Research Article

Some Histological and Histochemical Effects of Aqueous Extract of the the leaves of Neem on the Superior Colliculus and Lateral Geniculate Body of Adult Male Wistar Rats *(Rattus norvegicus)*

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Abstract

This study investigated the effect of the crude extract of the leaves of *Azadirachta indica* on the intracranial visual relay centers of adult male Wistar rats. It also investigated the effects of the extract on the activities of some enzymes of carbohydrate metabolism namely lactate dehydrogenase (LDH), succinate dehydrogenase (SDH) and NADH-diaphorase. This was with a view to evaluating its effect on the histology of the superior colliculus and lateral geniculate body of Wistar rats.

Thirty-six adult male Wistar rats with an average weight of 200g were distributed into 6 groups A to F. Groups A to E were treated orally with crude extract of Neem leaves at different repeated doses of (500, 400, 300, 200 and 100) mg/ml per kg body weight respectively for 3, 5, 7, 9 and 11 days. Each group had a pair –matched control. The rats were sacrificed on days 4, 6, 8, 10 and 12 days respectively and the superior colliculus and lateral geniculate body were examined microscopically after staining for histological and histochemical analyses. Group F served as the withdrawal effect group to determine the level of tolerance of the animals to the administered extract.

The results showed distortions in the histoarchitecture of the superior colliculus and lateral geniculate body of rats treated with crude extract of Neem leaves at the dose of 100mg/ml per kilogram body weight for 11 days. On microscopic evaluation, it was observed that the histochemical staining intensities showing the activity of LDH, SDH and NADH-diaphorase decreased in treatment groups of both the superior colliculus and lateral geniculate body implying that the administered extract had adverse effects on these two intracranial visual relay centers. The withdrawal effect group however show signs of recovery.

The study concluded that the crude extract of leaves of Neem had an inhibitive effect on the activities of enzymes of carbohydrate metabolism namely LDH, SDH and NADH-diaphorase as shown by the decrease in the staining intensities observed, and a degenerative effect on the neurons of the superior colliculus and lateral geniculate body of adult male Wistar rats.

Keywords: Neem, Superior Colliculus, Lateral Geniculate Body, Carbohydrate Metabolism

INTRODUCTION

The aqueous leaf extract of Neem is commonly used for the suppression and management of Malaria Khalid et al (1989).

Markinon (1997) reported the the aqueous extract of Neem is more effective and safer than chloroquine. Preliminary studies show that it has significant medicinal effects on several bacteria strains Rao (1969); Chopra (1956,).

Mhamoodin, one of Neem's metabolic products showed significant antibacterial activity against various gram positive and gram negative organisms Seddiqui (1942).

The staphylococcus aureus bacteria that causes peritonitis, cystitis and meningitis is killed or rendered harmless by small doses of leaf extracts Seddiqui (1942).

It has been shown that Neem extracts are toxic to herpes virus and quickly healed cold sores. Shingles is believed to be caused by the same virus that causes chicken pox, Schmutterer (1995) the virus affects the nerve endings, creating a painful burning sensation. Neem's ability to enclose viruses and prevent them from entering and infecting cells make it one of the only agents capable of reliving shingles Rao et al..(1997).

The seed and leaf extracts are effective against malaria parasites. (Khalid et al., 1989) who however reported that the alcoholic extracts of leaves and seeds of Neem were effective against both chloroquine resistant and sensitive strains of malaria parasite (Badani et al.. 1987). The methanol extract of the leaves exerted an antipyretic effect male rabbits (Okpanyi 1993), while the plant also possessed analgesic activity mediated through opoid receptors in laboratory animals. The aqueous extract of the leaves also possesses potent immune-stimulant activity as evidenced by both humoral and cell mediated responses. The leaf extract at 199mg/kg body weight after three weeks of oral administration is reported to cause higher IgM and IgG levels Banergee (1996).

The aqueous extract also reportedly decreased blood sugar levels significantly and affects adrenaline as well as glucose induced hyperglycemia (Murthy 1978). The aqueous extract when orally administered produced hypoglycemia in normal rats and decreased blood glucose levels in experimentally-induced hyperglycemia in rats (El-Hawarry 1990)

The aqueous leaf extract reduced hyperglycemia in streptozotocin-induced diabetes mellitus and these affects are possibly due to the presence of a flavanoid, quercertin (Singh et al.,, 1996). The leaf extract has been shown to reverse diabetic retinopathy in streptozotocin-induced diabetic Wistar rats (Halim et al..2002).

The overall aim of this study is to investigate its effects on the intracranial visual relay centres (superior colliculus and lateral geniculate body) of Wistar rats.

MATERIALS AND METHODS

Care of Animals

Thirty-six presumably healthy male Wistar rats (average weight 200g) were randomly distributed into six groups, A-E, each group with its matched pair control. The rats were kept in the animal holdings of the department of Anatomy and Cell biology, OAU Ile Ife, Nigeria, fed with rat pellets from Ladoke feeds in Ibadan and given water liberally. All rats were carefully assessed, screened and found to be healthy.

Preparation of the extract

Freshly collected Neem leaves 20g were homogenized with 1000ml distilled water in an electric blender (S-742) at room temperature. The homogenate was filtered through a sterilized cheese cloth. A cold extraction method was used to obtain the crude active agents of Neem leaves in a rotator evaporator. This extract was subjected to freeze drying to obtain a powdery substance which was dissolved in distilled water for administration to the animals repeatedly at the predetermined dosages orally.

Drug Administration

Group A, B, C, D, and E was treated with aqueous extract of Neem leaves (100/mg/ml stock solution) repeatedly orally at the doses of 500/mg, 400/mg, 300/mg, 200/mg and 100/mg per kilogram body weights of rat at 3, 5, 7, 9, and11 days respectively.
Group F served as withdrawal effect group allowed one week free of administration of Neem leaves extract **(NLX)**

Histological and Histochemical Techniques

Samples from the superior colliculus and lateral geniculate body of all the sacrificed animals were fixed in the formol calcium and processed with paraffin for the histological examination. Serials section at 5um thickness was obtained using a rotator microtome and stained with hematoxylin and eosin using Drury and Wallington (1956) method. The cresyl violet technique for demonstrating Nissl substance by the Alvarez (1970) was used for histology.

Lactate dehydrogenase was demonstrated using the method of Dietz and Lubrano (1967). Succinic Dehydrogenase was demonstrated using the method of Nachlas, Tsou, Cheng and Seligman (1957) while NADH-diaphorase was demonstrated using the method of Pearse (1972).

RESULTS

Photomicrograph of section the superior colliculus and lateral geniculate body of rats treated with 100mg/kg body weight crude extract of Neem leaves for 11 days showed disarrangement in the cytoplasm. Histological findings indicate that the extract affected the structure and integrity of the nuclei and cytoplasm.
In this study, neuronal assault was also observed in sections of the lateral geniculate body of treated rats by the lightly stained and vacuolated Nissl substance Figures 5a and 5b

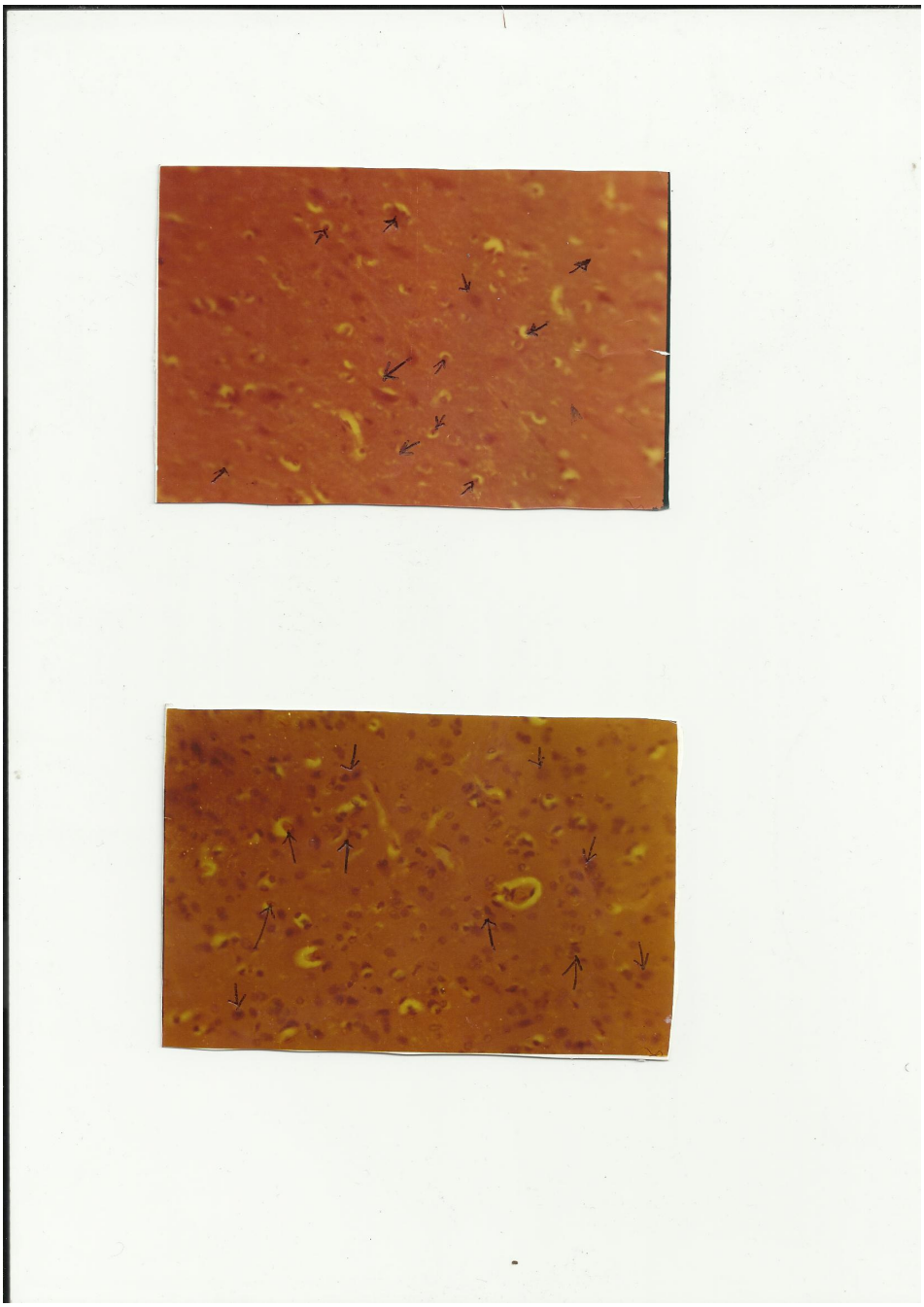
The histochemical staining intensities LDH, SDH and NADH-diaphorase decreased in treatment group of both superior colliculus and lateral geniculate body reflecting the deleterious effect of the administered extract on these two intracranial visual relay centres Figures 5, 6, & 7 and consequently on neuronal metabolism. The actual mechanism by which Neem mediate these effects are yet to be elucidated.

LEGENDS

FIGURE 1 and 2 presents photomicrograph of section the superior colliculus of control rats and rats treated with 100mg/kg body weight crude extract of Neem leaves for 11 days showing disarrangement in the cytoplasm (Figures 1b,1d and 2b) Contols (Figures 1a,1c and 2a). Arrows and arrowheads indicate nuclei of neurons.

Figure 2d presents a high neuronal population density indicating the reaction of the neuronal elements to Neem treatment for 11 days as compared to control (Figure 2c).Vacuolations were observed in the treament section of the superior colliculus of rats treated for 11 days (Figure 1d and 2b)

Figure 1a and 1b *400 Magnification



Figures 1c and 1d *400 Magnification

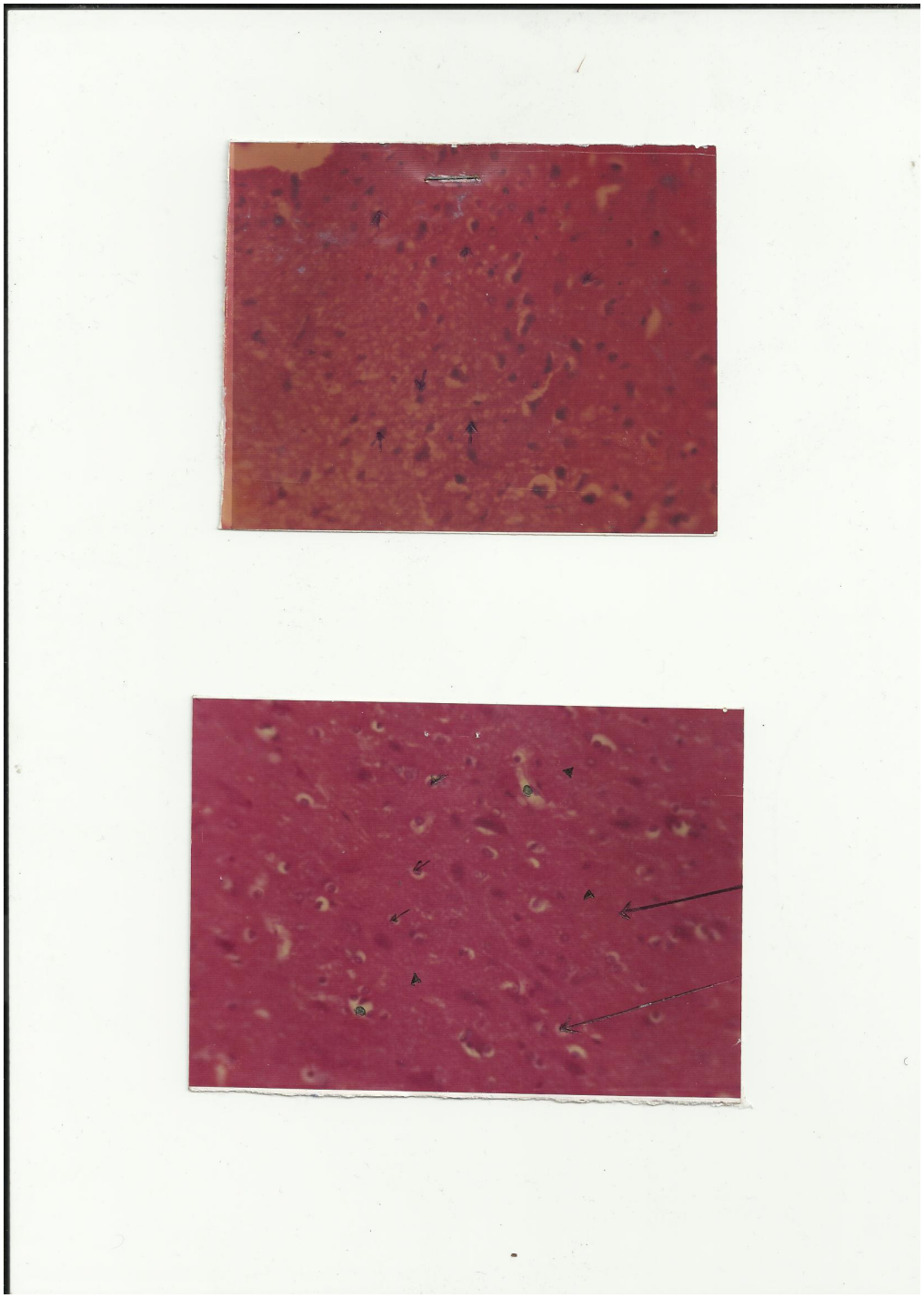


Figure 2a and 2b: *400 Magnification

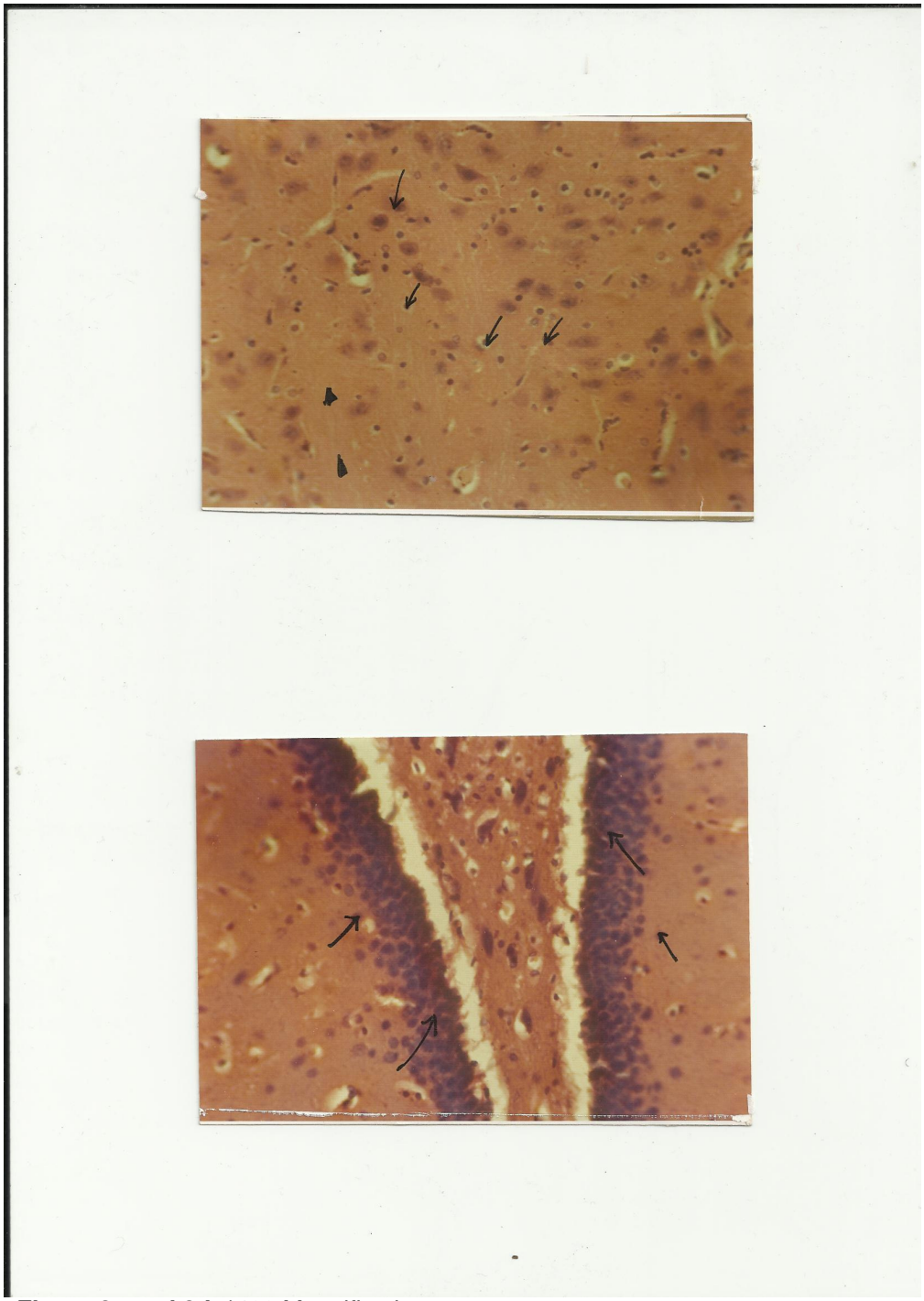


Figure 2c and 2d: *400 Magnification

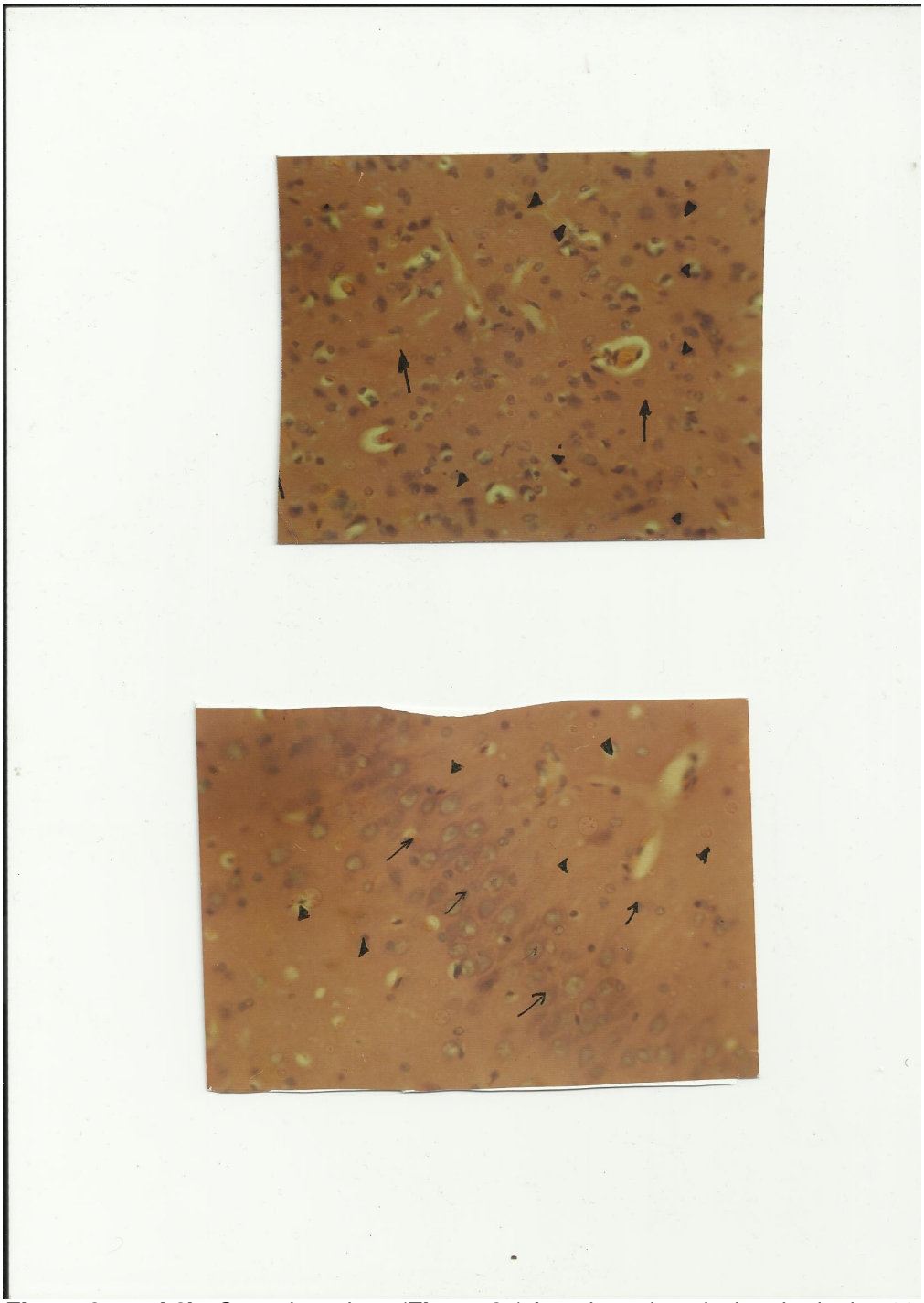


Figure 3a and 3b: Control sections (**Figure 3a**) from lateral geniculate body day 11 showing glia and neurons. *400 Magnification. Arrows show nuclei of glial cell. The arrangement regular in controls as compared to the irregularly arranged nuclei observed in treated section (**Figure 3b**). Figure 3b represents the treatment section from the superior colliculus day 11 showing irregular arrangement of nuclei and stroma.The integrity of the cells has been affected. Arrows show less intensely stained nuclei and the cellular population (density) appears reduced.

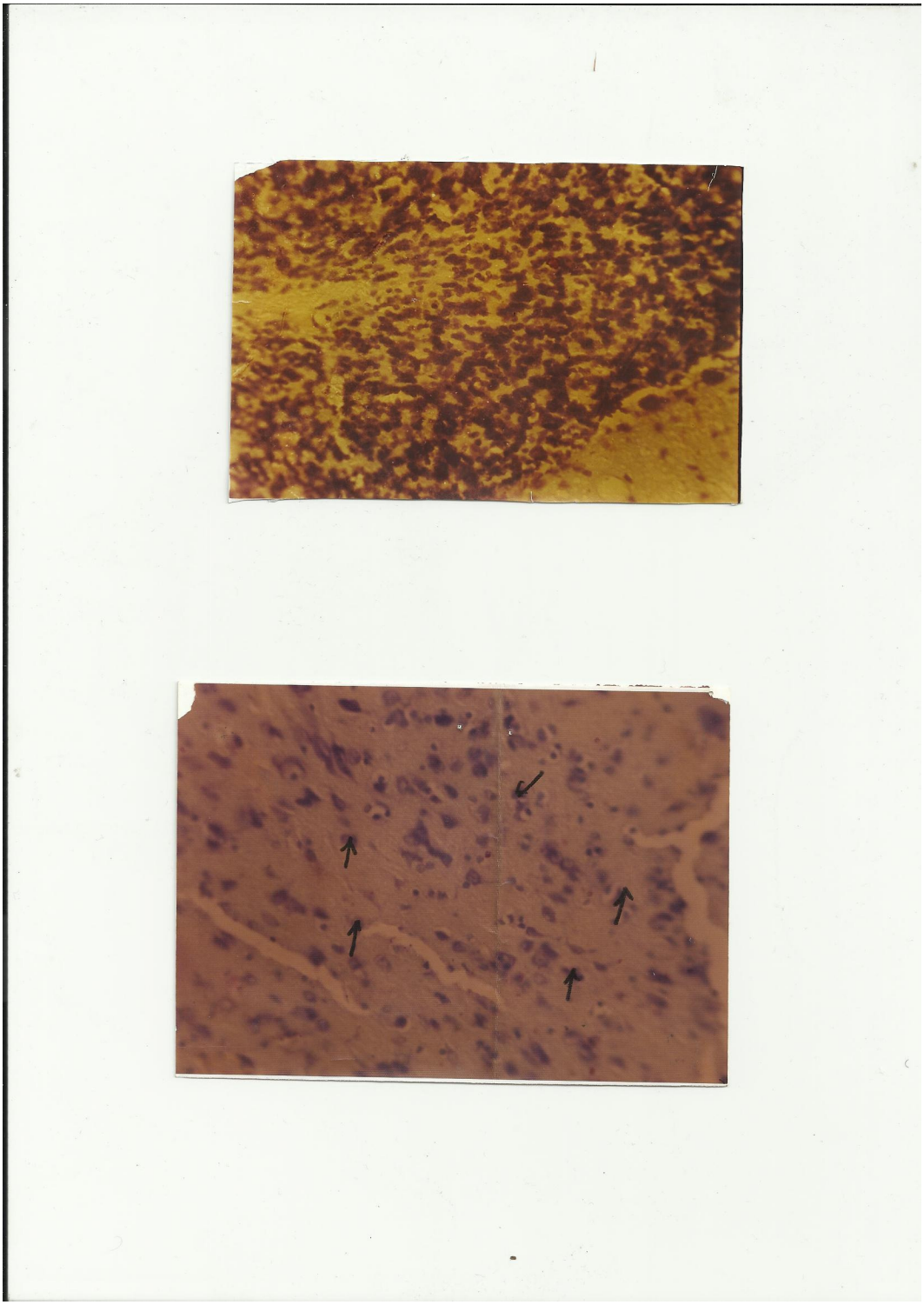


Figure 3c and 3d: Figure 3c (above) is the control section from the lateral geniculate body day 11 showing intensely stained Nissl substances. Cresyl etch violet method for Nissl bodies appear as dots (Figure 3d). *Nissl bodies/granules* are basophilic, cytoplasm structures that are concentrations of granular endoplasmic reticulum indicating the status of protein synthesis in cells.

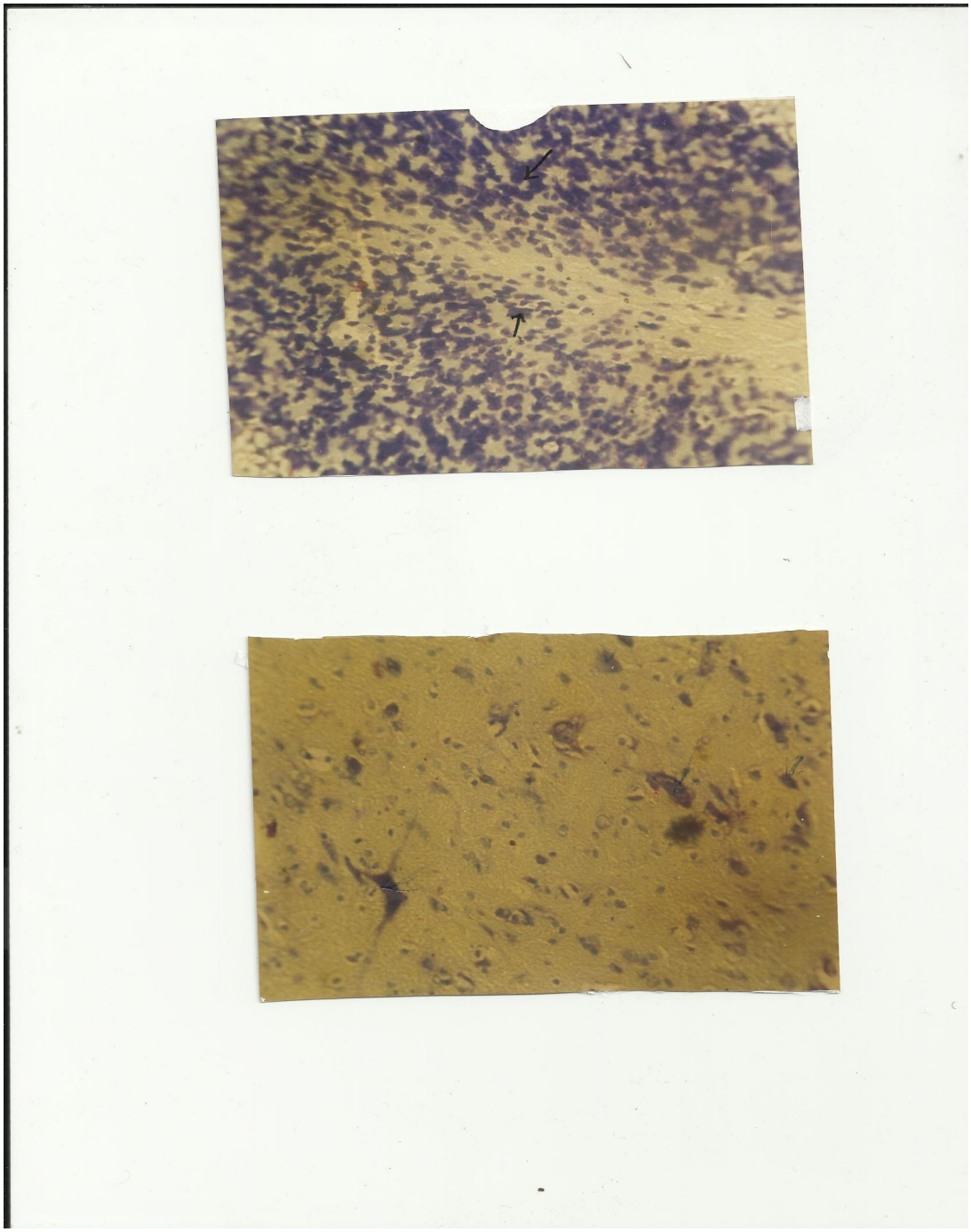


Figure 4a and 4b: **Figure 4a** (above) Control section from the lateral geniculate body day 11, showing Nissl stained bodies of neurons.Nissl substance is indicated by arrows and **Figure 4b** is less intensely stained.Note the differences in the cytoarchitecture. *400 Magnification

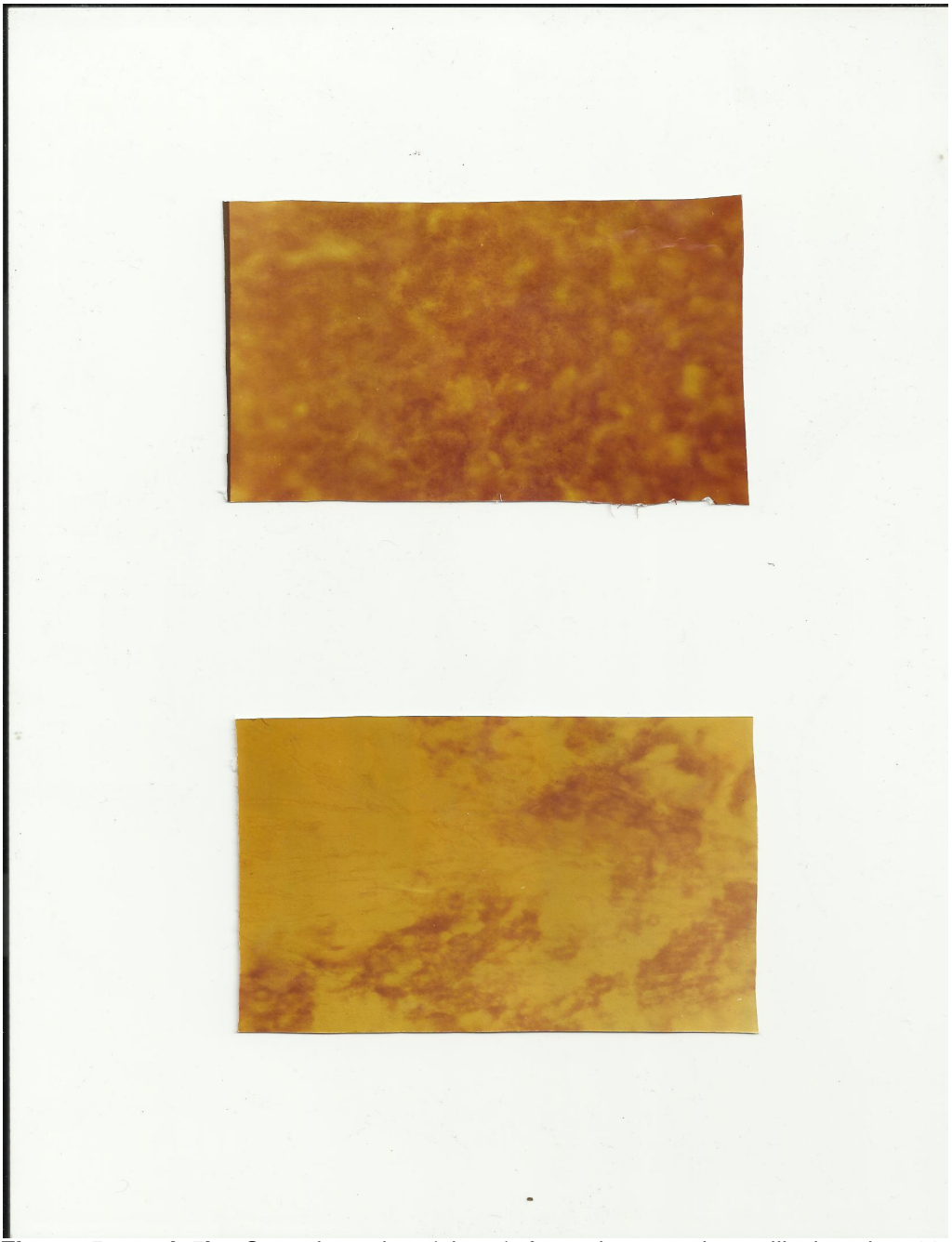


Figure 5a and 5b: Control section (above) from the superior colliculus day 11, showing the staining intensity of the activity of Lactate dehydrogenase (LDH) *400 Magnification

Figure 6a and 6b: Control section from the LGB day 11, showing the staining intensity of the activity of succinic dehydrogenase (SDH), *400 Magnification.
Figure 6b treatment section from the LGB day 11 showing the staining intensity of the activity of the SDH.The activity of SDH in the LGB is indicated by the purple formazan deposits, the enzyme activity is shown to be inhibited.

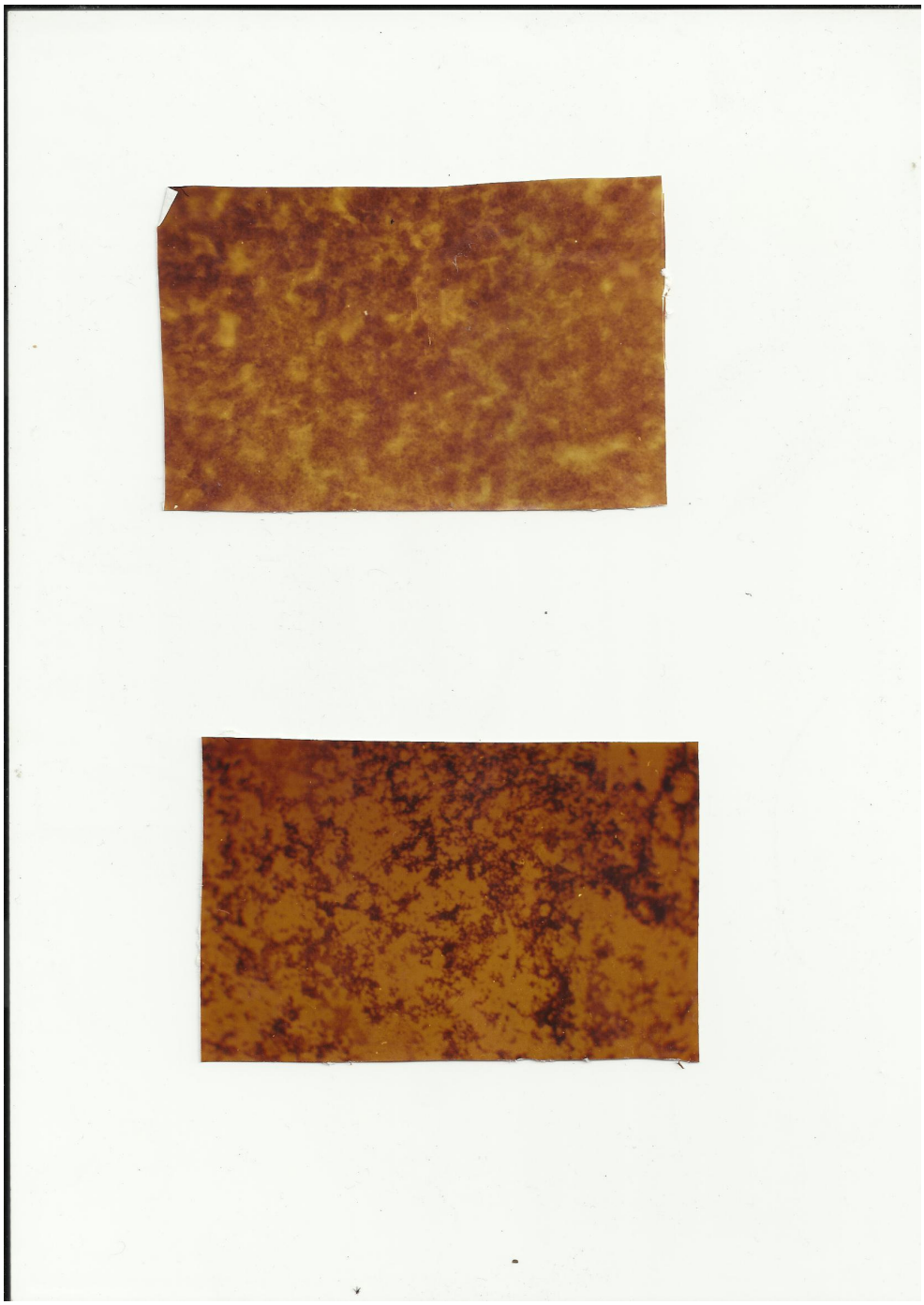


Figure 7a and 7b: 7a (above) the control section from the LGB day 11 showing the staining intensity of the activity of NADH-diaphorase compared to the treatment section Figure 7b (below)

Discussion

The results obtained in this study showed that oral administration of aqueous extract of Neem leaves to adult male Wistar rats caused histochemical changes, disarrangement of neurons and decreased staining of Nissl substances in the treatment groups. This may cause a reduction in the level of protein synthesis and neuronal activity in the intracranial visual relay centers. The histoarchitecture and arrangement of the sections in the treatment group in the present study varied from mild to marked distortions coupled with irregular arrangement of the chromatin pattern in the nuclei.

Consequently the pachychromatic pattern observed in day 11 treatment groups **(Figure 2d)** is consequent to the administered extract which reflects an abnormal level of metabolic activity. Which is in agreement with other studies (Biu et al 2010).

The effects of the administered crude extract was observed on the activities of some enzymes of carbohydrate metabolism especially the succinic dehydrogenase activity **(figures 6a and 6b)** in the lateral geniculate body of treatment sections suggest that the extract of Neem leaves impairs the process of carbohydrate metabolism through the Kreb cycle in the neuron.

CONCLUSION

In conclusion, this study establishes that Neem extract has an inhibitive effect on the activities of enzymes of carbohydrate metabolism namely LDH, SDH and NADH-diaphorase as shown by the decrease in the staining intensities observed, and a degenerative effect on the neurons of the superior colliculus and lateral geniculate body of adult male Wistar rat, following continuous consumption for days (11 days as in the case of this study), further experiments are however required to better understand the mechanisms underlying these effects.

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