Casp-3. The histological analysis of brain tissue in IR rats revealed a significant exacerbation of the loss of normal architecture and infiltration of inflammatory cells due to pre-SD diet. In conclusion, chronic SD intake is hazardously obesogenic, with Higher cardiac risk factors resulting from disrupted lipid metabolism. The findings may raise concerns regarding the potential health risks associated with prolonged intake of SD in humans, particularly among individuals who are obese or have neurological or other organ-related conditions.

**Key words:** Soft diet – Brain – Rat

## INTRODUCTION

Recently, there has been a revived emphasis on disease prevention and maintaining good health among global societies and the scientific/medical community. Together, these concerns offer critical considerations about how we may enhance health from an early age, and hence increase the quality of life.

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Adequate diet is associated with great health (Napoleão et al., 2021). However, there is inadequate information to determine the impact of food hardness on health. The firmness of food can impact the quantity of bites taken and the duration of chewing. The relevance of mastication has been reinforced in recent years, with evidence pointing to several good impacts on the whole body.

Oral stimulation, which refers to the act of tasting and chewing food, enhances the production of heat caused by diet while improving health. Furthermore, chewing enhances cognitive performance and has a relaxing impact (Furukawa et al., 2023). Dietary elements such as meal texture are considered to exert an impact on dietary patterns and the body's energy use.

Prior studies have demonstrated that rats who were given soft pellets had a greater increase in weight and adiposity compared to rats that were given control pellets, due to decreased postprandial thermogenesis (Bae et al., 2014). Ischemic stroke is presently recognized as a cerebrovascular illness worldwide characterized by a wide spectrum of morbidity and death rates.

Ischemia-reperfusion damage results in oxidative stress, which subsequently causes neuronal death and cognitive impairment, whereas circulatory regeneration causes detrimental oxidative consequences (Praveen Kumar et al., 2021). Excessive free-radical levels in the brain have been linked to a variety of neurological illnesses.

According to new reports, oxidative stress is a highly effective catalyst for causing tissue damage in the hippocampus. Prior research has demonstrated that the application of constraint stress leads to an elevation in oxidative stress inside the brain of rats. Oxidative stress has been related to increased tissue and DNA destruction (Ohno et al., 2012).

The brain is highly vulnerable to oxidative stress due to its relatively limited enzymatic defense mechanisms compared to other organs (Shagirtha et al., 2017). To date, the relation between the morphology and firmness of food staples and general well-being has not been extensively examined. In current work, we evaluated variations in brain histological appearance by evalu-

ating many oxidative and inflammatory variables between adult male rats given a hard diet and a group provided with a soft diet with identical nutritional composition.

## MATERIALS AND METHODS

### Obtaining ethical approval

The Ethics Committee "FMREC" of the Faculty of Medicine, Minia University, Minia, Egypt authorized this study with regards to the derivation of the rats, criteria for inclusion and exclusion, housing conditions, welfare, health status, as well as the comprehensive experimental design and methods. The approval number is 1148/04/2024.

### Animals

For this experiment, a total of twenty-four mature male albino Sprague-Dawley rats weighing around 150-200 gm (specific pathogen-free) were utilized. These animals were obtained from the animal Store at the Faculty of Medicine, Minia University. All animals were kept in hygienic, air-conditioned sections. Standard laboratory diet and water were allowed. A period of two weeks was allowed for adjustment to room temperature. The subjects were permitted to reside in stainless-steel mesh cages that provided individual housing, with alternating periods of 12 hours of darkness and 12 hours of light. After two weeks of accommodation, the rats were at random split up into three groups, each consisting of 8 rats:

1. Standard laboratory diet group (control): during the experiment, a group of 8 rats were fed commercially available conventional laboratory food for a duration of 4 weeks.
2. Soft diet (SD) group: 8 rats were served a commercially soft diet for 4 weeks.
3. Standard laboratory diet ischemic group (IR): a group of 8 rats were provided with standard laboratory food for a duration of 4 weeks before induction of brain ischemia-reperfusion (IR).
4. Soft diet ischemic group (SD+ IR): a group of 8 rats were provided with a commercially soft diet for a duration of 4 weeks before induction of brain ischemia-reperfusion (IR).

## Diet protocol

The standard laboratory diet group was nourished standard laboratory meal is composed of several ingredients: 5% fat (derived from maize oil), 65% carbs (15% corn starch and 50% sucrose), 20.3% proteins (20% casein and 3% DL-Methionine), 5% fiber, 3.7% salt mixture, and 1% vitamin combination. It was gotten from El-Gomhoria Company in Cairo, Egypt. (Noeman et al., 2011).

The soft diet group ingested standard laboratory chow mixed with water (standard laboratory chow, 1 g; water, 1.5 ml) (Han et al., 2018). We measured the individual body weight of rats in each group on a weekly basis.

## Body mass index (BMI)

The body length of all rats was measured, namely from the snout to the anus, and recorded in centimeters. The measurements were conducted on anesthetized rats using mild ether.

Body length may be measured by using a ruler to determine the distance between the bottoms of the lower incisors and the anus on the ventral surface. The rats were weighed in grams using an electronic balance (FY 2000). Each rat's weight was measured, and their nose-to-tail length was recorded, and their BMI was computed on a weekly basis. BMI was calculated using the method that takes into account body weight and length. The body mass index (BMI) is calculated by dividing the body weight (in grams) by the square of the length (in square centimeters). According to Novelli et al. (2007), an increase in BMI that is significantly higher than a control group is an indicator of obesity. Obesity is often defined as a considerable increase in body weight compared to control animals. This information was also in agreement with the conclusions of Li et al. (2008).

## Rat Model of brain ischemia-reperfusion

Occlusion of the common carotid artery caused brain ischemia. Rats were placed on their backs while under general anesthesia, which was performed using ketamine and xylazine at a dosage of 80 mg/kg and 5 mg/kg respectively, by intraperitoneal injection. (Struck et al., 2011). Their lower and upper limbs were glued.

A tiny midline cut was performed in the neck to expose the carotid arteries. The arteries were carefully separated from the vagal nerves and then visually presented on both sides. They were subsequently blocked using vascular clamps and locked for 30 minutes, following which the clamps were unconfined to allow reperfusion for 1 hour. Rats were euthanized one hour after reperfusion (Abed et al., 2021).

## The mass of the gastrocolic omentum

Rats underwent a surgical procedure in which an incision was made in the ventral abdominal area. The peritoneal omental adipose tissue was removed. The gastrocolic omentum was then weighed (Caesar and Drevon, 2008).

## Biochemical analyses

At the end of the experiment, the rats were subjected to an overnight fasting period prior to receiving intraperitoneal pentobarbital sodium anesthesia at a dosage of 60 mg/kg body weight and promptly decapitated. Specimens of blood were obtained from the jugular vein and promptly subjected to centrifugation. Sera were isolated and stored in aliquots at -80°C until utilized to estimate the lipid profile, The measurements of total cholesterol (TC), triglycerides (TGs), low density lipoprotein (LDL-c), and high-density lipoprotein (HDL-c) were conducted using enzymatic colorimetric procedures using Bio-5 Diagnostic kits from Egypt. The atherogenic index was calculated using the formula (Ikewuchi et al., 2014): atherogenic index =  $\log [\text{triglyceride}/\text{HDL cholesterol}]$ .

## Analysis of brain homogenates

The brain samples were weighed and then individually homogenized in a 10 mM potassium phosphate buffer with a pH of 7.4. The tissue weight to homogenization buffer ratio was 1:10. The homogenates were subjected to centrifugation at a speed of 5000 revolutions per minute for a duration of 10 minutes at a temperature of 4 degrees Celsius. The resulting supernatant was utilized to measure the total antioxidant capacity (TAC) by a colorimetric assay kit (Bio-diagnostic, Egypt) and tumor necrosis factor alpha (TNF-TNF-IX).

## Histological and Immunohistochemical examination

### *Histological study*

Histological investigation used light microscopy techniques. The right hemisphere of the cerebrum from all subjects was submerged in a 10% solution of formol saline for a duration of 12 hours prior to being handled and preserved in paraffin. Subsequently, slices with a thickness of 5 micrometers were sliced and treated with Hematoxylin and Eosin (H&E) stain (Aziz et al. 2020) and Toluidine Blue (TB), according to Ahmed and Fouad (2019).

### *Analysis using immunohistochemistry*

The paraffin sections were treated to remove the paraffin and then hydrated again, using slides coated with poly-L-lysine. Sections were immersed in 3% hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) to inhibit endogenous peroxidase. The process of microwave antigen retrieval was completed. The sections were treated with a primary antibody against Caspase-3 (Casp-3), which is a marker for apoptosis in the cytoplasm. This antibody was a rabbit polyclonal antibody obtained from Dako, Carpinteria California, USA. Additionally, an antibody against inducible nitric oxide synthase (iNOS), which is a marker for oxidative stress, was used. This antibody was also a rabbit polyclonal antibody obtained from Lab vision. Next, the biotinylated polyvalent secondary antibody was administered. Incubation of the slices with streptavidin peroxidase was subsequently performed. Afterward, the DAB substrate chromogen, namely diaminobenzidine tetrahydrochloride, was thereafter administered. Hematoxylin was used to counterstain the slides to be observed with a light microscope (Aziz et al., 2020).

## Morphometric analysis

Measurements were performed in 5 separate fields that did not overlap, taken from 5 distinct portions of 5 individual rats in each group, utilizing an image analyzer from Leica, Germany. The areas that exhibited positive staining for Toluidine blue (TB) and were also immunopositive for Casp-3 and iNOS were quantified as a percentage of the stained section. Furthermore, changes in area% of glial cells and deformed neurons were estimated (Kamel et al., 2020).

## Statistical Methods

The data were expressed as the mean value plus or minus the standard error of the mean (SEM) using GraphPad Prism 5 package. A one-way ANOVA was conducted to determine significant differences between groups. Multiple comparisons were then conducted using the Tukey-Kramer post hoc test. A significance level of  $P < 0.05$  was used to verify statistical significance.

## RESULTS

### **Evaluation of BMI and the weight of the gastrocolic omentum**

Rats that were fed a soft diet in both groups (SD and SD+IR) showed a significant higher BMI and gastrocolic omentum weight as compared to standard laboratory diet fed rats groups (control and IR) (Table 1).

### **Evaluation of the serum lipid profile**

As shown in Table 2, soft diet fed rats either alone or with brain ischemia-perfusion. The serum lipid profile exhibited atherogenic characteristics. There was a notable increase in the levels of TC (total cholesterol), TGs (triglycerides), and LDL-c

**Table 1.** The BMI and weight of the gastrocolic omentum (GCOF) in different groups.

Parameters		Groups	Control	SD	IR	SD+IR
wt. of GCOF (g)			5.4 ± 0.1	6.75 ± 0.3 a	5.9 ± 0.1 b	6.95 ± 0.2 ac
BMI	Initial		0.49 ± 0.05	0.47 ± 0.009	0.48 ± 0.02	0.45 ± 0.009
	Final		0.51 ± 0.04	0.71 ± 0.01 a	0.54 ± 0.01 b	0.69 ± 0.01 ac

Data are expressed as mean ± S.E.M. of 6 rats in each group. a: Significant from group I, b: Significant from group II, c: Significant from group III respectively,  $P < 0.05$ . Control: Standard laboratory diet group, SD: Soft diet group, IR: Standard laboratory diet ischemic group, SD+IR: Soft diet ischemic group, BMI: Body mass index.

**Table 2.** Lipid profile concentrations in the different studied groups.

Parameters	Groups	Control	SD	IR	SD+IR
TC (mg/dl)		49.49 ± 2.5	55.75 ± 2.7 a	43.51 ± 2.1 b	59.1 ± 3.1 ac
TGs (mg/dl)		50 ± 2.5	67 ± 3.2 a	48.5 ± 2.7 b	61.4 ± 3.1 ac
HDL-c (mg/dl)		18.8 ± 0.9	20.25 ± 1.2	19.8 ± 0.7	21.8 ± 1.1
LDL-c (mg/dl)		19.95 ± 0.8	25.6 ± 1.7 a	17.85 ± 0.7 b	27.3 ± 1.3 ac
Atherogenic index		0.42±0.02	3.3±1.2 a	0.39±0.01 b	2.8±0.9 ac

Data are expressed as mean ± S.E.M. of 6 rats in each group. a: Significant from control group, b: Significant from SD group, c: Significant from IR group respectively,  $P < 0.05$ . Control: Standard laboratory diet group, SD: Soft diet group, IR: Standard laboratory diet ischemic group, SD+IR: Soft diet ischemic group.

(low-density lipoprotein cholesterol), but the level of HDL-c (high-density lipoprotein cholesterol) remained relatively unchanged. However, there was a considerable increase in the atherogenic index as compared with standard laboratory diet fed rats alone or with brain ischemia-reperfusion.

### Assessment of the level of oxidative stress and indicators of inflammation in the tissue

Within the present investigation, SD+IR group showed the highest brain TNF- with the lowest brain TAC concentration amongst all the studied experimental groups. Additionally, IR group showed a significant higher level of brain TNF- TAC level when compared with both groups, control and SD. Our results showed the 7 feeding soft diet also caused significant higher level of brain TNF- $\alpha$  with lower brain TAC level when compared with rats supplied standard laboratory diet (Table 3).

### Histological results

#### *Histological results using H&E staining*

The sections of the control group exhibited typical neuronal configuration. The homogenous acidophilic neuropil proved variant cortical neurons, pyramidal, granular, and glial cells together with

intact blood vessels in between. Pyramidal cells had pyramidal cell bodies with basophilic cytoplasm, long apical dendrites, open face nucleus, and prominent nucleolus. Granular cells were noticed with large vesicular nuclei. Neuroglial cells with dense nuclei can also be detected (Fig. 1).

Some fields of the SD group detected a little disruption in the cerebral cortex, with a few shrunken pyramidal neurons exhibiting intensely pigmented pyknotic nuclei. Normal granular cells were seen, and the acidophilic neuropil displayed blood vessels through dilated peri-vascular space (Fig. 2).

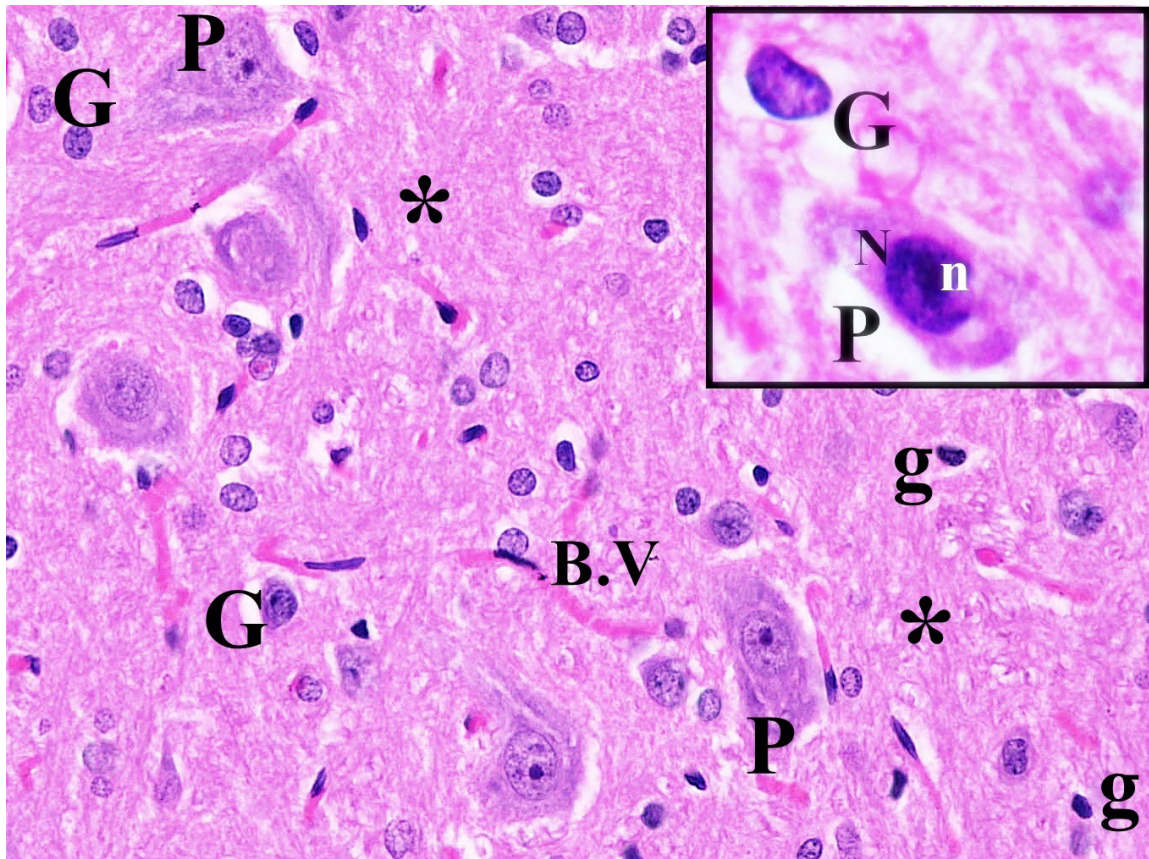
Sections belonging to the IR group demonstrated multiple deformed neurons. The granular cells were rounded and small while the pyramidal cells were shrunken with pyknotic nuclei and were surrounded by clear areas. Among the vacuolated neuropil, blood vessels appeared dilated and congested (Fig. 3).

Most of the sections of SD+IR group recruited separation of the pia mater beside rarified neuropils which have dilated congested blood vessels that are surrounded by a broad peri-vascular space. The cerebral cortical tissues exhibited a disruption in their typical structure, with the cell bodies of neurons displaying nuclei that were deeply stained and surrounded by vacuolations (Fig. 4).

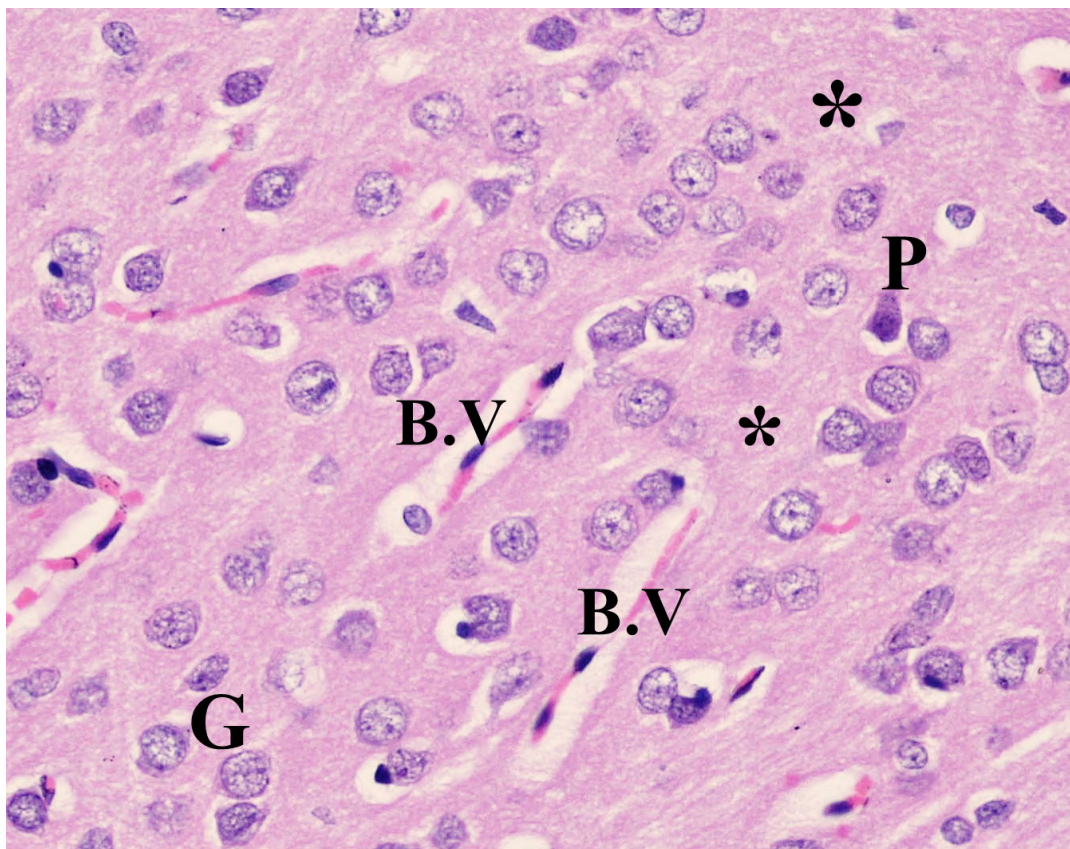
**Table 3.** Oxidative and inflammatory markers in the different studied groups.

Parameters	Groups	Control	SD	IR	SD+IR
TAC ( $\mu\text{M}/\text{mg}$ tissue)		55.75 ± 2.1	40.49 ± 1.5a	33.51 ± 1.3 ab	29.1 ± 1.1 abc
TNF- $\alpha$ ( $\text{pg}/\text{mg}$ tissue protein)		58.5±2.4	76.9±2.5 a	92.1±2.7 ab	103.9±3.1abc

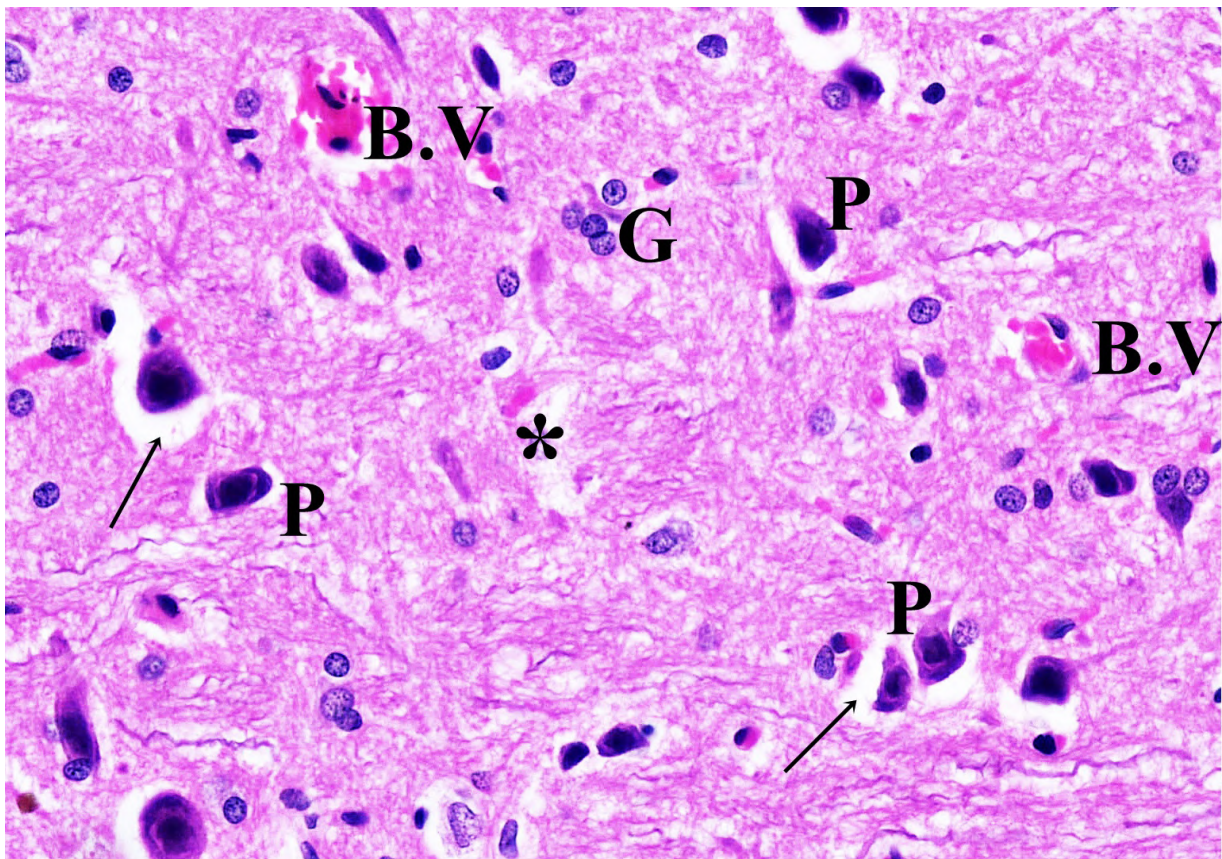
Data are expressed as mean ± S.E.M. of 6 rats in each group. a: Significant from control group, b: Significant from SD group, c: Significant from IR group respectively,  $P < 0.05$ . Control: Standard laboratory diet group, SD: Soft diet group, IR: Standard laboratory diet ischemic group, SD+IR: Soft diet ischemic group. TNF- $\alpha$ : Tumor necrosis factor alpha; TAC: Total anti-oxidant capacity.



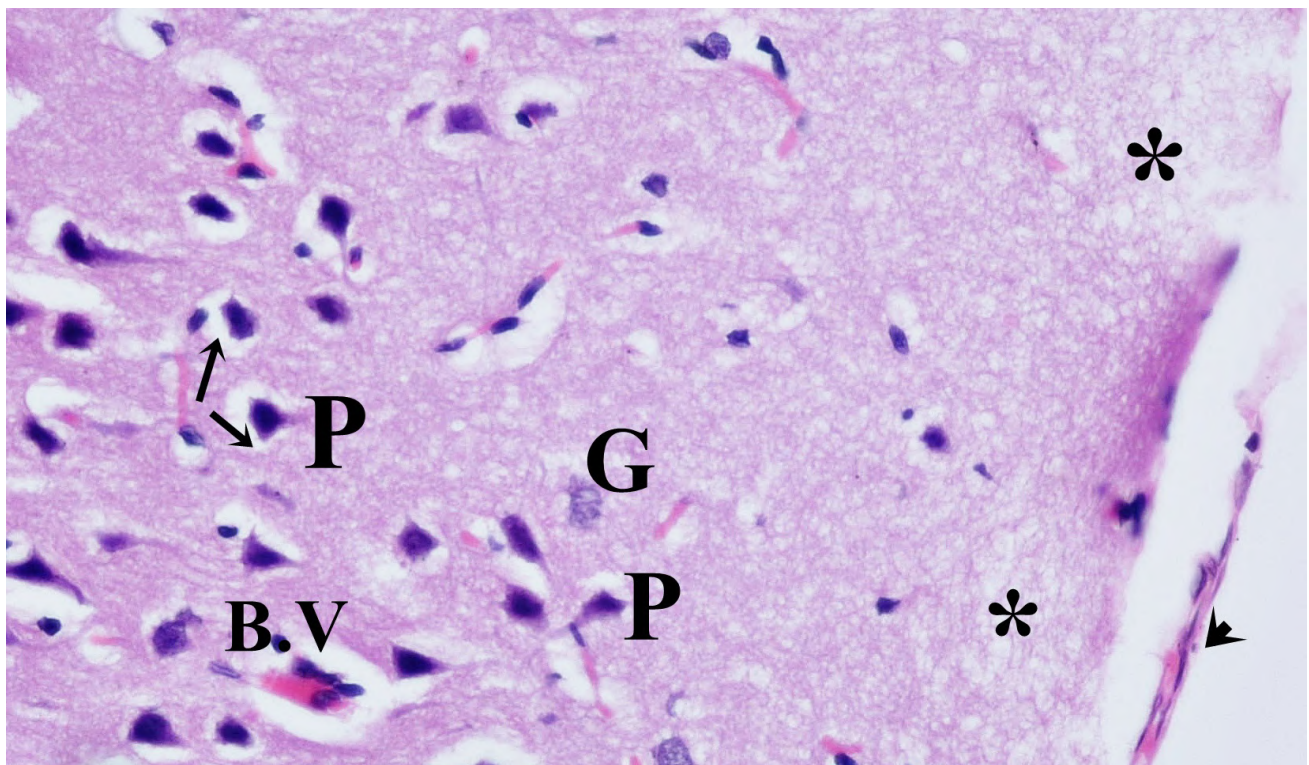
**Fig. 1.-** A portion of the cerebral cortex from the control group: the cortex exhibits multiple pyramidal (P), granular (G), and glial (g) neurons in the neuropil (\*). Notice the blood vessels (B.V) in between the cells. Inset: granular cell (G) and pyramidal (P) cell with open face nucleus (N), prominent nucleolus (n), and basophilic cytoplasm. (H&E staining, x400, Inset x1000).



**Fig. 2.-** Section of the SD group: cerebral cortex shows rounded granular cells (G), shrunken pyramidal cells (P) and blood vessels (B.V) with dilated perivascular space in the neuropil (\*). (H&E staining, x400).



**Fig. 3.-** Section of the IR group: the cerebral cortex shows congested dilated blood vessels (B.V), shrunken granular (G) and pyramidal (P) cells in a vacuolated neuropil (\*). Notice the pyramidal cells (P) with dark stained nucleus and pericellular halos (↑). (H&E staining, x400).



**Fig. 4.-** Section of the SD+IR group: the cerebral cortex shows Pia matter separation (arrowhead), congested expanded blood vessels (B.V), degenerated granular (G) and irregular pyramidal (P) cells with clear halos (↑) in a vacuolated neuropil (\*). (H&E staining, x400).

### ***Histological findings obtained using Toluidine blue staining***

The control group exhibited cortical neurons that are undamaged and intact with an open-faced nuclei encircled by pale cytoplasm that have basophilic Nissl's granules (Fig. 5A). In contrast, the SD group showed some cortical neurons with intense stained's show educointy (Fig. 5B). However, the IR 8 group displayed that the most cortical cells have reduced color intensity (Fig. 5C). In SD+IR group, numerous cortical neurons appeared irregular and degenerated with faint stained (Fig. 5D).

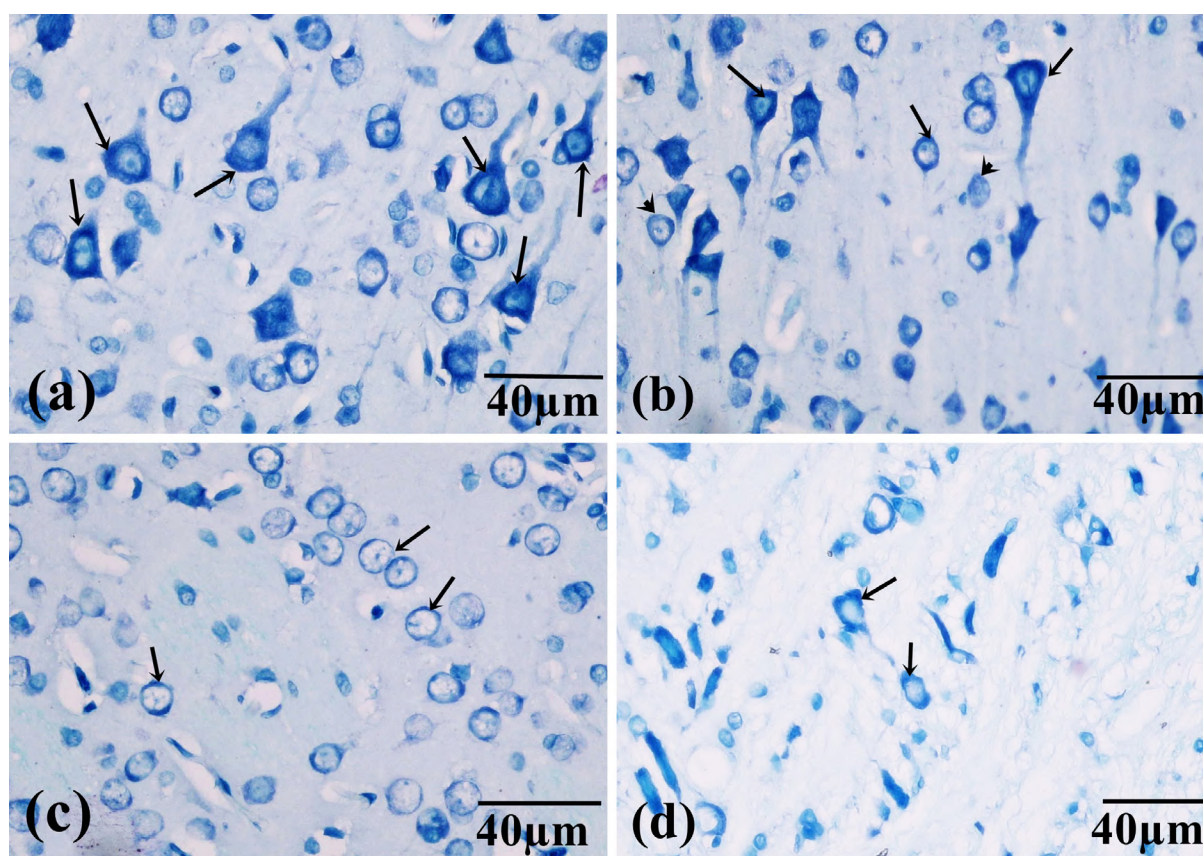
### **Immunohistochemical analysis**

The level of Casp-3 immunoreactivity was analyzed to determine the apoptotic and anti-apoptotic condition of the cerebral cortical neurons. The control group exhibited absence of Casp-3 immunoreactivity in the cytoplasm of cortical neurons (Fig. 6A). While in the SD

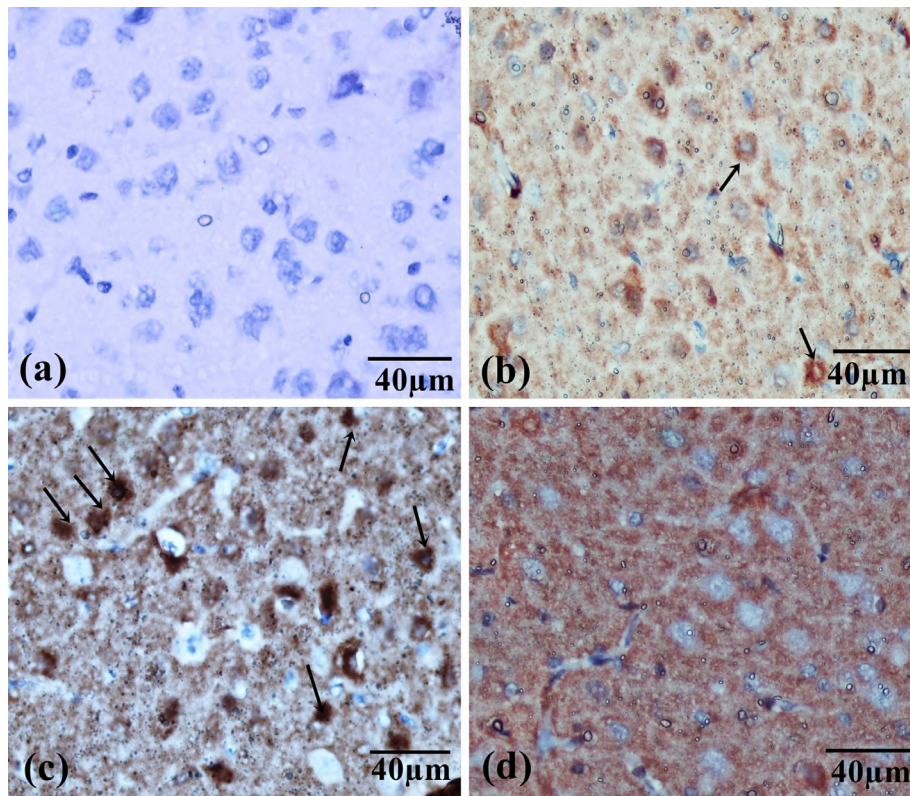
group, positive brown staining for casp-3 was seen in the cytoplasm of some apoptotic cortical neurons (Fig. 6B).

However, within the IR group, many cortical neurons had a favorable immune response to Casp-3 (Fig. 6C). In SD+IR group; most of the cortical neurons were positive immunoreactive for Casp-3 (Fig. 6D).

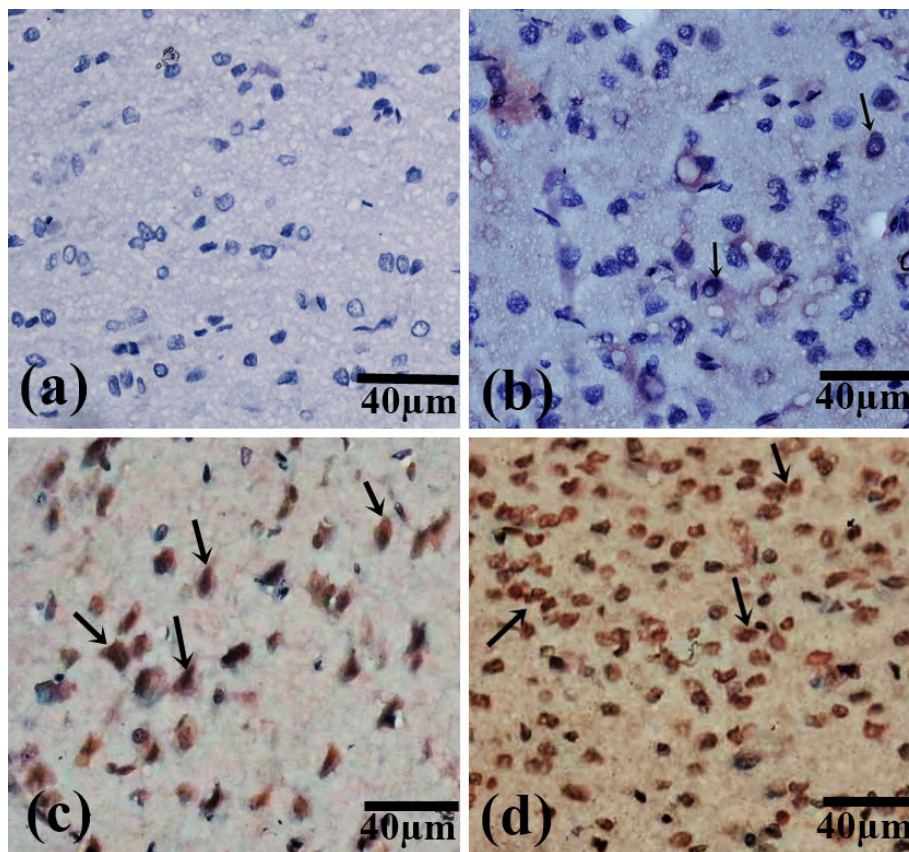
iNOS expression analysis was implemented to assert the impact of oxidative stress. Compared to the control group (Fig. 7A), iNOS immunoreactivity was slightly elevated in the SD group (Fig. 7B). Moreover, IR group enhanced iNOS expression (Fig. 7C). while in the SD+IR group further enhanced iNOS expression in most of the cortical neurons (Fig. 7D). The results were validated using morphometric and statistical analysis, which involved quantifying the number of immuno-positive neurons for both casp-3 and iNOS, as presented in Table 4.



**Fig. 5.-** Cross-section of the rat cerebral cortex, illustrating the variations across various groups: **a)** Control group it displays undamaged cortical neurons with exposed nuclei surrounded by transparent cytoplasm with basophilic Nissl's granules. (↑). **b)** SD group show some cortical neurons with intense stained Nissl's granules (↑), others show reduced color intensity (arrowhead). **c)** IR group show most of the cortical neurons with reduced color intensity (↑). **d)** SD+IR group show degenerated cortical neurons with faint Nissl's granules (↑). (Toluidine blue, x400). Scale bars = 40 µm.



**Fig. 6.-** The representative photomicrographs demonstrate the presence of apoptotic neuronal cells in several groups tested by using caspase-3 immunostaining of cortical neurons to determine immunoreactivity. **(a)** Control group; showing -ve immunostaining among different types of neurons. **(b)** SD group; showing some positive neurons. **(c)** IR group; showing multiple +ve neurons. **(d)** SD+IR group; showing numerous +ve neurons. (Caspase-3 immunostaining, x400). Scale bars = 40 µm.



**Fig. 7.-** Presence of iNOS immunoreactivity in the neuronal cells of the various groups under study. **(a)** Control group; showing -ve immunostaining among different types of neurons. **(b)** SD group; showing some positive neurons with faint immunostaining. **(c)** IR group; showing strong immunostaining. **(d)** SD+IR group; showing dense immunostaining. (iNOS immunostaining, x400). Scale bars = 40 µm.

**Table 4.** Changes in area% of TB & casp-3 and iNOS in different studied groups.

Parameters	Groups	Control	SD	IR	SD+IR
Area % of TB		32.2 ± 1.8	24.4 ± 1.6a	22.8 ± 1.4ab	19.8 ± 0.9abc
Area % of Casp-3		00±00	30.24±2.2a	39.51±2.9ab	88.48±5.3abc
Area % of iNOS		00±00	24.21±1.8a	52.31±3.7ab	70.28±4.1abc

Data are expressed as mean ± S.E.M. of 6 rats in each group. a: Significant from control group, b: Significant from SD group, c: Significant from IR group respectively,  $P < 0.05$ . Control: Standard laboratory diet group, SD: Soft diet group, IR: Standard laboratory diet ischemic group, SD+IR: Soft diet ischemic group, TB: Toluidine blue, Casp-3: Caspase-3, iNOS: inducible nitric oxide synthase.

**Table 5.** Changes in area% of glial cells & distorted neurons in different studied groups.

Parameters	Groups	Control	SD	IR	SD+IR
Mean area of glial cells		3.40±0.09	6.85±0.19a	9.48±0.13ab	17.61±0.14abc
Mean area of distorted neurons		00±00	22.18±1.29 a	56.02±1.80 ab	123.45±1.99 abc

Data are expressed as mean ± S.E.M. of 6 rats in each group. a: Significant from control group, b: Significant from SD group, c: Significant from IR group respectively,  $P < 0.05$ . Control: Standard laboratory diet group, SD: Soft diet group, IR: Standard laboratory diet ischemic group, SD+IR: Soft diet ischemic group.

### Morphometric results

According to the data shown in Tables 4 and 5, the average percentage of TB regions was considerably less in the SD, IR, and SD+IR groups compared to the control group ( $P < 0.05$ ). Meanwhile, the levels of Casp-3, iNOS immunostaining, area% of glial cells and deformed neurons were considerably elevated in all groups ( $P < 0.05$ ).

### DISCUSSION

Stroke is the second leading cause of death and the third primary cause of disability. Most ischemic strokes are caused by a blockage of the middle cerebral artery. Rodent models have been created to replicate human focal ischemia stroke (Komatsu et al., 2021). In the current investigation, H&E-stained slices of the regular laboratory diet ischemia group (IR) revealed congested dilated blood vessels and reduced neurons in a vacuolated 9 neuropil. These findings agreed with Alafify and Essawy (2023) who reported that the pathogenesis of IR injury is referred to damage to blood-brain barrier and inflammatory response. Tissue injury can take the pattern of selective neuronal necrosis, resulting in damage to neurons, glia, and blood vessels.

In addition, sections exposed to the toluidine blue stain for the assessment of neuronal cell loss showed a significant decrease in the intensity of

Nissl's granules in the cytoplasm of the neurons in the IR group as compared to that of the control group. Similar findings were shown by Elsayed et al. (2021) as an IR manifestation. An evident increase in the Casp-3 immunoreaction was seen in the rats subjected to ischemia-reperfusion (IR) compared to the control group.

This may refer to elevated caspase-3 activity in the cerebrum after ischemia-reperfusion injury in rats which resulted in neuronal apoptosis (Zhang et al., 2020). Also, a significant increase the iNOS immunoreaction was perceived. This may be related to an elevation in the nitric oxide (NO). Along with the neuronal NO deleterious effect in ischemia, NO production during reperfusion has a substantial role in the incident of reperfusion injury (Gürsoy-Özdemir et al., 2000).

The brain is very susceptible to both external and internal dangers, and an ischemic stroke initiates a prolonged inflammatory reaction characterized by the activation of glial cells and the infiltration of leukocytes. During the inflammatory response, there is an increase in the synthesis of cytokines, which occurs both in the brain and in the peripheral areas of the body (Clausen et al., 2020).

In the current work, the result clearly demonstrated that the brain TNF- $\alpha$  concentration significantly increased in the IR group. Multiple studies

have investigated the associations between caloric consumption and health conditions (Napoleão et al., 2021). Nevertheless, the association between the form and toughness of food staples and overall healthiness has not been well examined.

Within the scope of this research, the consistent consumption of a soft diet on a daily basis led to a notable rise in the weight of the fatty tissue surrounding the colon, along with an imbalance in the processing of fats, resembling the characteristics of metabolic syndrome and a substantial presence of lipids that promote the development of atherosclerosis. These latter symptoms are considered factors that increase the likelihood of developing atherosclerosis, cardiovascular illnesses (CVDs), and stroke.

In the current study, BMI and gastro-colic omentum weight were used as markers of adiposity. So, rats fed a soft diet (SD) became obese after 4 weeks when compared to rats on a regular laboratory diet, which was most likely owing to increased fat accumulation. Fat deposition is caused by a disparity between energy intake and spending.

This may be because the lack of chewing in SD fed rats can hinder the process of digestion and contribute to a range of health issues, including obesity, resulting from decreasing energy expenditures. The same results were reported by (Furukawa et al., 2023; Tada and Miura, 2018). There are two probable causes for the relationship between mastication and obesity:

One is that those with impaired masticatory function consume less vegetables and fruits and more high-energy foods than those with normal mastication, which leads to obesity (Tada and Miura, 2014).

Another is that decreased chewing with SD causes obesity-causing events due to a decrease in diet-induced thermogenesis and a short number of chewing cycles, which reduces chewing's influence on obesity prevention (Robinson et al., 2014).

Obesity is characterized by the release of elevated quantities of pro-inflammatory adipokines by hypertrophic adipocytes. This leads to the development of inflammation, dyslipidemia, and the accumulation of fat in abnormal locations.

Moreover, elevated levels of low-density lipoprotein (LDL) in the blood can be taken up by macrophages and deposited in the inner layer of blood vessels, influencing atherosclerosis. This indicates that dyslipidemia is a widely recognized main contributor to cardiovascular disorder (Hedayatnia et al., 2020).

Multiple research studies have revealed that correlation impairment includes higher TG and LDL levels (Trandafir et al., 2022), as demonstrated in the current study in SD-fed rats. The liver has three main sources of fatty acids, all of which can undergo alterations in obese individuals. Initially, there is an augmentation in the movement of fatty acids from adipose tissue to the liver.

An elevated amount of adipose tissue, specifically in the visceral region, leads to increased fatty acid transport to the liver. Multiple studies have demonstrated that hepatic fatty acid synthesis is elevated in individuals with obesity (Björnson et al., 2017).

The liver's uptake of triglyceride-rich lipoproteins functions as an additional origin of fatty acids. Chewing is linked to a rise in blood flow to the brain, which impacts the development of the brain after birth, the aging process, and the ability to move. Poor masticatory function (because of tooth loss or prolonged consumption of SD) is followed by broad anatomical and functional brain alterations in areas responsible for sensory, motor, cognitive, and emotional processing.

In the current study, the sections achieved from the SD fed rats revealed some shrunken pyramidal cells, a decreased intensity in some neurons. On the other hand, in the SD + IR group, a severe deterioration was detected in the form of vascular congestion, neuronal degeneration, apoptosis, and a significant drop in the area % of TB.

Ischemic energy depletion, together with a decrease in cerebral blood flow and the formation of oxidants, causes the release of glutamate. This release leads to the activation of iNOS and enhanced activity as shown in the current study in SD group and more aggravated in SD+IR group. The current work demonstrates that the production of inflammatory cytokines, such as TNF- $\alpha$ , leads to the activation of iNOS (Garry et al., 2015).

The excessive synthesis of iNOS has been involved in several disease processes, such as tissue damage and cellular programmed cell death (apoptosis), resulting from inadequate blood supply (ischemia) and inflammation (Pang et al., 2020). Increased NO production by iNOS activates downstream pathways resulting in neuronal death (Ansari et al., 2024) as presented in the current study by increased expression of Casp-3 in SD group and more aggravated in ischemic groups.

Therefore, it may be concluded that SD has a negative impact on cerebral IR. These findings align with previous research by Furukawa et al. (2023), which showed that prolonged consumption of a high-sugar diet resulted in decreased levels of brain-derived neurotrophic factor (BDNF) protein. BDNF is one of the most efficient promoters of neurogenesis in the brain, and it is necessary to enhance neural precursor cell proliferation.

Oxidative stress is characterized as a disparity between pro-oxidants (such as ROS) and antioxidant defenses. Previous research demonstrated that masticatory motor activity motions, such as biting, reduce oxidative stress in the brain caused by constraint stress (Ohno et al., 2012). The current data in SD rats imply that feeding a soft-food 12 diet increases oxidative stress, as evidenced by a substantial drop in TAC concentration due to a decrease in chewing.

In addition, the upregulation of iNOS shown in the present immune-histochemical study in the SD group produces excessive NO causing oxidative damage to cerebral tissues (Rampelotto et al., 2023).

## CONCLUSION

Based on the current statistics, one may infer that long-term feeding of SD causes weight gain, dyslipidemia with oxidative, inflammatory and apoptotic changes in the brain; it also aggravates the pathological changes that occur with brain ischemia-reperfusion.

## Authors' contributions

All authors made nearly equal contributions to the work, including interpreting the data and writing the report. The writers who contributed to the histo-pathologic examination were the first and third.

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