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Effects of Ethanolic Extract of *Brassica juncea* (Mustard Seed) on the Brain and Kidney Tissues of Albino Wistar Rats

Imeobong Joseph Inyang[1] Aniekan-Augusta Okon Eyo[1*] Tomilola Margaret Olajide[1] Abel Essien[2]

1. Department of Medical Laboratory Science, College of Medical Sciences, University of Calabar, P.M.B. 1115, Calabar, Nigeria
2. Department of Histopathology, University of Calabar Teaching Hospital, P.O. Box 1278, Calabar, Cross River State, Nigeria

* E-mail of the corresponding author: ask4ani2003@yahoo.com

Abstract

Mustard seeds (*Brassica species*) are widely used as medicinal crops and spices. They are a rich source of oil and protein containing as high as 46 – 48% oil and 43.6% protein in whole seed meal. Historically, mustard seeds are mentioned in ancient Sanskrit writings dating back five thousand years. The ancient Greeks and Romans also used the seeds for medicinal purposes. However, not much has been documented on the neurotoxic and nephrotoxic effects of mustard seeds. A study designed to investigate the possible toxicity of mustard seeds on brain and kidney tissues was carried out. Twenty albino wistar rats of mixed sexes, aged two months and weighing 100 - 140g were divided into four groups of five rats labeled A to D. Groups A and B were orally administered different concentrations of ethanolic extract of mustard seeds, 2000mg/kg and 4000mg/kg body weight respectively daily for two weeks while groups C and D served as pre and post-treatment controls and received no extract. Kidney and brain tissues of rats were histologically processed and stained using Haematoxylin and Eosin technique and examined microscopically. Sections revealed that the ethanolic extracts of *Brassica juncea* had visible histological effects and altered the histoarchitecture of the brain and kidney tissues of the test groups. The results suggest that prolonged ingestion of extract is toxic to tissues at the concentrations investigated.

Keywords: Mustard seeds, albino wistar rats, ethanolic extract

1. Introduction

Medicinal plants are those whose parts or seeds can be used for therapeutic purposes (Ayoola *et al.* 2006). According to the World Health Organization (WHO), about one billion people rely on herbal medicine to some extent and has listed twenty-one thousand plants with reported medicinal uses from around the world (Wikipedia 2009, Ensminger *et al.* 1983). More than half of medications in most African countries, particularly Nigeria, come directly or indirectly from indigenous plant seeds (Usfoelda *et al.* 2010). Mustard plant is one of such plants that has enjoyed wide medicinal usage.

Mustard seeds are the tiniest part of the mustard plant which flourishes in the cold weather, moist soil and general temperate conditions (Wikipedia 2009). The plant grows as a shrub while the seeds are about two millimeters in diameter and used as spices in many countries. There are approximately 40 different varieties of mustard plants, but the three principal ones which also vary in colour are *Brassica hirta or alba* (yellow-white), *B. nigra* (black) and *B. juncea* (brown) (Elvin-Lewis 2001). Black and brown mustard seeds return higher yields than their yellow counterparts. Mustard seeds can be traced to different areas of Europe and Asia with the yellow-white variety originating in the Eastern Mediterranean regions, the brown from the foothills of the Himalayas and the black from the Middle East (Wikipedia 2009). Canada is currently the largest producer of mustard seeds, but seeds can also come from other countries including United States, Hungary, Great Britain and India (AAFC 2009).

The unique healing properties of mustard seeds could be attributed to its high content of vitamins and minerals including iron, magnesium, manganese, selenium, phosphorus, calcium, niacin, fibre and zinc. It is also a good source of protein, omega-3 and fatty acids (Billman 2013). The primary chemical constituent of mustard seeds is an enzyme, myrosinase which reacts with a glucosinolate, known as sinigrin, from the black or brown mustard seeds when ground and mixed with water, vinegar or other liquids (Wikipedia 2009). This reaction produces a phytonutrient called isothiocyanate which has been studied widely for its anticancer effect (Elvin-Lewis 2001). In animal studies, particularly those involving kidney, brain and colorectal cancers, intake of isothiocyanate from

mustard seed has been shown to inhibit growth of existing cancer cells while protecting against the formation of new ones (Lai & Roy 2004). Other phytochemical constituents of these seeds include alpha-linolenic acid, erucic acid, palmitic acid, nitric oxide and thiamine (AAFC 2009). Mustard seeds contain irritants that may cause reactions mimicking allergic reactions, thus inducing non-IgE mediated cell degranulation (Rastogi *et al.* 2004).

Mustard seeds are increasingly being used in cooking and processed and pre-packaged foods as flavouring agent, seasoning and water binding agent for texture control. This study was undertaken to ascertain the effect of prolonged intake of mustard seeds on the histological architecture of the brain and kidney tissues of the consumer.

2. Materials and methods

2.1 Animal subjects and mustard specimen collection

Twenty albino wistar rats of mixed sexes aged 1 – 2 months and weighing 100 – 140kg were obtained and housed in the animal house of the Physiology Department, College of Medicine, University of Calabar. The animals were randomly distributed into four groups of five rats each. They were acclimatised for two weeks under standard condition of temperature with a 12 hour light/dark cycle. The rats were fed with standard diet and water throughout the study period. Mustard seeds were obtained and authenticated by the Botany Department of University of Calabar.

2.2 Preparation of extracts

The mustard seeds (Brassica juncea) were ground into powder with an electric blender. One thousand two hundred grams of the ground seeds was soaked in 4000ml of 80% ethanol, stirred and left for 72 hours in a refrigerator at 4^0C. the mixture was sieved and filtered with Watman No.1 filter paper. The filtrate obtained was placed in the water bath at 40^0C to evaporate yielding 105g of extract.

2.3 Preparation of animals

The rats were distributed into four groups, A,B,C and D. Groups C and D served as pre and post-treatment controls respectively. The rats in the pre-treatment group were sacrificed before administration of the extracts. Rats in groups A and B were treated with 2000mg/kg and 4000mg/kg body weight of extracts respectively, daily by oral gavage for two weeks. Twenty-four hours after the last administration, the animals were anaesthesized under chloroform and dissected. The kidney and brain tissues were surgically removed, the kidney tissues fixed in 10% neutral buffered formalin while the brain tissues were fixed in 20% formal saline for histological evaluation. The fixed kidney and brain tissues were processed and embedded in paraffin wax. Tissue blocks were cut into 5µm sections and stained with Haematoxylin and Eosin (H&E) technique. The sections were examined microscopically for visible alterations.

3. Results

Alterations to the histological architecture of both kidney and brain tissues of the experimental rats were observed even at the lower dose (2000mg/kg) of the ethanolic extract of Brassica juncea. The tissue sections were analyzed as follows;

3.1 Kidney: Control

Sections of tissues with preserved architecture showed the cortex and medulla. Within the cortex were prominent glomeruli with distinct Bowman's capsule and cellular messengium. The medulla showed prominent and closely packed tubules (Plate 1).

3.2 Group A: 2000mg/kg

Sections of kidney tissues showed prominent glomeruli with distinct Bowman's capsule. The Bowman's space was expanded as a result of contraction/shrinkage of the messengium (Plate 2).

3.3 Group B: 4000mg/kg

Sections showed prominent glomeruli with distinct Bowman's space. The tubules were lined by cuboidal cells with distinct lumen. The messengium was hypocellular with sclerosis (Plate 3).

3.4 Brain: Control

Sections of the cerebrum showed intact cell layers with prominent cells having eosinophilic cytoplasm and triangular shaped nuclei. Also seen were microglial cells with ovoid nuclei and thin rim of cytoplasm (Plate 4).

3.5 Group A: 2000mg/kg

Sections of brain tissues showed pyramidal cells with pyknotic nuclei and abundant eosinophilic cytoplasm. Reactive microglial cells were also seen and atrophic neurons signifying toxic effects of extract (Plate 5).

3.6 Group B: 4000mg/kg

Sections showed degenerated and atrophic neuronal cell bodies with gliosis. The pyramidal cells had pyknotic nuclei (Plate 6).

4. Discussion

The results of this study showed that ethanolic extract of mustard seeds (*B. juncea*) had some adverse effects on the brain and kidney of albino wistar rats even at the lower experimental dose of 2000mg/kg body weight. Medicinal plants have widespread use in alternative medicine in Nigeria and, particularly in cases of diseases not amenable to treatment by modern method (Ayoola *et al.* 2006, Su *et al.* 2000, Rawal *et al.* 2009), with little or no attention being paid to the possible adverse effects or otherwise of these herbs in the body. The regulation of their dosages still poses a challenge. Mustard oil has high levels of erucic acid which is thought to have toxic effect on the heart at high enough doses in laboratory animals (Salter 2013, Seth *et al.* 2009) Including oils in the diet that are high in alpha-linolenic acid has been thought to protect the heart and prevent cardiovascular diseases, but recent reviews cast doubt on this. Two Indian studies on the health effects of mustard oil have shown conflicting results. One found that mustard oil had no protective effect on the heart and the authors reckoned that the benefits of alpha-linolenic acid were overweighed by the harm of erucic acid (Rastogi *et al.* 2004), while another study found that mustard oil had a protective effect on the heart and the benefits of alpha-linolenic acid overweighed the harm of erucic

acid (Ghafoorunissa 1998). In this study, the kidney and brain tissues of the animals were adversely affected as their histological architecture was significantly altered (Plates 2, 3, 5 and 6). According to the Hodge and Sterner scale for toxicity classes (CCOHS 2005), a substance whose dose of 500 to 5000mg/kg gives an oral LD_{50} is considered toxic in rats. In general, if the immediate toxicity is similar in all the animals tested, the degree of immediate toxicity will probably be similar for humans. Thus mustard seeds (*B. juncea*) extract at doses of 2000 to 4000mg/kg for two weeks is toxic for the albino wistar rats and may be toxic for humans if exposed for the same period since both species have similar organs that function in the same way.

5. Conclusion

Brassica plants are highly regarded for their nutritional value and potent anticancer properties. They have been used in the treatment of many ailments including arthritis. This study has shown that prolonged oral intake of mustard seed extract is toxic in albino wistar rats at high enough doses of 2000 to 4000mg/kg body weight. It is inferred that these doses could also be toxic in humans. Suggestion is made therefore, for further studies to determine the dose at which mustard seeds extracts would be toxic to humans.

Acknowledgement

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References

Agriculture and Agri-Food Canada (AAFC). Canadian seed – from little comes much. Available: http://www.marquecanadabrand.agr.gc.ca/fact-fitche/4706-eng.htm (August 25, 2014).

Ayoola, G. A., Odukoya, O., Coker, H. A. B. & Shodiya, A. (2006). Phytochemical screening of free radical scavenging activity of some Nigerian medicinal plants. *Journal of Pharmacological Science,* 8, 133 – 136.
Billman, G. E. (2013). The effects of omega-3 polyunsaturated fatty acids on cardiac rhythm; a critical assessment. *Pharmacology and Therapy,* 140(1), 53 – 80.
Canadian Center for Occupational Health and Safety (2005). Hodge and Sterner scale of toxicity.
Elvin-Lewis, M. (2001). Should we be concerned about herbal remedies? Journal of Ethnopharmacology, 75(2-3),141 – 164.
Ensminger, A. H., Ensminger, M. E., Kondale, J. E. & Robson, J. R. K. (1983). *Food and Nutrition Encyclopedia.* Regus Press, Clovis, California.
Ghafoorunissa, P. (1998). Requirements of dietary fats to meet nutritional needs and prevent the risk of artherosclerosis – An Indian perspective. Indian Journal of Medical Research, 108, 191 – 202.
Lai, P. K. & Roy, J. (2004). Antimicrobial and Chemopreventive properties of herbs and spices. *Current Medicines and Chemicals*, 11(11), 1451 – 60.
Rastogi, T. Reddy, K. S. Vas, M., Spiegelman, D., Prabhakaran, D., Willet, W. C., Stampter, M. J. & Ascherio, A. (2004). Diet and risk of ischemic heart disease in India, *American Journal of Clinical Nutrition*, 79(4), 582 – 592.
Rawal, A. K., Manohar, G. M. & Saibal, K. B. (2009). Neuroprotective effects of *Rubia cordifolia, Fagoniacritis lim* and *Tinaspora cordifolia* as potent anti-inflammatory agents. *International Journal of Applied Research on Natural Products,* 2, 19 – 26.
Salter, A. M. (2013). Dietary fatty acids and cardiovascular disease. *Animal,* 7(1), 163 – 171.
Seth, P., Kumara, R. J., Madhavan, S. & Singh, A. K. (2009). Prevention of renal ischemia-reperfusion-induced injury in rats by picroliv. *Biochemistry and Pharmacology*, 59(10), 1315 -1322.
Su, L. Chen, Y. C. & Hu, J. D. (2000). Comparisons between different doses of *Astragalus membrabaceus* and *Sativa miltiorrhiza* in rats proteinuria. *Chinese Journal of New Clinical Remedies*, 19, 205 - 208.
Usfoelda, A., Yina, N. & Sunmonu, T. (2010). Invivo studies on antidiabetic plants. *Journal of Clinical Biochemistry and Nutrition,* 47, 98 – 106.
Wikipedia (2009). Mustard seed. Available: http://en.wikipedia.org/wiki/mustard_seed (August 20, 2014).

Table 1. Observation table for Group A

Days	Daily activities	Weight	Food intake	Water intake	Sleeping habit
1	Normal	No loss/gain	Normal	Normal	Normal
2 - 5	Normal	No loss/gain	Normal	Normal	Normal
6 - 9	Sluggish	No loss/gain	Normal	Normal	Normal
10 - 13	Sluggish	No loss/gain	Normal	Increased	Increased
14 - 15	Very sluggish	No loss/gain	Reduced	Increased	Increased

Table 2. Observation table for Group B

Days	Daily activities	Weight	Food intake	Water intake	Sleeping habit
1	Normal	No loss/gain	Normal	Normal	Normal
2 - 5	Sluggish	Slight loss	Normal	Increased	Increased
6 - 9	Very Sluggish	Prominent loss	Reduced	Excess	Increased
10 - 13	Very Sluggish	Prominent loss	Reduced	Excess	Increased
14 - 15	Weak	Prominent loss	Reduced	Excess	Increased

Table 3. Weight before and after extract administration

Group	Weight (g) before administration	Weight (g) after administration	Observation
A	100 – 140	100 – 140	No weight loss
B	120 – 140	100 – 110	Prominent weight loss
Control	100 – 140	140 – 170	Prominent weight loss

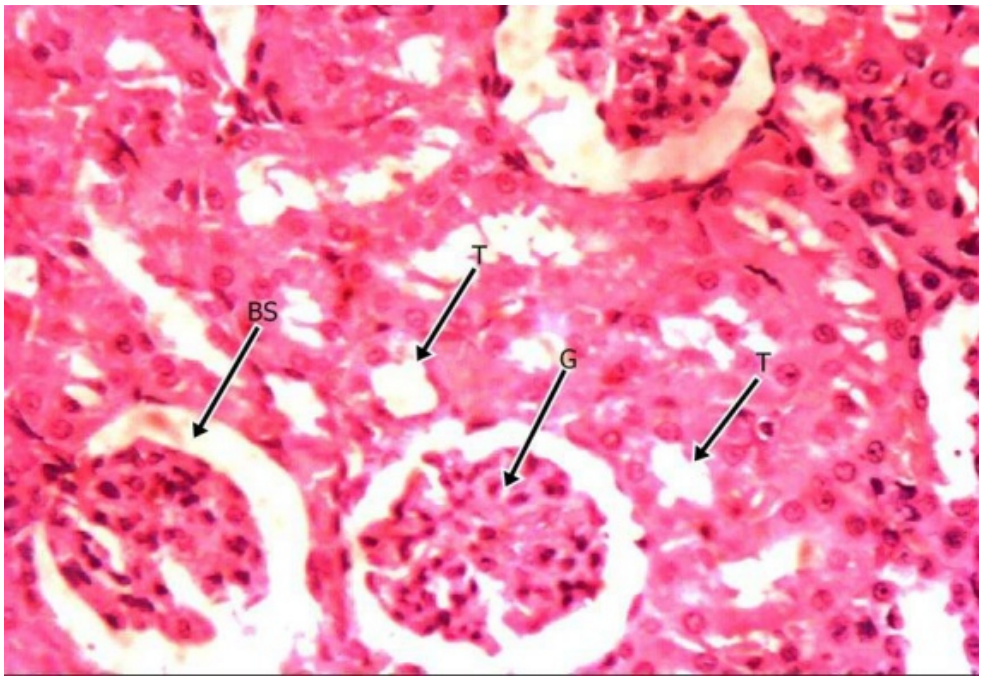

Plate 1. Photomicrograph of the control group of albino wistar rat kidney tissue (X400). Glomerulus(G), Bowman's space(BS), Tubules(T).

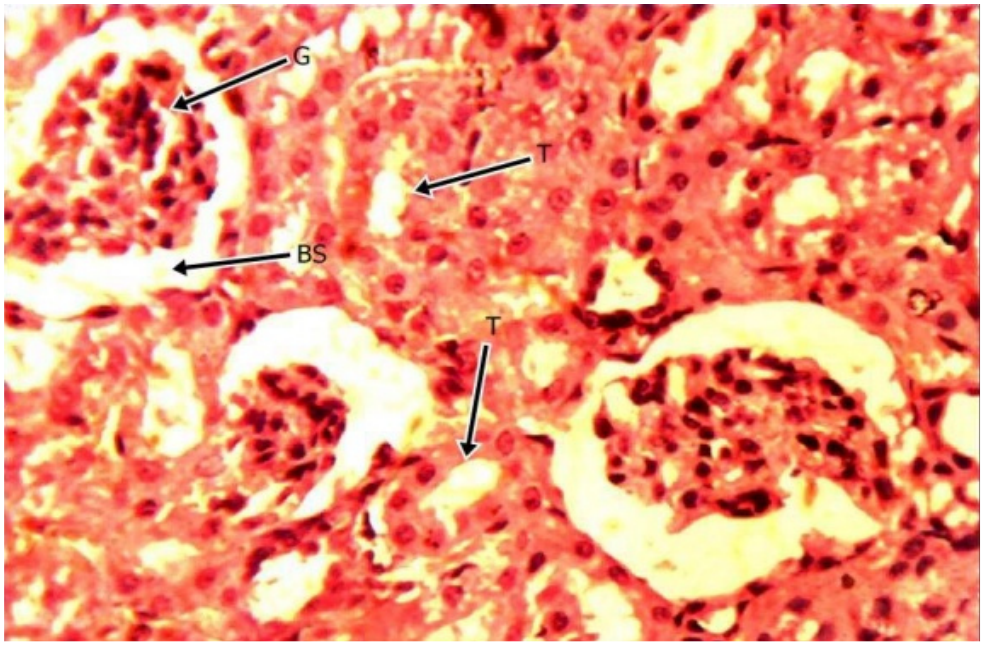

Plate 2. Photomicrograph of Group A (2000mg/kg) albino wistar rat kidney tissue (X400) treated with mustard seeds *(B. juncea)* extract. Glomeruli(G), Bowman's space(BS), Tubules(T).

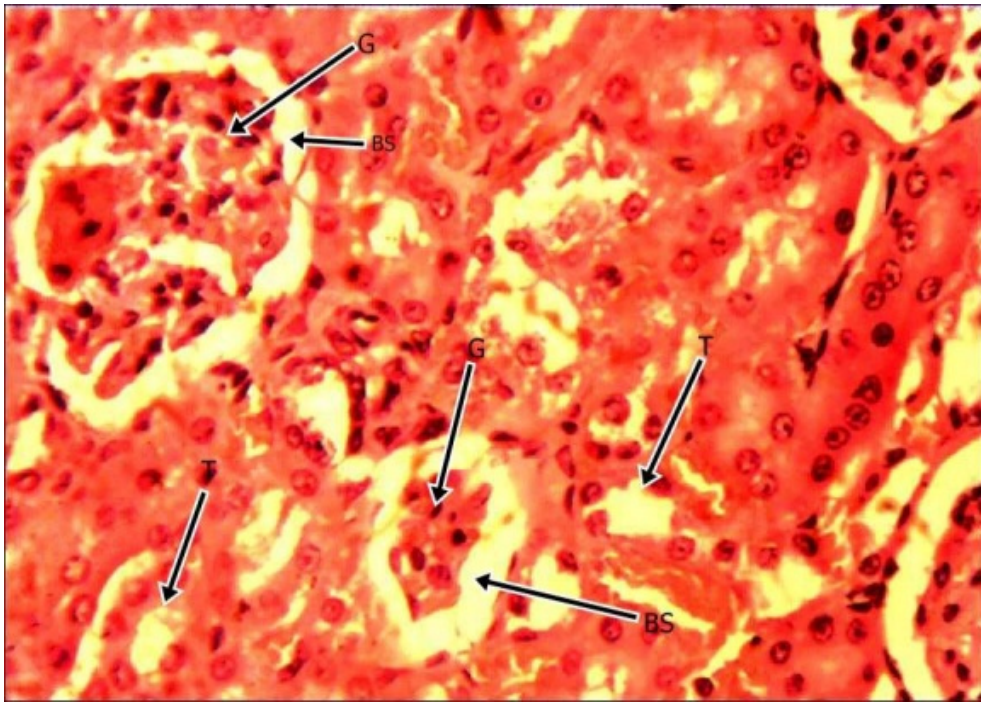

Plate 3. Photomicrograph of Group B (4000mg/kg) albino wistar rat kidney tissue (X400) treated with mustard seeds *(B. juncea)* extract. Glomeruli(G), Bowman's space(BS), Tubules(T).

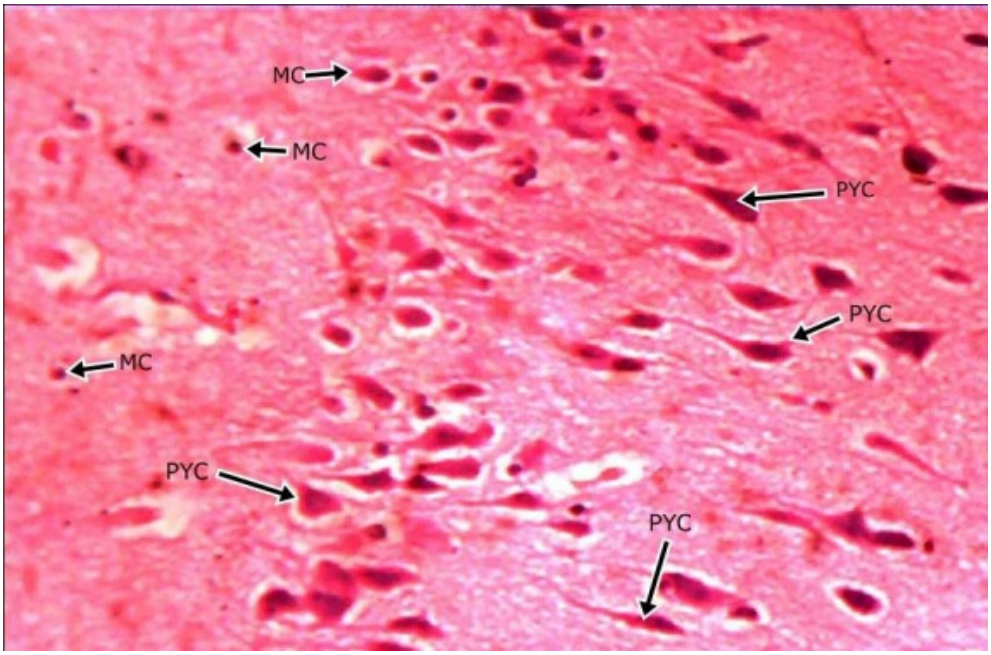

Plate 4. Photomicrograph of cerebrum of the control group of albino wistar rat (X400). Pyramidal cell bodies(PYC), Microglia cells(MC).

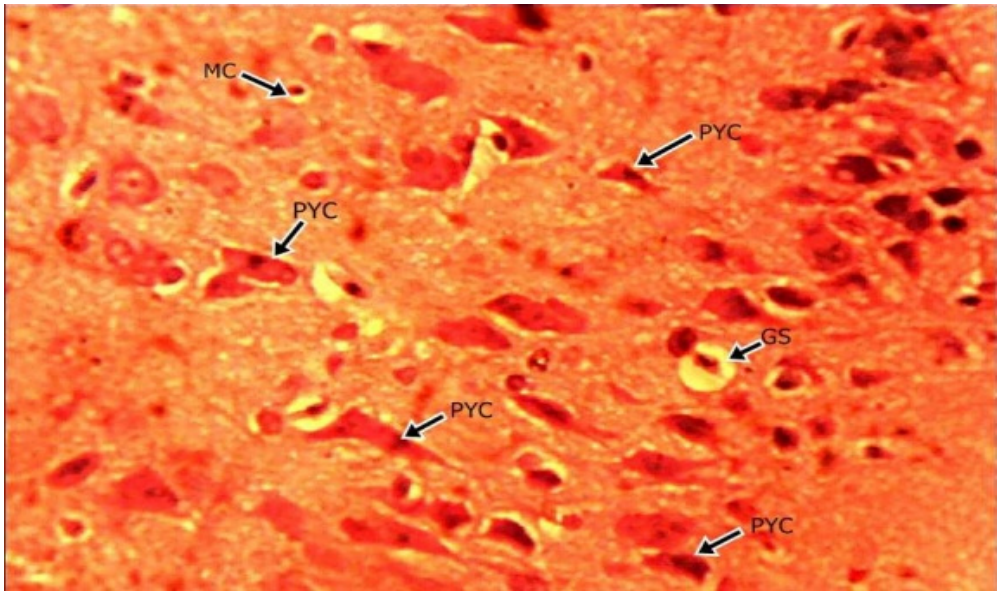

Plate 5. Photomicrograph of cerebrum of Group A (2000mg/kg) albino wistar rat (X400) treated with mustard seed *(B.juncea)* extract. Pyramidal cell bodies(PYC), Microglia cells(MC), Gliosis(GS).

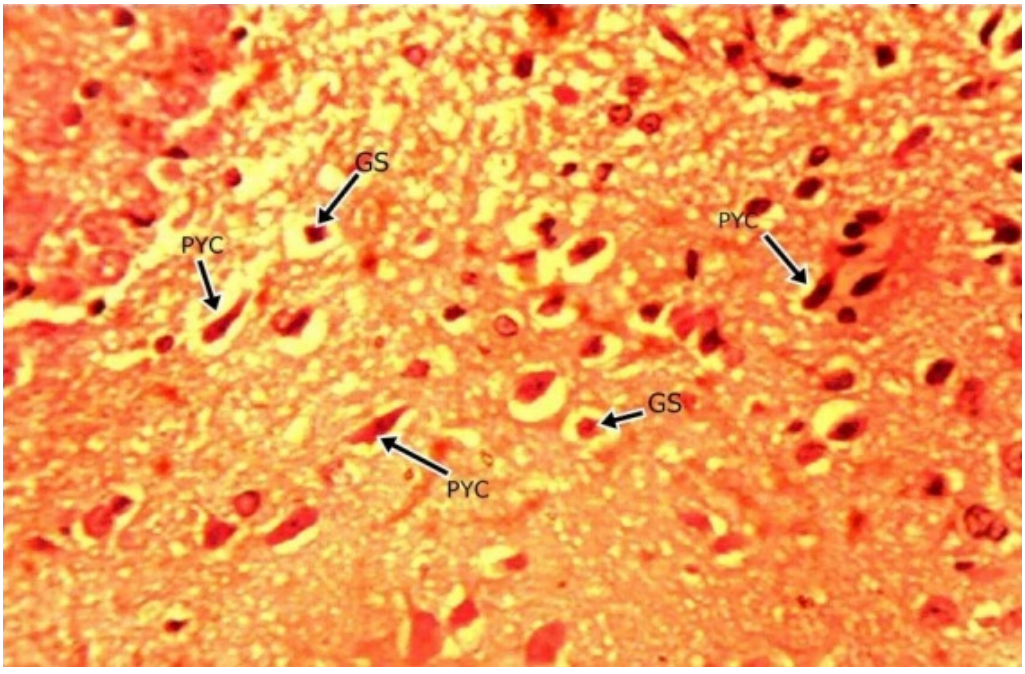

Plate 6. Photomicrograph of cerebrum of Group B (4000mg/kg) albino wistar rat (X400) treated with mustard seed *(B. juncea)* extract. Pyramidal cell bodies(PYC), Microglia cells(MC), Gliosis(GS).