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Gabapentin and loperamide co-administration effect on the neuropathic pain in alloxan diabetic female albino mice

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ABSTRACT: The present study aimed to evaluate the effect of gabapentin-loperamide co-administration on induced diabetic neuropathic pain in female albino mice. The study involved 30 female albino mice, divided into six groups: Group A served as the negative group and received distilled water; Group B served as the positive group (induced diabetic neuropathic pain); for 14 days, Group C included induced mice that received solely oral gabapentin (2.5 mg/kg); Group D included induced mice that received gabapentin 2.5 mg/kg for 14 days orally and loperamide 0.05 mg/kg for 14 days orally. Group E includes induced mice treated with gabapentin 1.8 mg/kg for 14 days orally and loperamide 0.05 mg/kg for 14 days orally, Group F includes induced mice treated with gabapentin 1.2 mg/kg for 14 days orally and loperamide (0.05 mg/kg) for 14 days orally. Results illustrated that Group D showed a significant decrease in the levels of substance-p and interleukin-6 (10.92 ±0.74 and 165.8 ±8.53, respectively) in comparison with those levels in Group B (108.78 ±4.46 and 1083.34 ±76.35, respectively), which may be attributed to the increase in the analgesic effect of gabapentin due to the reduction in its elimination from the body by decreasing small intestine movement and increasing absorption, which led to keeping it in the body for a long time.

KEYWORDS: Diabetic neuropathy; gabapentin; interlukine-6; loperamide; substance P.

1. INTRODUCTION

Diabetes is a metabolic disorder characterized by elevated blood glucose levels due to various factors, such as deficiencies in insulin production, action, or both [1]. Consequently, cells fail to respond to insulin, preventing glucose absorption by the body's cells [2, 3]. As a complications for diabetes, blood arteries that nourish nerves may be damaged as a consequence of high blood glucose that prevents the nourishment of nerves [4]. Long-term consequences of diabetes include retinopathy, nephropathy, and peripheral neuropathy, which can result in amputations and foot ulcers [5]. A variety of clinical and subclinical symptoms affecting the peripheral nerve system (PNS) are associated with diabetic neuropathy (DN), a consequence of diabetes mellitus (DM) [6]. The International Association for the Study of Pain's (IASP) defines neuropathic pain (NP) as "pain caused by a lesion or disease of the somatosensory nervous system in humans." [7]. It was reported that high blood glucose and cholesterol levels lead to diabetic neuropathy, which damages nerves [8]. The pathologic process of diabetic neuropathy involves three key alterations: first, inflammation; second, activator protein 1, the principal activator of nuclear factor kappa B; and third, mitogen-activated protein kinases. Secondly, advanced glycosylation end-products, polyol, hexosamine, protein kinase C, and glycolysis all appear to be implicated in the oxidative stress brought on by hyperglycemia[9].

Gabapentin (GBP) is an γ-aminobutyric acid (GABA) structural analog [10]. It works by block Ca^{2+} and Na^{+} channels lead to no action potential and no nerve impulse. Furthermore, gabapentin inhibits exocytosis and the release of neurotransmitters from presynaptic terminals and used to treat central neuropathic pain and partial seizures [11]. Loperamide is a phenylpiperidine opioid that at therapeutic doses targeted primarily the intestinal mucosa's μ-opioid receptors in the myenteric plexus, it reduces intestinal transit time [12]. It was reported earlier that systemic and local (intraplantar) administration of

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loperamide alleviated mechanical allodynia (i.e., mechanical hypersensitivity to non-noxious tactile stimuli) during the development and maintenance phases of neuropathic pain in rats. A previous study suggested that the intraperitoneal or subcutaneous administration of loperamide also alleviated neuropathic heat hyperalgesia [13].

Substance P (SP), an 11-amino acid neuropeptide that is expressed by the immune system, peripheral nervous system, and central nervous system (CNS). Through its interactions with G protein-coupled neurokinin receptors (NKRs), SP elicits its biochemical effects [14]. Numerous cell types, such as immunological cells, fibroblasts, neurons, and endothelial cells of blood vessels and lymphatics, have these receptors on their surface [15]. SP functions by either the cAMP or IP3/DAG pathway via a G protein-coupled receptor, depending on the type of cell, SP facilitates the dorsal horn's transmission of pain sensations to the central nervous system [16, 17]. Acetylcholine and serotonin from post-synaptic neurons trigger smooth muscle contraction in the gastrointestinal system, which is enhanced by SP [18]. Interleukin-6 (IL-6) is an immune-regulatory cytokine that protects the host against injuries and infections, but prolonged IL-6 production dysregulation leads to the emergence of numerous illnesses [19]. IL-6 interferes with nociceptive transduction, conduction, and transmission, which contributes to the regulation of pain and has a significant role in the pathophysiology of pain and the physiology of nociception [20]. The present work aimed to assess the effect of co- administration of loperamide with gabapentin to increase its analgesic effect by measuring the levels of IL-6 and SP.

2. RESULTS AND DISCUSSION

2.1 Evaluation of SP

Results obtained in Table 1 revealed that the levels of SP were significantly increased ($p< 0.05$) in the group of mice receiving an alloxan for DM induction (group B), which was 108.78 ±4.46 pg/ml in comparison with the control group (group A), which showed a level of 8.42 ±0.57 pg/ml. Alloxan in the present study has been used to induce diabetes mellitus in experimental animals. It is well known for being a diabetogenic substance, meaning that it can be used to induce type I diabetes. Alloxan induces the pancreatic B-cell of the islet to selectively necrotize [21]. The levels of SP showed a significant reduction in the treated groups (C, D, E, and F) in comparison with the induced group. Additionally, significant differences were also obtained among the groups of mice that received treatments in that the level of SP in group F showed significantly higher levels than those of both groups C and E, which was further reduced significantly in group D compared with all other groups, as illustrated in Table 1. On the other hand, groups C showed no significant difference from group E in the levels of SP.

The previous studies revealed that the elevation in the SP levels can be considered one of the markers of diabetic neuropathic pain [22, 23], which is consistent with the results of the current study, which indicated that the increment in the levels of SP occurred due to damage to the nerve endings of the paw due to diabetes that caused neuropathic pain, which led to increased pain and that caused the release of SP from the peripheral terminals of sensory nerve fibers [24, 25].

The results demonstrated that the administration of gabapentin in groups C, D, E, and F showed a significant reduction in SP levels, which is in agreement with previous studies which demonstrated that the analgesic activity of gabapentin has been achieved by inhibiting calcium influx, which causes a reduction in the release of excitatory neurotransmitters such as glutamate and SP that participate in the pathway of pain signaling [26, 27]. Additionally, the analgesic effect of gabapentin that appeared in the reduction of SP levels showed to be increased with the increase in the dose used, which provided an explanation for the significant reduction in groups F, E and D, subsequentially.

The interesting results that were obtained in this study are the synergistic effect of loperamide on gabapentin that led to an analgesic effect with lower doses of gabapentin, as illustrated in Table 1. The levels of SP in group C that received 2.5 mg gabapentin were nearly similar to those in group E, in which mice received 1.8 mg gabapentin with 0.05 mg loperamide, which is confirmed statistically by the non-significant difference in SP levels between group C and E. Another piece of evidence that confirms the role of loperamide in improving the analgesic effect of gabapentin is the significant differences in the levels of SP between groups C and D, given that both groups received the same dose of gabapentin, and the only difference is the concomitant administration of loperamide with gabapentin in group D, which is assumed to be the possible cause of this significant difference.

Several studies reported that the effect of gabapentin improved when it was used concomitantly with loperamide, which may lead to a toxic effect [28, 29]. Loperamide alone has no analgesic property [30],

but it may affect the analgesia's efficiency and duration [31] and also suggested to has a potential effect in alleviating pain and controlling anxiety [32]. On the other hand, the route of administration used for administering gabapentin in the current study is the oral route, which is affected by the intestinal movement, and using loperamide, known to cause a decrease in the intestinal movement that allows for longer transit time [33] for gabapentin that may enhances its absorption and augments its analgesic activity [34], which is reflected by the significant reduction in SP levels in group D and causes the levels of SP to return to levels nearly similar to those of controls, which is confirmed by the non-significant difference in their levels between group A and D.

Table 1. Evaluation of SP in induced diabetic mice after 14day of being treated with Gaba at a dose and Gaba - loperamide combinations:

Groups	Sub-p (pg/ml)
Control group A /negative group	8.42 ±0.57 d
Group B/ Diabetic positive group	108.78 ±4.46 a
Group C/Diabetic Negative (Treated only with GBP 2.5mg)	21.5 ±1.07 c
Group D/ Diabetic Negative (Treated with GBP 2.5mg+ lop 0.0.5mg)	10.92 ±0.74 d
Group E /Diabetic Negative (Treated with GBP 1.8mg +lop0.05mg)	23.2 ±1.37 c
Group F/Diabetic Negative (Treated with GBP 1.2mg + lop 0.05mg)	34.14 ±2.08 b
LSD value	9.618 **
P-value	0.0001

GBP: Gabapentin, Means having with the different letters in column differed significantly, ** (P≤0.01).

2.2. Evaluation of IL-6:

With a pattern similar to that obtained with SP levels, IL-6 levels showed to be significantly increased ($p<0.05$) in induced group (1083.34 ±76.35 pg/ml) in comparison with controls (125.02 ±6.71 pg/ml) and these levels become reduced significantly with treatment by either gabapentin alone or by the combination of gabapentin and loperamide as illustrated in Table 2. Several studies showed a results similar to those obtained in the current study in which they concluded that the levels of IL-6 increased in diabetic patients [35] and this elevation also associated with the development of peripheral neuropathy [36] and correlated with sensory nerve action potentials and compound muscle action potentials leading to peripheral nerve axonal damage [37, 38]. Moreover, previous literature stated that gabapentin has the ability to reduce the levels of IL-6 in a dose-dependent manner [39] which is consistent with the results of the present study in groups C, D, E, and F that may be owned to the possible role of gabapentin in increasing IL-10 and Heme oxygenase (HO)-1 protein expression. IL-10 was first described as an inhibitor of cytokine synthesis, and it was subsequently demonstrated that IL-10 attenuates nociceptive effects by inhibiting spinal glia activation and the production of pro-inflammatory cytokines in an animal model which is supported by findings of Rusciano who reported that In vivo, in a rat model of neuropathic pain, gabapentin increased the levels of IL-10, an anti-inflammatory cytokine capable of inhibiting interferon-gamma (INFγ), and the expression of pro-inflammatory cytokines like TNF-α, IL-1, IL-6, IL-8, and IL-12 [26].

As postulated in Table 2, the lowest levels of IL-6 obtained with the group of mice receiving a combination of 2.5 mg gabapentin with 0.05 mg loperamide (Group D) exceeded the decrease in IL-6 levels that were obtained in mice receiving gabapentin alone (Group C) and showed levels that did not significantly differ from those of controls, which indicates that the use of loperamide with gabapentin increases its analgesic effectiveness. These findings were confirmed by the non-significant difference between the group that received 2.g mg alone and the group received 1.2 mg gabapentin with 0.05 mg loperamide, which indicates that the use of loperamide increases the potency of gabapentin and reduces the dose required to achieve the desired therapeutic outcome. The explanation of this synergistic activity is the previously mentioned cause, which includes the effect of loperamide on the intestinal movement [33] that prolongs the transit time of gabapentin, which improves its activity [34] in addition to its potential effect in alleviating pain and controlling anxiety [32].

Table 2. Evaluation of interleukin-6 in induced diabetic mice after 14day of being treated with Gaba at a dose of and Gaba -loperamide combinations.

Groups	IL-6 (pg/ml)
Control group A/negative group	125.02 ±6.71 d
Group B/ Diabetic positive group	1083.34 ±76.35 a
Group C/Diabetic Negative (Treated only with GBP 2.5 mg)	440.92 ±18.66 b
Group D/ Diabetic Negative (Treated with GBP 2.5mg/kg + lop 0.05 mg	165.8 ±8.53 d
Group E/Diabetic Negative (Treated with GBP 1.8mg+ lop 0.05mg)	301.48 ±15.47 c
Group F/Diabetic Negative (Treated with GBP 1.2mg+ lop 0.05mg)	421.94 ±19.34 b
LSD value	42.694 **
P-value	0.0001

GBP: Gabapentin, Means having with the different letters in column differed significantly, ** (P≤0.01).

3. CONCLUSION

It was concluded that the use of loperamide in combination with gabapentin causes an increase in the potency of gabapentin as an analgesic against diabetic neuropathic pain as it reduces the dose of gabapentin required to achieve the desired therapeutic outcome, as indicated by the significant reduction in the markers of diabetic neuropathic pain that were subjected to the present study, which include SP and IL-6.

4. MATERIALS AND METHODS

4.1. Experimental Animals

The research was granted approval by the institutional animal care and use committee, specifically the College of Veterinary Medicine at the University of Baghdad. The approval, with reference number 2573 and dated November 20, 2023, underwent a thorough review and was subsequently granted approval. The research involved Thirty female Swiss albino mice, maintained at the University of Baghdad's College of Veterinary Medicine. The mice were fed a conventional rodent meal, and their conditions were carefully controlled to maintain a temperature of 20°C, a light/dark cycle of 14 hours and 10 hours, and regular air replacement using ventilation vacuums and weekly litter replacement.

4.2. Experimental designs

A case control study was conducted on thirty female albino mice weighing 25g divided into six groups as the following:

- **Group A**: five mice were served as negative group received distilled water (control group).
- **Group B**: five mice were served as positive group (induced diabetic neuropathic pain) who received alloxan without any treatment.
- **Group C**: five mice were received oral gabapentin treatment (2.5 mg) for 14days.
- **Group D**: five mice received oral gabapentin treatment (2.5 mg) and loperamide (0.05mg) for 14 days.
- **Groupe E**: five mice received oral gabapentin treatment (1.8mg) and loperamide (0.05mg) for 14 days.
- **Group F** five mice received oral gabapentin treatment (1.2 mg) and loperamide (0.05mg) for 14 days.

4.3. Induction of Diabetes

The alloxan solution was prepared by dissolving in citrate buffer because it is unstable at neutral pH [40]. In order to prepare the citrate buffer stock solution, 4.964g of sodium citrate and 6.363 of citrate acid were dissolved. The solution was then adjusted to the final desired pH of 4 using HCl or NaOH, and distal water was added until 1L was reached to which an alloxan was added. Mice were fasted for 18 h from (3 pm-9 am) then were given three intraperitoneal injections of 180 mg/kg of alloxan in consecutive order at intervals of 48 h to produce a kind of insulin-dependent diabetes mellitus known as alloxan- induced diabetes mellitus [41]**.** A week after the third dose, the blood glucose concentration was determined by using a glucose meter and there was a significant increase in the level of glucose in all induced groups as illustrated in Table 3 which is consistent with the proposed protocol [40].

Table 3. levels of blood glucose after diabetes Induction by alloxan 180mg/dl.

Groups	Glucose levels (mg/dl) Mean ± SD
Control group A	75.4 ±3.27 e
Group B	264.6 ±16.92 c
Group C	222.6 ±14.57 d
Group D	293.8 ±17.96 c
Group E	374 ±22.42 b
Group F	400.4 ±26.51 a
LSD value	36.028 **
P-value	0.0001

Means having with the different letters in column differed significantly ** (P≤0.01).

4.4. The assessment of neuropathic pain

The observations recorded after diabetes induction regarding the walking and movement difficulties which indicate neuropathic pain existence [42, 43] that alas accompanied by an elevation in the SP levels as illustrated in Table 1 that considered as an indicator of that pain since it was reported that SP recruits leucocytes to the peripheral terminals of nociceptors where they release neuroactive mediators that contribute to neuropathic pain [44]

4.5. Dose calculations:

A 2 mg loperamide stock solution was prepared and diluted with 12 ml of distilled water to obtain a concentration of 0.167 mg/ml, from which 0.3 ml were obtained to get a dose of 0.05 mg per 25 g of mice body weight [45], which is used for groups D, E, and F. Regarding gabapentin, a series of dilutions was conducted to create stock solutions of gabapentin with varying concentrations. A dose per 25-mg mouse body weight was obtained by preparing a stock solution by dissolving 100 mg (1 tablet) of gabapentin in 20 ml of distal water to obtain a concentration of 5 mg/ml. From that stock solution, a volume of 0.5, 0.36, and 0.24 ml was obtained to get a dose of 2.5, 1.8, and 1.2 mg, respectively [46]. The dose of 2.5 mg was used in groups C and D, while doses of 1.8 mg and 1.2 mg were used for groups E and F, respectively.

4.6. Sample collection and biochemical measurement

Mice were euthanized and blood sample were collected and from which a serum were obtained and stored in a fridge for freezing for 24 hrs. the levels of IL-6 and SP were determined by using an ELISA technique according to the manufacturer instructions [47].

4.7. Statistical analysis

The data obtained in the present study were stored and statistically analyzed by using the Statistical Analysis System (SAS) (2018) to assess the effect of the treatment used on the studied parameters. Results were expressed as mean ± standard deviation (SD). The least significant differences (LSD) test (two-way analysis of variation) (ANOVA) was used to compare the means in this study, in which $p \leq 0.05$ was considered a statistically significant difference [48, 49].

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