Official Publication of the Faculty of Basic Medical Sciences University of Ibadan, Nigeria

Arch. Bas. App. Med. 4 (2016) 87 –94
www.archivesbamui.com

ARCHIVES OF BASIC & APPLIED MEDICINE

Research Article

Alpha-2 adrenergic and Cyclo-Oxygenase Mechanisms in Lipopolysaccharide-induced neuropathic pain in Rats

Onasanwo S.A., Edesiri. T.P., Adebimpe-John E.O

Department of Physiology, College of Medicine, University of Ibadan, Ibadan, Nigeria

Accepted in Revised form: March 2016

Abstract

The mechanism by which tissue injuries produces a state of neuropathic pain represent one of the most intensely investigated areas in the biomedical science over several cascades. Hence, a substantial body of evidence gathered from a variety of disease points to a critical interaction between the sympathetic nervous and the inflammatory cascade. The present study explored the probable action of the synergistic effects of adrenergic neurotransmission and cyclo-oxygenase inhibition in lipopolysaccharide-induced neuropathic pain in male Wistar rats. Wistar rats (225–250) g was used for this study. Neuropathic pain was induced with the systematic administration of 250µg/mg lipopolysaccharide (LPS). The effects of sulindac sulfide, yohimbine, and naphazoline on the movement were accessed using the Morris water maze model. Histological analysis using Nissl staining techniques and biochemical assay were evaluated. Peripheral administration of LPS was showed to reduce cognitive and locomotor process. The result of the LPS only showed a significant decreased in protein, GSH, SOD concentration and increased in MDA level when compared to control. However, drugs co-administered were shown to significantly ameliorate the decrease in antioxidant defense. Histology shows that the damage caused by LPS to the brain neurons was markedly reduced by the administration of the synergistic action of the adrenergic receptor pathway and COX inhibition pathway. These findings suggest that the synergistic action of both pathways might be beneficial in the management of neuropathic pain.

Keyword: *Neuropathic Pain, Adrenergic Neurotransmission, Cyclo-Oxygenase, Lipopolysaccharide, Nissl Staining.*

*INTRODUCTION

Neuropathic pain generated by disorders of the peripheral and central nervous system, can be particularly severe and disabling. Prevalence estimate indicates that 2% to 3% of the population in the developed world suffers from neuropathic pain. The International Association for the Study of Pain (IASP) defines neuropathic pain to be initiated or caused by a primary lesion or dysfunction in the nervous system and due to disorder peripheral or central nerves (Merskey and Bogdug, 1994). Neuropathic pain was used to describe only pain related to peripheral neuropathies, and central pain (CP) to lesions of the central nervous system associated with pain. Neurogenic pain embraced all causes, both peripheral and central. However, evidence based guideline for the pharmacological management of neuropathic pain may be needed.

The multimodal approaches to the management of neuropathic pain: the role of topical analgesia have been reviewed (Oscar, 2007), Although, the role of COX-2 in inflammatory diseases has been confirmed in both animal models and in humans (Taylor, 1999; Clemett and Goa, 2000; Bianchi and Broggini, 2002; Broom et al., 2004); its role in neuropathic pain is yet to be properly defined. There have been several lines of experimental evidence to suggest that the up-regulation of COX-2 mRNA and protein occurs in injured human and rat nervous system tissues in multiple neuropathic pain models (Ma and Eisenach, 2002; Durrenberger et al., 2004; Takahashi et al.,2004). However, the efficacy of COX-2 inhibitors in preclinical studies of neuropathic hypersensitivity remains equivocal (Lashbrook et al., 1999; Broom et al., 2004; Padi and Kulkarni, 2004), and no randomised, placebo-controlled studies of COX-2 inhibitors in clinical neuropathic pain have been reported. However, it has been reported that COX-2 may indeed play an important role in the maintenance of neuropathic pain following nerve injury (Zhao et al., 2007), but only certain COX-2 inhibitors, such as GW406381, are effective in this paradigm. Evidence based guideline for the pharmacological management of neuropathic pain may be needed. Yohimbine has been reported to produce antinociception they demonstrated that yohimbine produces dose-related antinociception in formalin test in rats which is mediated in part by agonistic action at 5-HT1A receptor (Shannon and Lutz., 2000).

Chronic neuroinflammation can be produced by infusing Lipolysaccharide (LPS) into the fourth ventricle of young rats resulting in the activation of microglia within the hippocampus, piriform and entorhinal cortices (Hauss-Wegrzyniak *et al.*, 1999). Lipolysaccharide selectively binds to a signal-transduction receptor complex (CD14/toll-like receptor 4) that is expressed only by microglia (Lehnardt *et al.*, 2003). By activated microglia through LPS infusion, young rats can show pathological, biochemical and behavioural

**Author for Correspondience:* + 234 8060711936, *E-mail:* samphil2002@yahoo.com;

changes that are similar to those observed in several neurodegenerative diseases associated with neuroinflammation. These include impaired spatial memory, reduction of N-Methyl-D-aspartate receptor1 (NMDAR1), astrocytosis, elevated cytokines and pro-inflammatory transcription factors (Hauss-Wegrzyniak *et al.,* 1998; Rosi *et al.*, 2004, 2005).

The mechanism by which tissue injuries produces a state of neuropathic pain represent one of the most intensely investigated areas in the biomedical science over several cascades. Hence, a substantial body of evidence gathered from a variety of disease points to a critical interaction between the sympathetic nervous and the inflammatory cascade. In the recent years, the pharmacological industry has shown considerable interest in new drugs originating from natural sources. Chronic neuroinflammation is implicated in several neurodegenerative diseases, such as Alzheimers disease (AD), traumatic brain injury, autism, Down syndrome, HIV-dementia, and demyelinating, such as multiple sclerosis and amyotrophic lateral sclerosis and many contributes to learning and memory deficits associated with these diseases. Though much study has been reported separately on the role of adrenergic pathway and COX inhibitor pathway in the modulation of pain, there has not been notable research work, to the best of our knowledge, on the central synergistic activity of adrenergic pathway and cyclo-oxygenase (COX) inhibition pathway in the modulation of LPS-induced neuropathic pain in male Wistar. rats.

Hence, this research study is aimed at determining the probable central synergistic activity of adrenergic pathway and cyclo-oxygenase (COX) pathway in the modulation of LPS-induced neuropathic pain in male Wistar rats.

The objectives of this study are to determine: (i) The synergistic effects of adrenergic receptor and COX inhibitor on the neurobehavioral indices or response (such as escape latency time, distance covered and swimming speed) of LPS-induced neuropathic pain in male Wistar rats using Morris water maize test model. (ii) The synergistic effects of adrenergic receptors and COX inhibition on the histology of the brain tissues of LPS-induced neurons damages in male Wistar rats using Nissl staining techniques. (iii) The synergistic effects of adrenergic receptor and COX inhibition on the brain tissues of LPS-induced neurons damages in male rats using biochemical assay.

METHODOLOGY

Chemicals and reagents: The following drugs and chemicals were used all purchased from Sigma-Andrich: Yohimbine**,** Naphazoline**,** Sulindac sulphide and lipopolysaccharide. Formalin was sourced locally, and of analytical grade.

Choice of animals: Male Wistar rats weighing 220-250g were used in this study, and were given access to water to food and water *ad libitum*. Experimental procedures were carried out in strict compliance with the ethical guidelines for investigation of experimental animal.

Animals were divided into sixteen groups of six rats per group, and pre-treated with distilled water (negative control) orally, lipoplyssacharide only (positive control, i.p.), naphazoline (0.2mg/kg, i.p.), yohimbine (0.2mg/kg, i.p.) and sulindac sulfide (100µg/kg, p.o.) until the seventh day when neuropathic pain was induced with lipopolysacchide (250µg/kg, i.p.) for four hours. Also, Neuropathic pain was induced with Lipopolysacchide (LPS) (250µg/kg) and after four (4) hours were post treated with varying dose of distilled water (negative control) given orally, LPS only (positive control) given intraperitoneally, Naphazoline (0.2mg/kg) given intraperitoneally, Yohimbine (0.2mg/kg) intraperitoneally, and Sulindac sulfide (100µg/kg) given orally until the seventh (7th) day.

Behavioural test

Morris Water Maze Test: The water maze test was performed as described by Morris and co-workers (Morris, 1984). A circular pool (height: 70cm, diameter: 122cm) was filled with water, made opaque by dissolving food colorings and maintained at 22~25°C. An escape platform (height: 14.5 cm, diameter: 4.5 cm) was then submerged 0.5~1 cm below the surface of the water in the north-eastern quadrant of the pool. On training trials, the rats were placed in the pool of water and allowed to remain on the platform for 10sec and were then returned to their cage during the second-trial interval. The rats that did not find the platform within 60s were placed on the platform for 10 s at the end of trial. 24hrs after 6 trials (two times per day for 3 days), rats will be given LPS. Four hours after the treatment of LPS (designated as day 1), they were allowed to swim until they sought the escape platform. Escape latency, escape distance, swimming speed and swimming pattern of each rats will be monitored for 3 days (1 time/day) by a camera above the center of the pool connected to a SMART-LD program, with slight modification**.**

Histomophometric analyses using Nissl staining: The histological analysis was performed by method described by Nissl and co-workers (Nissl, 1894). The Nissl-staining method is based on the interaction of basic dyes such as cresyl violet, thionine, toluidin blue, methylen blue or anylin with the nucleic acid content of cells. These dyes can bind to the DNA content of the cell nuclei, but also to the RNA (Scott and Willett, 1966) that is highly concentrated in rough endoplasmic reticulum and ribosomes (Nissl substance) in the cytoplasm. Since neurons are very active protein synthesizing cells, the cytoplasm of these cells contain high concentration of rough endoplasmic reticulum (Knowles *et al.*, 1996; Kosik and Krichevsky, 2002). Due to this special characteristic of neurons, the Nissl staining can specifically stain the cytoplasm of neurons without recognizing the perikarya of other cellular elements in the brain.

Biochemical Analysis: Rats were humanely sacrificed through cervical dislocation and the brain were collected and homogenized. Each brain tissue homogenate was centrifuged at 10,000g for 10 minutes at 4^0C, the pellet was discarded and the supernatant was immediately separated into different eppendorfs for the different biochemical assays. The Protein concentrations of various samples were determined by Lowry et al (1951) using Bovine Serum Albulmin as a standard. A breakdown product of lipid peroxidation thiobarbituric acid reactive substances (TBARS) was asayed as malondialdehyde (MDA) and measured according to the method of Buege and Aust (1978). The method of Jollow et al (1974) was followed in estimating the level of the reduced gluthatione (GSH) in the Brain post mitochondrial supernatant and the levels of total SOD activity in the tissues were determined by the method of Misra and Fridovich (1972).

RESULTS

Effects of sulindac sulfide, yohimbine, and naphazoline on lipopolysaccharide-induced neuropathic pain on cognitive deficits in rodent learning and memory tasks.

The result of the acquisition training (Tables 1 and 2 visible platform trial) of six trials (two times per day) for 3 days to measure the latency to mount the platform (escape latency) and distance across the platform (path-length) showed a gradual reduction in percent time along the days between the groups during the training trail. The results were averaged across two trials per day for 3days. The result of the probe trial (Tables 3 and 4), non-visible platform trial) to measure the latency to locate the platform (platform cross) showed a gradual increase in the lipopolysaccharide-treated group (positive control) when compared to other groups that has been pre-treated with sulindac sulfide yohimbine, and naphazoline that had a reduction the escape latency along the experimental days. The path-length to reach the platform (swimming distance) showed a gradual reduction in percent time along the days between the groups during the experimental period. The LPS-treated group travelled a further distance to reach the platform than the control group did.

Effects of sulindac sulfide, yohimbine, and naphazoline on single dose of lipopolysaccharides-induced neuropathic pain on SOD level, MDA and in Gluthatione

We examined the effect of pre- treatment and post-treatment with sulindac sulfide, yohimbine, and naphazoline on the SOD level MDA and GSH level on LPS-induced neuropathic pain. As shown in Tables 5 and 6, treatment with sulindac sulfide and yohimbine) and (sulindac sulfide, yohimbine and naphazoline) inhibited LPS induced neuropathic pain. This results shows that neuropathic pain was stimulated by a significant reduction in the level of the reduced glutathione and SOD. The synergistic effects of the drug (sulindac sulfide and yohimbine) and (sulindac sulfide, yohimbine and naphazoline) treatment significantly reduced lipid peroxidation and regenerated intracellular GSH and SOD content in the brain neurons. In the case of negative control animals (LPS group) which were significantly increased.

DISCUSSION

Degeneration of nerves and inflammation, is seen as a direct effect following nerve injury, and may play an important role in the early stages of the development of neuropathic pain states (Wu *et al.*, 2002; Ma and Eisenach, 2002). In this research study, we checked the synergistic effect of combined therapy of 3 standard drugs (sulindac sulfide, yohimbine, and naphazoline) in the elevation of neuropathic pain. Injured peripheral nerves tend to express adrenergic sensitivity; while sympathetic agonists may increase the ectopic impulses generated from these types of efferent axons (Galer, 2001). The sympathetic nerve terminals containing alpha-2 receptors are shown to be inhibited from releasing norepinephrine upon adrenergic agonist binding, potentially resulting in a reduction in pain and allodynia (Galer, 2001; Davis *et al.*, 1991).

Table 1:
Effects of sulindac sulfide, yohimbine, and naphazoline on single dose of LPS-induced (250µg/kg) neuropathic pain on the escape time distance and swimming speed in brain, following pre-treatment for 7 days.

Parameters/Treatment	Escape Latency Time (sec)	Distance Covered (cm)	Swimming Speed (m/s)
Control	14.8 ± 0.401	208 ± 19.0	14.2 ± 1.45
LPS ONLY	55.0 ± 3.12	1099 ± 137***	32.0 ± 2.29***
SDS	31.7 ± 5.35	597 ± 92.9	21.0 ± 1.18##
YMB	37.2 ± 5.96	589 ± 77.7	19.5 ± 7.23##
NPZ	32.2 ± 2.96	643 ± 123#	23.0 ± 2.93*##
SDS + YMB	27.5 ± 7.92	589 ± 161##	19.3 ± 1.15##
SDS + NPZ	38.8 ± 4.84	794 ± 134**	20.2 ± 2.23##
SDS + YMB + NPZ	24.8 ± 3.15	506 ± 442##	18.5 ± 138###

*Data are presented as the mean ± S.D. p value < 0.0001(n = 6 to 8 groups)**.** Statistical analyses were performed by one-way ANOVA test, followed by Student Newman-Keuls test for multiple comparisons.***p≤0.001, **p≤0.01 when compared with control, ###p≤0.001 when compared with LPS*

Table 2: Effects of sulindac sulfide, yohimbine, and naphazoline on single dose of LPS (250µg/kg) -induced neuropathic pain on the escape time distance and swimming speed in brain, following post-treatment for 7 days.

Treatment/ Parameters	Escape Latency Time(sec)	Distance Covered(cm)	Swimming Speed (m/s)
CONTROL	96.2 ± 0792	182 ± 20.7	11.8 ± 1.96
LPS ONLY	50.8 ± 2.96***	1139 ± 739***	34.8 ± 3.51***
SDS	32.2 ± 3.03*#a	705 ± 125**#	22.0 ± 2.88##
YMB	31.8 ± 3.23*#a	705 ± 125*##	21.8 ± 3.09##
NPZ	35.7 ± 5.23 ac*#	679 ± 112*#	22.5 ± 1.43##
SDS + YMB	21.0 ± 4.16***	455 ± 83.3###	19.0 ± 2.78##
SDS + NPZ	26.3 ± 176##	595 ± 81.6##	21.3 ± 2.78##
SDS + YMB + NPZ	19.5 ± 0.671###	364 ± 80.4###	16.8 ± 291###

*Data are presented as the mean ± S.D. p < 0.0001(n = 6 to 8 groups) .Statistical analyses were performed by one-way ANOVA test, followed by Student Newman-Keuls test for multiple comparisons***p≤0.001, **p≤0.01 when compared with control, ###p≤0.001 when compared with LPS. [a]p≤0.05 when compared with SDS+YMB+NPZ, [b]p≤0.05 when compared with SDS+NPZ, [c]p≤0.05 when compared with SDS+YMB.*

Table 3: Effects of sulindac sulfide, yohimbine, and naphazoline on single dose of LPS-induced (250µg/kg) neuropathic pain on the escape time distance and swimming speed in brain, following pre-treatment for 7 days.

Parameters/Treatment	Escape Latency Time (sec)	Distance Covered (cm)	Swimming Speed (m/s)
CONTROL	10.0 ± 0577	651 ± 113	20.5 ± 0.719
LPS ONLY	52.7 ± 3.40***	1115 ± 136*	45.5 ± 4.99***
SDS	29.0 ± 7.76ac#	521 ± 119#	21.5 ± 1.95###
YMB	25.0 ± 5.05ac#	625 ± 152#	21.5 ± 2.08###
NPZ	38.7 ± 6.11abc#	760 ± 89.8#	31.7 ± 3.47##
SDS + YMB	19.2 ± 2.43###	404 ± 67.1##	20.5 ± 0.719###
SDS + NPZ	25.0 ± 5.05##	521 ± 119#	21.5 ± 2.08###
SDS + YMB + NPZ	15.0 ± 1.00###	404 ± 67.1##	20.2 ± 27###

*Data are presented as the mean ± S.D. p value < 0.0001(n = 6 to 8 groups) .Statistical analyses were performed by one-way ANOVA test, followed by Student Newman-Keuls test for multiple comparisons.***p≤0.001, **p≤0.01 when compared with control, ###p≤0.001 when compared with LPS. [a]p≤0.05 when compared with SDS+YMB+NPZ, [b]p≤0.05 when compared with SDS+NPZ, [c]p≤0.05 when compared with SDS+YMB.*

Table 4: Effects of sulindac sulfide, yohimbine, and naphazoline on single dose of LPS (250µg/kg) -induced neuropathic pain on the escape time distance and swimming speed in brain, following Post-treatment for 7 days.

Parameters/Treatment	Escape Latency Time (sec)	Distance Covered (cm)	Swimming Speed (m/s)
CONTROL	12.7 ± 0.843	223 ± 27.3	9.00 ± 1.37
LPS ONLY	53.0 ± 2.11***	1141 ± 178***	30.8 ± 2.32***
SDS	26.2 ± 2.89abc*#	621 ± 61.1abc*#	24.0 ± 1.53*#
YMB	34.8 ± 6.94abc*#	562 ± 157abc*#	23.2 ± 1.56*#
NPZ	25.8 ± 1.97abc*#	755 ± 143abc*#	25.5 ± 32.77*
SDS + YMB	9.83 ± 14###	186 ± 21.4##	20.2 ± 1.46*#
SDS + NPZ	23.7 ± 5.70*#	215 ± 11.2##	22.0 ± 1.46*#
SDS + YMB + NPZ	9.50 ± 1.59###b	161 ± 12.2###	13.0 ± 1.15##

*Data are presented as the mean ± S.D. p < 0.0001(n = 6 to 8 groups) .Statistical analyses were performed by one-way ANOVA test, followed by Student Newman-Keuls test for multiple comparisons***p≤0.001, **p≤0.01 when compared with control, ###p≤0.001 when compared with LPS. [a]p≤0.05 when compared with SDS+YMB+NPZ, [b]p≤0.05 when compared with SDS+NPZ, [c]p≤0.05 when compared with SDS+YMB.*

Table 5: Effects of sulindac sulfide, yohimbine, and naphazoline on single dose of LPS (250µg/kg) -induced neuropathic pain on GSH, SOD and MDA levels in the brain, following pre-treatment for 7 days.

Groups	Protein Assay	MDA	GSH	SOD
CONTROL	1.40 ±0.26	2.20 ±0.07	8.16 ±0.03	1.47 ±0.02
LPS ONLY	1.03 ±0.01	4.79 ±0.24	5.10 ±0.46	0.60 ±0.05
SDS	1.03 ±0.02	4.09 ±0.37	7.91 ± 0.69	1.18 ±0.02
YMB	10.3 ±0.01	4.05 ±0.42	6.74 ±0.41	1.02 ±0.01
NPZ	1.02 ±0.003	3.91 ±0.42	5.89 ±0.33	1.13±0.06
SDS+YMB	1.03 ±0.02	3.98 ±0.41	8.04 ±0.01	1.41 ±0.01
SDS+NPZ	1.03 ±0.01	3.98 ±0.29	7.52 ±0.51	1.25 ±0.07
SDS+YMB+NPZ	1.16 ±0.005	3.74 ±0.35	9.57 ±0.15	1.41±0.01

Data are presented as the mean ± S.D. p value < 0.0001(n = 6 to 8 groups) .Statistical analyses were performed by one-way ANOVA test, followed by Student Newman-Keuls test for multiple comparisons.

Table 6: Effects of Sulindac sulfide, Yohimbine, and Naphazoline on single dose of LPS (250µg/kg) -induced neuropathic pain on GSH SOD level and MDA levels in brain, following post treatment for 7days in wistar rats

GROUPS	PROTEIN ASSAY	MDA	GSH	SOD
CONTROL	1.53 ± 0.02	2.07 ± 0.11	9.16 ± 0.04	1.53 ± 0.02
LPS ONLY	0.62 ± 0.08***	6.40 ± 0.42***	5.85 ± 0.58***	0.70 ± 0.05***
SDS	1.02 ± 0.002**abc	3.80 ± 0.54##*	7.86 ± 0.70a#*	1.21 ± 0.04### *** ac
YMB	1.02 ± 0.003#*abc	4.25 ± 0.14##**	6.743 ± 0.41*abc	1.04 ± 0.02### ***abc
NPZ	1.01 ± 0.002#*abc	3.35 ± o.153*###	6.38 ± 0.09*abc	1.13 ± 0.06### ***ac
SDS+YMB	1.39 ± 0.05###	3.13 ± 0.32###	8.29 ± 0.25a#	1.46 ± 0.03###
SDS+NPZ	1.27 ± 0.01###a	3.19 ± 0.72###	7.92 ± 0.33a#	1.28 ± 0.09###a
SDS+YMB+NPZ	1.50 ± 0.03###	2.87 ± 0.29###	10.60 ± 0.15###	1.15 ± 0.06###

Data are presented as the mean ± S.D. p value < 0.0001(n = 6 to 8 groups)**.** Statistical analyses were performed by one-way ANOVA test, followed by Student Newman-Keuls test for multiple comparisons. ***p≤0.001, **p≤0.01 when compared with control, ###p≤0.001 when compared with LPS. [a]p≤0.05 when compared with SDS+YMB+NPZ, [b]p≤0.05 when compared with SDS+NPZ, [c]p≤0.05 when compared with SDS+YMB.

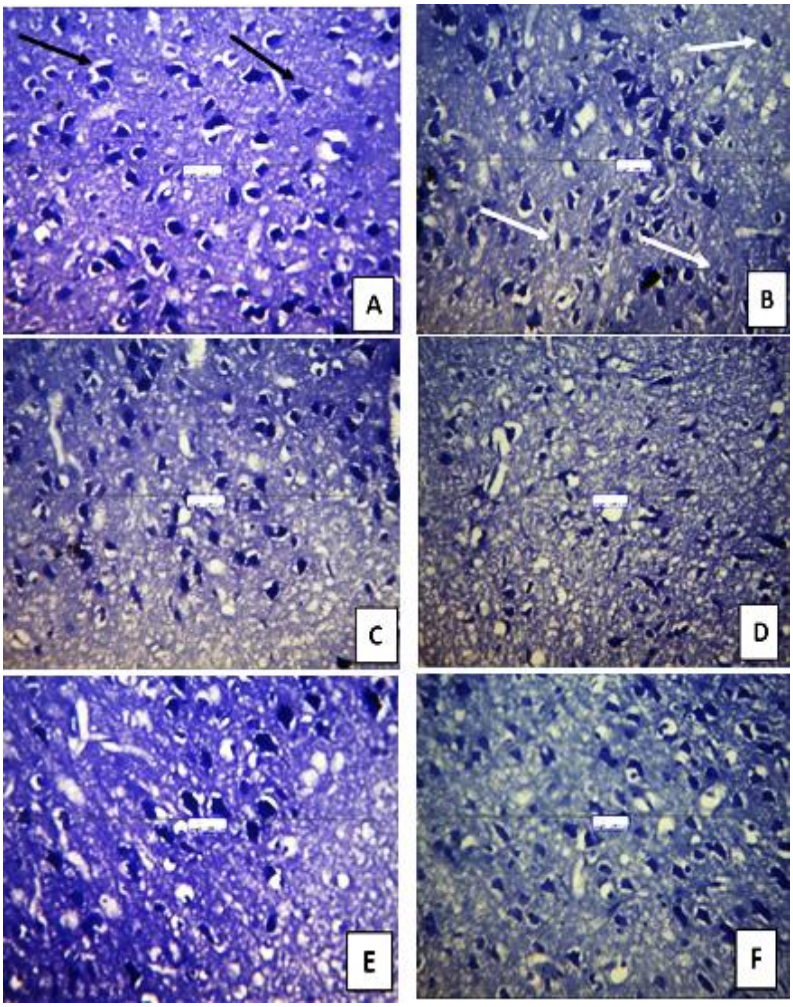

Plate 1: The effects of pre-treatment with varying concentrations of the drugs on the neurons using Nissl-staining. **A-**Photomicrograph of brain cerebral hemisphere of control (untreated) showing strong positive staining to nissl substance in normal distribution while the architecture appears normal (black arrows) during Nissl staining (X 400). **B**-Photomicrograph of brain cerebral hemisphere of lipopolysaccharide-treated showing sharp edges of axons degeneration and about 26 increased numbers of neurons damages per unit area was seen. However, the focal area of pale staining (white arrows) shows degenerating pyramidal cell during Nissl staining (X400). **C**-Photomicrographs of brain cerebral hemisphere of sulindac sulfide-treated showing strong positive staining to Nissl substance in normal distribution, and the architecture in the pre-treatment group shows 19% increase of neuronal damages. **D**-Photomicrographs of brain cerebral hemisphere of yohimbine-treated showing strong positive staining to Nissl substance in normal distribution, and the architecture in the pre-treatment group shows 19% increase of neuronal damages **E**-Photomicrographs of brain cerebral hemisphere of naphazoline-treated showing strong positive staining to Nissl substance in normal distribution, and the architecture in the pre-treatment group shows 22% increase of neuronal damages **F**-Photomicrographs of brain cerebral hemisphere treated with sulindac sulfide and yohimbine showing strong positive staining to Nissl substance in normal distribution, and the architecture in the pre-treatment group shows 14% increase of neuronal damages

Most likely, the pain relief mechanism also involves the hyperpolarization of nicotinic ganglia by alpha-2 adrenergic stimulants (Walland 1984). There have been several lines of experimental evidence that suggested the up-regulation of COX-2 mRNA and protein in an injured human and rat nervous system tissues in multiple neuropathic pain models (Ma and Eisenach, 2002; Durrenberger *et al.,* 2004; Takahashi *et al.*,2004). However, it has been reported that COX-2 may indeed play an important role in the maintenance of neuropathic pain following nerve injury (Fei-Yue *et al.,* 2007), but that only certain COX-2 inhibitors (like GW406381) is effective in this paradigm. Morris water maize model was used as the main model. This neuropathic pain induced in this experimental work is in agreement with previous work as reported (Bastos *et al.,* 2013; Karen *et al.,* 2012) in which lipopolysaccharide was used to induce neuropathic and inflammatory pain models. The involvement of the probable action of the probable synergistic action of the adrenergic receptor agent and the cyclooxygenase inhibitor used in this study was to ascertain the dynamic mechanistic pathways of alleviating neuropathic pain. It has been reported that the adrenergic sensitivity of cutaneous nociceptors develops as early as 7 days after partial nerve injury (Sato et al., 1991).

Thus, the increased sensitivity of cutaneous nociceptors to norepinephrine may contribute to the hyperalgesia in the early stages of the nerve injury (Tracey et al.,1995). Much later, alpha-2 adrenergic receptor will be expressed in the dorsal root ganglion cells which will induce norepinephrine sensitivity and possibly contribute to hyperalgesia and ongoing pain. This effect was observed and blocked by yohimbine and more effective in the combination.

In this study, rats were tested for escaped latency time, distance covered, and swimming speed. And this Morris water maize procedure used was able to test learning and short term memory of the rats. The more efficiency of (sulindac sulfide+yohimbine+naphazoline) was associated with a highly significantly shorter distance covered, swimming speed and escape latency time and thus, improving the neurological function which has been significantly impared by the intraperitoneally induced LPS. The results suggest that the combination of this drug (sulindac sulfide +yohimbine+naphazoline) could be useful in the management of neuropathic pain in human considering its excellent safety profile. In addition, the synergistic interaction between the drugs observed in the pre-treatment group could be suggested to build the immunological cells of the brain against endogenous pyrogens.

Also, considering this observation, it could be suggested that the synergistic interaction of the two (sulindac sulfide+yohimbine) and the three (sulindac sulfide+yohimbine+naphazoline) combined drugs shows an efficacy treatment reduction in neuropathic pain and this could probably be by stimulating the specific area of the dorsal horn of the spinal cord and could also be by affecting the amydala, hypothalamus and emotional modulation area of the brain as reported by various researchers in the field of neurophysiological sciences.

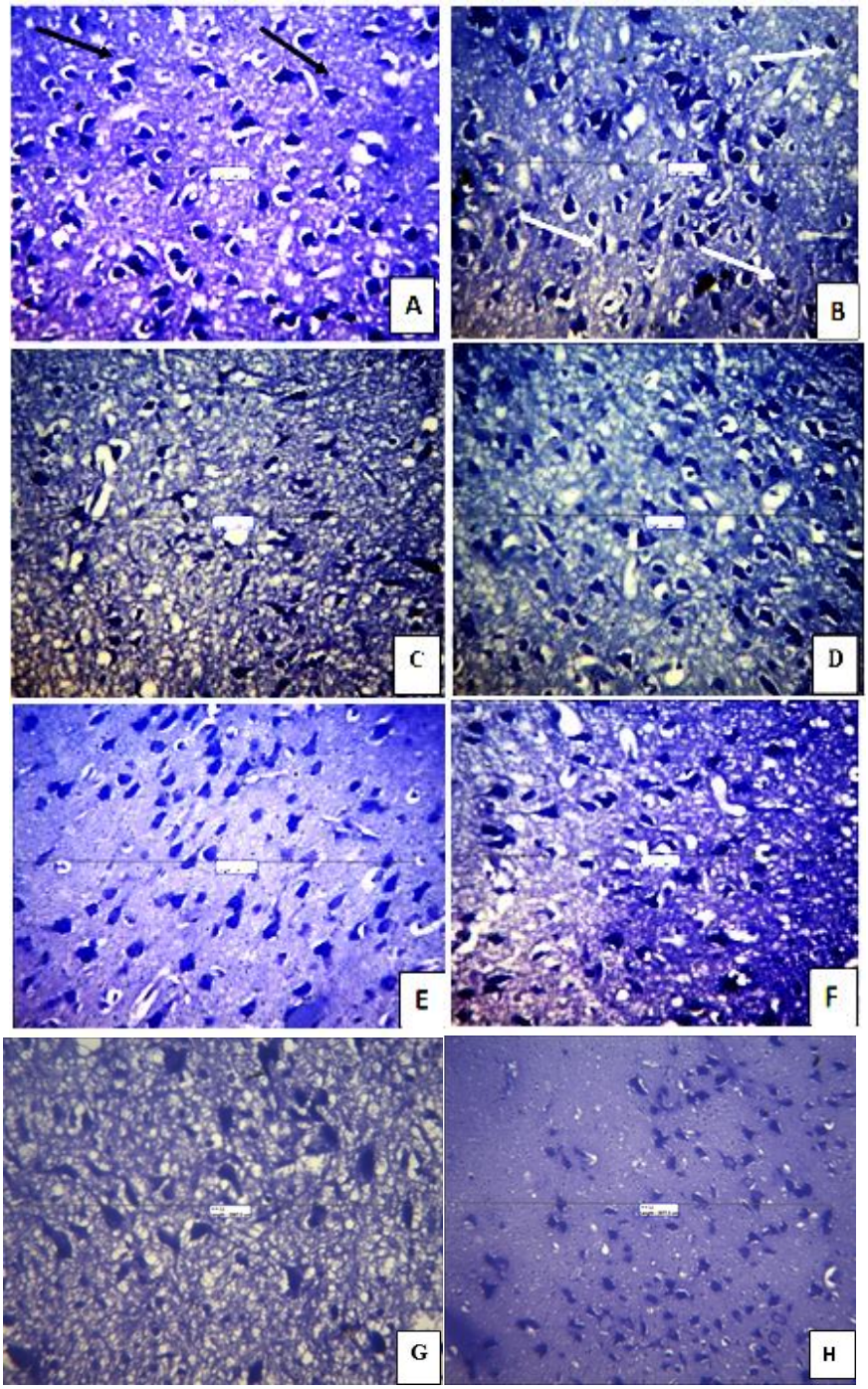

Plate 2
Effect of post-treatment with varying concentrations of the drugs on the neurons using Nissl-staining. **A-**Photomicrograph of brain cerebral hemisphere of control (untreated) showing strong positive staining to nissl substance in normal distribution while the architecture appears normal (black arrows) during Nissl staining (X 400). **B-** brain cerebral hemisphere of lipopolysaccharide-treated showing sharp edges of axons degeneration and about 26 increased numbers of neurons damages per unit area was seen. However, the focal area of pale staining (white arrows) shows degenerating pyramidal cell during Nissl staining (X 400). **C-** brain cerebral hemisphere of **sulindac sulfide-treated** showing strong positive staining to Nissl substance in normal distribution, and the histoarchitecture in the post-treatment, it shows 13% increase of neuronal damages during Nissl staining (X 400).. **D-** brain cerebral hemisphere of **yohimbine-treated** showing strong positive staining to Nissl substance in normal distribution, and the architecture in the post-treatment group shows 13%increase of neuronal damages during Nissl staining (X 400). **E-** Photomicrographs of brain cerebral hemisphere of **naphazoline-treated** showing strong positive staining to Nissl substance in normal distribution, and the architecture in the post-treatment, shows 14% increase of neuronal damages during Nissl staining (X 400). **F-**Photomicrographs of brain cerebral hemisphere treated with **sulindac sulfide and yohimbine** showing strong positive staining to Nissl substance in normal distribution, and the architecture in the post-treatment, shows 10% decrease of neuronal damages during Nissl staining (X 400). **G & H-** brain cerebral hemisphere treated with **sulindac sulfide and naphazoline** showing strong positive staining to Nissl substance in normal distribution, and the architecture in the post-treatment, it shows 14% decrease of neuronal damages during Nissl staining (X 400).

This results support the concept that the activation of noradrenergic bulbospinal neuron through the antangonistic effects of yohimbine and COX pathway inhibitor mediates stimulation produced anti-nociceptive (SPA) evoke from the nucleus raphe magnus (NRM) and the nucleus reticularis paragigantocellularis (NRPG) of LPS-induced neuropathic pain in male wistar rats. Additionally, it also suggests that the noradrenergic component involves an alpha-2 noradrenergic receptor. However, from the results, it was shown that the standard drugs (naphazoline, yohimbine, and sulindac sulfide) administered was able to alleviate the neuropathic pain induced by the inflammatory model (lipopolysacchacharide) but not to the level of the co-administered drugs (i.e in the combined drugs therapy). The co-administration of (yohimbine+sulindac sulphide) and (yohimbine+sulindac sulphide+naphazoline) was more potent as observed from the study to antagonize the increased neuropathic pain produced by the intraperitoneally induced LPS.

In the biochemical analysis of brain tissues, it is well established that oxidative stress plays a major role in age-related neurodegenerated disease (Anderson *et al.,* 2004). Thus, the brain is among the major organs generating large amounts of reactive oxygen species and is especially susceptible to oxidative stress. Glutathione (GSH) and superoxide dismutase (SOD) are some of the major antioxidant involved in neuroprotective fro oxidative stress offers protection from highly reactive superoxide anions and convert them to H_2O_2. From this study, the enzymatic and non-enzymatic activities (GSH, SOD, and Protein concentration) were observed to be significantly decreased in LPS-induced rats. This depletion could be suggested to be probably as a result of phospholipase (A2) dependent release of Arachidonic

acids, which may cause damage to the GSH depleted cells through its metabolism by lipooxygenase.

However, drugs administered were shown to significantly ameliorate the decrease in antioxidant defense. The results clearly demonstrate a protective synergistic effects of the 2 combined drugs (sulindac sulfide and yohimbine) and the 3 combined drugs (sulindac sulfide, yohimbine and naphazoline) combined drugs and its mediated through attenuation of oxidative stress, thus suggesting a therapeutic potential of the combined drugs in the management of neuropathic pain.

Malondialdehyde (MDA) is one of the most frequently used indicators of Lipid Peroxidation (LPX) processes. This study shows a statistically significantly increase in the MDA level during the course of this study, thus, suggesting an induced oxidative stress by LPS-induced neuropathic pain. The synergistic effects of the drug (sulindac sulfide and yohimbine) and (sulindac sulfide, yohimbine and naphazoline) treatment significantly reduced lipid peroxidation and regenerated intracellular GSH content in the brain neurons. It is also worthy to note that the reduction observed in GSH and SOD in lps-induced wistar rats might be a reflection of the decreased in protein concentration observed in this study as GSH groups play a critical role in enzyme catalysis.

In the histomorphometric photomicrograph of the brain tissues, it has been known that histological changes in the brain are the histological bases of changes in the neurophysiological functions. Furthermore, normal brain structure and the maintenance of its internal microenvironment are important for normal neurological function in terms of neuro-behavioural responses or management. Thus, the histomorphometric photomicrograph of the brain neurons shows a normal histoarchitecture in the control group and decerased in neuronal damages in the pre and post-treatement group when compared to the LPS-induced neuropathic pain alone.

From the results obtained, it shows that the brain neuronal process was improved in the pre- and post-treatment groups when compared to LPS only. More improvement was observed in the (sulindac sulfide+yohimbine) and (sulindac sulfide+yohimbine+naphazoline) group when compared with LPS only.

In conclusion, this present study support previous findings on the anti-nociceptive activities of alpha-1 and alpha-2-adrenergic receptors. It could be suggested herein that the dynamic mechanistic route through which neuropathic pain can be alleviated is basically through the synergistic action of the 3 combined standard drugs (sulindac sulfide, yohimbine, and naphazoline) administered. Results obtained from this present study revealed that the synergistic actions of the 3 combined drugs (sulindac sulfide, yohimbine and naphazoline) administered might have improved the neuropathic pain by reducing oxidative stress, through antioxidant potentials, in the LPS-induced neuropathic pain Wistar rats. Histological assessment also shows that the damage caused by LPS to the brain neurons was also markedly reduced by the administration of the synergistic action of the adrenergic receptor pathway and COX inhibitor pathway. Finally, these findings suggest that the synergistic action of both pathways might be beneficial in the management of neuropathic pain. However, further studies are needed to better understand the benefits of the synergistic effects of adrenergic neurotransmission and COX inhibitor pathway in the management of neuropathic pain

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