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STEVIA EXTRACT AND MK-801 ATTENUATE NALBUPHINE TOLERANCE AND DEPENDENCE IN MICE: ROLE OF GLUTAMATE, NITRIC OXIDE, AND OXIDATIVE STRESS

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This study aimed to investigate for the first time, the potential role of stevia aqueous extract alone and in combination with MK-801 on nalbuphine-induced tolerance and dependence in mice using biochemical and histological tools. In this study, Repeated administration of stevia extract (300 mg/kg, p.o) along with nalbuphine (10 mg/kg, s.c.) attenuated the development of tolerance, as measured by the hot plate test, and dependence, as assessed by naloxone (5 mg/kg, i.p.) - precipitated withdrawal manifestations. Concomitantly, increase in brain glutamate level, malondialdehyde level, and serum nitrite level-induced by repeated administration of nalbuphine to mice or by administration of naloxone to nalbuphine-dependent mice were inhibited by co-administration of stevia extract. Also, co-administration of the stevia extract inhibited the decrease in brain intracellular reduced glutathione level and glutathione peroxidase activity induced by both treatments. The inhibitory effect of the extract on nalbuphine-induced tolerance and dependence and on naloxone-induced biochemical alterations in nalbuphine-dependent mice was enhanced by concurrent i.p. administration of the NMDA receptor antagonist, MK-801 (0.25 mg/kg). histopathological results revealed that stevia extract co-administration produced decrease in nerve cell degeneration, vacuolation, apoptosis and enhance neuropil appearance. This histopathological improvement increased by concurrent administration of MK-801 with stevia extract. These results provide evidence that stevia extract appears to have a therapeutic potential in opioid tolerance and dependence, through inhibition of nalbuphine-induced elevation in glutamate level, NO overproduction and oxidative stress.

Keywords: Nalbuphine, Tolerance, Dependence, Stevia, MK-801, Glutamate.

INTRODUCTION

Nalbuphine (NLB) is a mixed synthetic opioid of the phenanthrene series. It has kappa-receptor agonist and partial mu-receptor antagonist activities. It is available and used as an analgesic without the undesirable side effects of pure mu-opioid agonists like morphine. Also, it has been used for decades for relieving moderate to severe pain[1].

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However, Long-term use of nalbuphine may lead to the development of tolerance and dependence[2].

Several studies have proposed that glutamate (GLU) and its N-methyl D-aspartate (NMDA) receptors have a role in opioid dependence and tolerance development[3-5].

It was proven that Activation of NMDA receptors leads to activation of nitric oxide (NO) synthase and subsequent NO production[6]. As a result, NO has a role in opioid dependence and tolerance[7]. Also, MK-801, an NMDA receptor antagonist, administration has been shown to reduce both opioid dependence and tolerance[8].

Moreover, Opioids have been demonstrated to cause oxidative stress in the brain[9]. Free radical scavengers used before treatment reduced the severity of the withdrawal signs produced by opioids[10].

Stevia rebaudiana (Bertoni) is known as a candy leaf, sweet leaf, or honey leaf. Its sweetness derives from steviol glycosides, which are sweeter than sucrose by 100–300 times, and it has multiple beneficial activities, in addition to antioxidant properties[11].

Stevia aqueous extract has been demonstrated to contain steviol glycosides, mainly stevioside and rebaudioside, tannins, alkaloids, glycosides, flavonoids, quinone, saponins, and triterpenes[12].

Stevia extract was discovered to reduce the amount of NO that rats' lipopolysaccharide-stimulated macrophages produced[13]. Additionally, it has been discovered that stevia extract can reduce the amount of pro-inflammatory cytokines (mast cell protease 1, Tumor necrosis factor-alpha (TNF-α), Interleukin-1 (IL-1), and IL-6) produced by lipopolysaccharide-stimulated macrophages in rats[14].

Stevia extract has been demonstrated to significantly reduce oxidative stress by lowering lipid peroxidation levels in diabetic rats' kidney and liver[15]. Also, it was reported that stevia extract decreased the amount of malondialdehyde (MDA) that rats exposed to carbon-tetrachloride injections deposited[16]. Furthermore, it has been noted that stevia extract exhibits hepatoprotective properties against the harmful effects of carbon tetrachloride in rats[16].

Stevia extract has been found to have free radicals scavenging effect and a substantial protective effect in tissue against hydrogen peroxide-induced damage and delayed the oxidation process[17&18].

This investigation aimed to determine whether stevia extract can inhibit NLB-induced dependence and tolerance and the possible contribution of glutamate, NO, and oxidative stress in these effects.

MATERIALS AND METHODS

Chemicals and kits

NLB hydrochloride was obtained as ampoules from Amriya pharmaceuticals company (Egypt), dizocilpine (MK-801) hydrogen maleate and NX hydrochloride were purchased as powder from Sigma Chemical Co. (USA).

Stevia extract

Dried stevia was used for the extraction by hot water. The dried plant - stems and leaves - were crushed manually. So, stem fragments ranged in size from 3 to 7 cm, whereas leaves easily disintegrated into smaller fragments. One Kg of dried plant material was mixed with 5 litres of water distillate heated to 80°C. The vessel was sealed After stirring and incubated for 1 hr at 80°C, then filtered using a 150 µm nylon filter bag. The filtered extract was cooled by pouring the fluid back and forth using two vessels till it reaches 40°C. The extract was comparatively devoid of big particles and equivalent in color to weak coffee or really strong tea. Then the extract was poured into large vessels and leaved to dry in open air for 3 days[19].

Animals

Male Balb-c mice that all experiments were conducted on them were obtained from the animal house of Sohag University weighing 22–30 g, kept under a 12 hrs light/dark cycle at 25°C, 40–60% relative humidity in stainless steel cages, and allowed food and water (laboratory chow). Before the examination, the animals spent a week acclimatizing to the environment of the study. Mice were split into 4 groups, with 8 animals in every group. The experiments described here were permitted by

the Sohag Institutional Animal Care and Use Committee (approval NO. 12-7-2022-1).

Protocol of treatment

Male mice were used and randomly allocated into 4 groups (8 mice each).

Group (I) (negative control): Mice received subcutaneous (S.C.) saline solution only for 14 days.

Group (II) (positive control; model for NLB dependence and tolerance): Mice were injected S.C. with 10 mg/kg NLB twice daily for 14 days. Mice received NLB On day 15 followed by a NX injection 2 hrs later.

Group (III): Mice were pretreated, with stevia extract 300 mg/kg orally (P.O.) 30 min prior each NLB injection utilizing a stomach tube for 14 days. On day 15, mice were injected with NLB and stevia extract followed by a NX injection after 2 hrs, in order to study the effects of stevia extract on NLB dependence and tolerance (SE+NLB) development.

Group (IV): Mice were pretreated with stevia extract 300 mg/kg orally (P.O.) utilizing an oral tube 30 min prior every NLB injection for 14 days, along with MK-801 (non-competitive NMDA receptor antagonist) intraperitoneal (I.P.) in a dose of 0.25 mg /kg. On day 15 mice received NLB, stevia extract, and MK-801 injection followed by a NX injection 2 hrs later. This group served to assess the effect of MK-801 and stevia extract on the development of NLB dependence and tolerance (SE+MK-801+NLB).

1- Study of NLB tolerance development by hot plate test

For assessing centrally mediated analgesia, hot plate test was utilized[20], by measuring the time mouse was taken to jump with all four feet or to lick its hind paws within a Plexiglas cylinder placed on a 55°C hot plate surface. This reaction time served as the endpoint and the increase in hot plate latency served as an assessment to the analgesic activity of the tested drug. Mice were examined prior to medication administration on the hot plate for establishing a consistent control response level for 4 days. If the animals did not respond within 30 seconds, they were removed off the hot plate and excluded immediately, to avoid tissue damage and then retested after 30 min. The NBL's anti-nociceptive effect was determined post the initial injection by 60 min. The anti-nociceptive effect was estimated on the 1st, 3rd, 5th, 7th, 11th, and 14th days.

2- Study NLB dependence development by induction withdrawal syndrome

On day 15 of the treatment by NLB, every mouse was administered with 5 mg/kg NX I.P, 2 hrs after the first injection. Every mouse was put in a transparent acryl cylinder (35 cm in height, 20 cm in diameter) immediately after the injection with NX to observe withdrawal manifestations (teeth chattering jumping, rearing and paw tremor) for 30 min.

3- Biochemical tests

Animals were deceased by decapitation after the recording of symptoms of withdrawal. From each animal, brain tissues and blood were obtained for histopathological and biochemical analysis.

The serum was separated by centrifugation using a high-speed centrifuge (MPw-350, Warsaw, Poland) at 3000 r.p.m for 10 min, and stored at -80°C to measure the level of serum nitrite.

Brains were removed from each animal, isolated and bisected into to hemispheres. The left ones were fixed for 48 hrs in 10% neutral-buffered formalin to be used for histopathological examination. The right hemispheres were immediately preserved in liquid nitrogen to be used in ELISA.

The right hemispheres rinsed in ice-cold saline carefully blotted, weighed, then homogenized using a glass homogenizer that contains 2 ml of saline or phosphate buffer (pH 7.4), following that, the homogenate was split into 2 equal amounts. The first amount underwent 10 min centrifuging and the supernatant was utilized to estimate the MDA level and GSH-Px activity. the second amount was added to an equal amount of perchloric acid (1 mol/l) and mixed by vertex. The mixture was placed for 5 min at room temperature. The supernatant was collected carefully after centrifugation for 10 min, and utilized for estimating the levels of GLU and intracellular reduced GSH.

Glutamate assay

GLU content was measured using a commercially available ELISA kit Bio-

diagnostic kit (Cairo, Egypt) in accordance with the manufacturer's guidelines. For each assay, a standard reference curve was created.

Serum nitrite assay

Determination of nitrite level (one of NO oxidation's stable byproducts) in serum was carried out using Bio diagnostic kit (Cairo, Egypt) in accordance with the manufacturer's guidelines.

Malondialdehyde assay

The content of MDA (an indicator of lipid peroxidation) was measured using a commercially available sandwich ELISA kit Bio-diagnostic kit (Cairo, Egypt) in accordance with the manufacturer's guidelines.

Intracellular reduced glutathione assay

The content of GSH was measured using a commercially available sandwich ELISA kit Bio-diagnostic kit (Cairo, Egypt) in accordance with the manufacturer's guidelines.

Glutathione peroxidase activity assay

The content of GSH-PX was measured using a commercially available sandwich ELISA kit Bio-diagnostic kit (Cairo, Egypt) in accordance with the manufacturer's guidelines.

Statistical analysis

Statistical analysis was conducted by a Prism computer program (GraphPad Software Inc. V5, San Diego, CA, USA). Results were presented as mean ± SEM. One-way analysis of variance (ANOVA) was used for analyzing the statistical difference between 3 or more groups, followed by Tukey-Kramer Multiple Comparisons Test. A two-tailed probability values (p)< 0.05 were deemed statistically significant.

Brain histopathological examination
Hematoxylin & Eosin staining

The left hemispheres were obtained, rinsed in physiological saline and fixed in 10% formalin saline for 48 hrs. The preserved specimens were dehydrated in ascending grades of alcohol, cleared with xylene, and embedded in paraffin wax. Paraffin blocks were sectioned at 5 um thickness using microtome (Leica RM 2125) and stained with hematoxylin & eosin for general histological study. Stained sections were exanimated and photographed.

RESULTS

Effect of stevia extract on the development of NLB tolerance to analgesia

Nalbuphine-treated mice showed a significant ($p\leq$ 0.01) increase in analgesic latency in hot plate test in comparison with negative control mice (Fig. 1). Repeated administration of NLB with dose of 10 mg/kg subcutaneously twice daily for 14 days to mice caused a progressive decrease in analgesic latency in hot plate test, indicating NLB tolerance development (Fig. 1).

Mice Pretreatment with stevia extract in dose of 300 mg/kg orally 30 min prior every NLB injection significantly ($p\leq$ 0.01) inhibited the development of NLB tolerance to analgesia. This indicated from improvement of analgesic latency in hot plate test compared to NLB alone treated group (Fig. 1).

Effect of dizocilpine (MK-801) on stevia extract-induced inhibition of the development of NLB tolerance to analgesia

Concurrent MK-801 0.25 mg/kg I.P. administration with stevia extract 300 mg/kg P.O. to mice 30 min prior every NLB injection for 14 days significantly ($p\leq$ 0.01) enhanced the stevia's inhibitory effect on the development of NLB tolerance to analgesia (Fig. 1).

Effect of stevia extract on NX-induced withdrawal symptoms in NLB-dependent mice

Intraperitoneal administration of NX 5 mg/kg on day 15 of treatment, 2 hrs following the first NLB injection induced withdrawal symptoms including teeth chattering, rearing, jumping, and paw tremor in NLB-dependent mice (Fig. 2).

Mice pretreatment with stevia extract 300 mg/kg P.O. 30 min prior every NLB injection significantly ($p\leq$ 0.01) inhibited these withdrawal symptoms development compared to NLB- treated group indicating attenuation of dependence expression (Fig. 2).

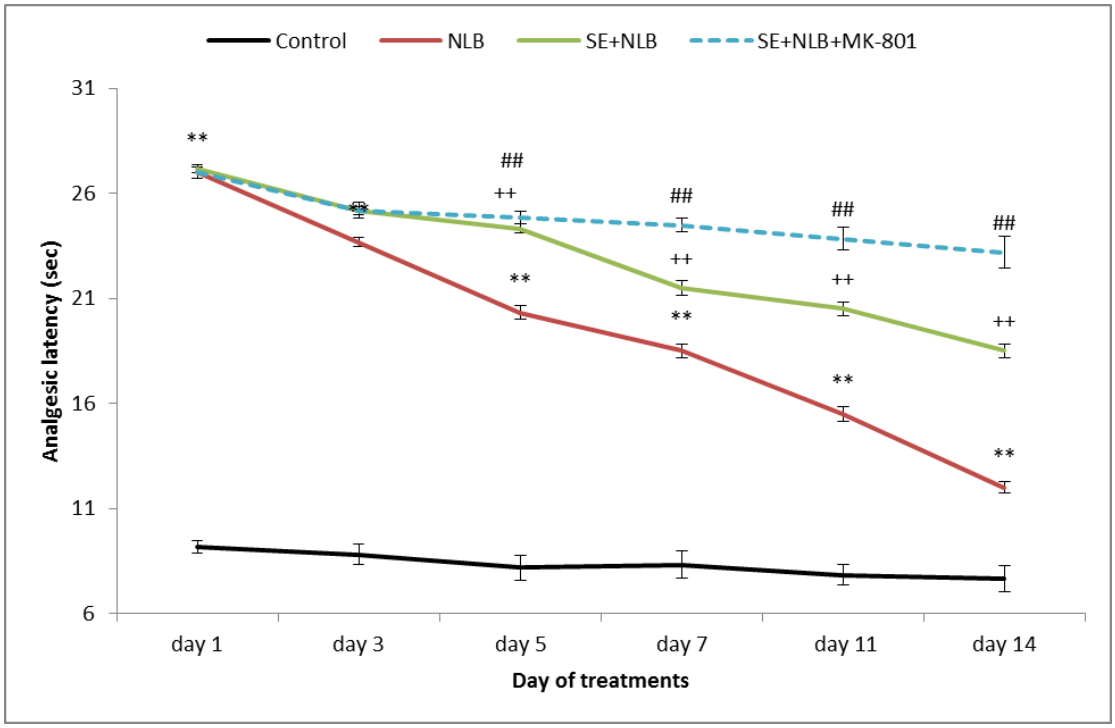

Fig. 1: Effect of stevia extract (SE) alone and combined with dizocilpine (MK-801) on the development of NLB tolerance to analgesia in mice. Mice were injected with 300 mg/kg SE P.O. alone and in combination with MK-801 (0.25 mg/kg), 30 min prior S.C. injection of 10 mg/kg NLB twice daily for 14 days. Values are means ± SEM of 8 observations. **$p \leq 0.01$ vs. control values; ++$p \leq 0.01$ vs. NLB values; ##$p \leq 0.01$ vs SE+NLB.

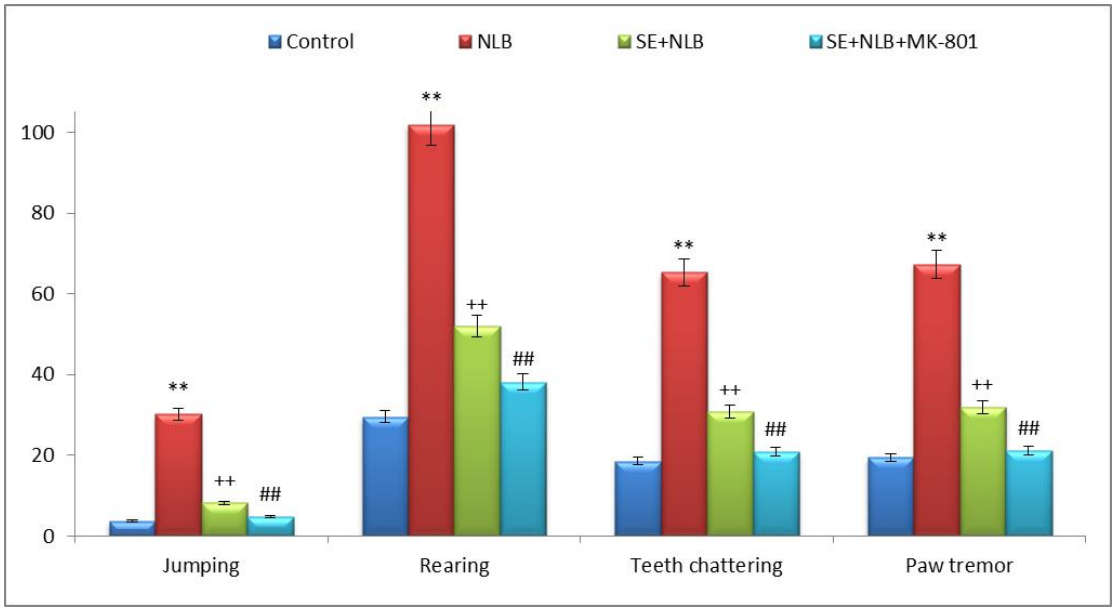

Fig. 2: Effect of stevia extract (SE) alone and combinedwith dizocilpine (MK-801) on NX-induced withdrawal sypmtoms in NLB-dependent mice. Mice were treated with SE (300 mg/kg P.O.) alone and combined with MK-801 (0.25 mg/kg) 30 min prior S.C. NLB (10 mg/kg) injection twice daily for 14 days. NX was injected on day 15, 2 h following the initial NLB injection into mice. Values are means ± SEM of 8 observations. **$p \leq 0.01$ vs.control values; ++$p \leq 0.01$ vs. NLB values; ##$p \leq 0.05$ vs. SE+NLB.

Effect of dizocilpine (MK-801) on stevia extract-induced inhibition of NX-induced withdrawal symptoms in NLB-dependent mice

The inhibitory effect of stevia extract on NLB withdrawal symptoms development after the NX challenge significantly ($p \leq 0.01$) increased by concurrent injection of MK-801 0.25 mg/kg I.P. compared to stevia treated group (Fig. 2).

Effect of stevia extract on NX-induced changes in brain glutamate, serum nitrite level, and brain oxidative stress biomarkers (MDA level and intracellular GSH as well as GSH-Px Activity) in NLB-dependent mice

Intraperitoneal administration of NX 5 mg/kg on day 15 of treatment, 2 hrs following the initial NLB injection caused significant ($p \leq 0.01$) increase in brain glutamate level, brain MDA level and serum nitrite level. This treatment also caused significant ($p \leq 0.01$) decrease in intracellular GSH-Px activity and GSH level compared to negative control group (Fig. 3 (a, b, c, d and e)).

Mice Pretreatment with stevia extract 300 mg/kg, P.O. 30 min prior every NLB injection significantly ($p \leq 0.01$) inhibited the elevation of brain glutamate, brain MDA level and serum nitrite level induced by NX- challenge in NLB-dependent mice. Also, this treatment significantly ($p \leq 0.01$) reversed the decline in GSH level and GSH-Px compared to NLB alone treated group (Fig. 3 (a, b, c, d and e)).

Effect of MK-801 on stevia extract induced inhibition on NX-induced changes in brain glutamate, serum nitrite level, and brain oxidative stress biomarkers (MDA Level and intracellular GSH as well as in GSH-Px Activity) in NLB-dependent mice

The inhibitory effect of stevia extract 300 mg/ kg, P.O. on the elevation of brain glutamate, brain MDA level and serum nitrite level induced by NX challenge in NLB-dependent mice was significantly ($p \leq 0.01$) enhanced by concurrent I.P. administration of MK-801 0.25 mg/kg. In addition, the concurrent administration of these agents with stevia extract significantly ($p \leq 0.01$) boosted its enhancing effect on GSH level and GSH-Px activity in mice brain tissues (Fig. 3 (a, b, c, d and e)).

Histopathological results

Negative control group

Examining H&E-stained sections of the negative control group revealed that the cerebral cortex is formed of inner white matter and outer gray matter. The gray matter is formed of nerve cells arranged in layers. The neuropil contains blood vessels and neuroglial cells. The layers of cortical neurons are in the following order: molecular, outer nuclear, pyramidal, inner nuclear and pleomorphic. Nerve cells appear with vesicular nuclei and acidophilic cytoplasm. The white matter is formed of nerve fibers and neuroglial cells (Fig. (4. A)).

Nalbuphine-treated group

Examining H&E-stained sections of the NLB-treated group revealed that some nerve cells appeared shrunken with dense nuclei and increased perineural space. The neuropil showed multiple vacuolations with increased perivascular space (Fig. (4. B)).

Stevia extract + nalbuphine treated group

Examination of this group revealed some improvement in the form of: some nerve cells appeared with regular vesicular nuclei. Others appeared shrunken with pyknotic nuclei. The neuropil showed decreased vacuolations compared to the previous group (Fig. (4. C)).

Stevia extract + MK-801 + nalbuphine treated group

Examination of this group showed many nerve cells with vesicular nuclei, and other nerve cells appeared degenerated with vacuoles in some parts of neuropil (Fig. (4.D).

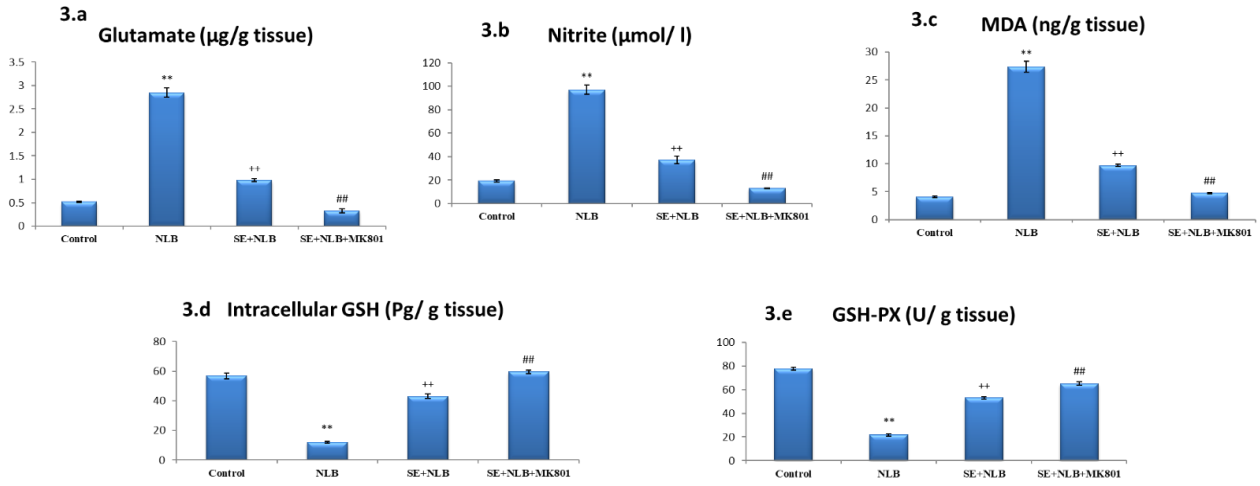

Fig. 3: Effect of stevia extract alone and in combination with dizocilpine on NX-induced change in (a) brain glutamate level, (b) brain MDA level, (c) serum nitrite level as well as (d) brain intracellular reduced GSH and (e) brain GSH-Px activity on NLB-dependent mice. Mice were treated with SE (300 mg/kg P.O.) alone and combined with MK-801 (0.25 mg/kg) 30 min prior S.C. NLB (10 mg/kg) injection twice daily for 14 days. NX was injected on day 15, 2 h following the initial NLB injection into mice. Values are means ± SEM of 8 observations. $**p \leq 0.01$ vs. control values; $++p \leq 0.01$ vs. NLB. values; $\#\#p \leq 0.05$ vs. SE+NLB.

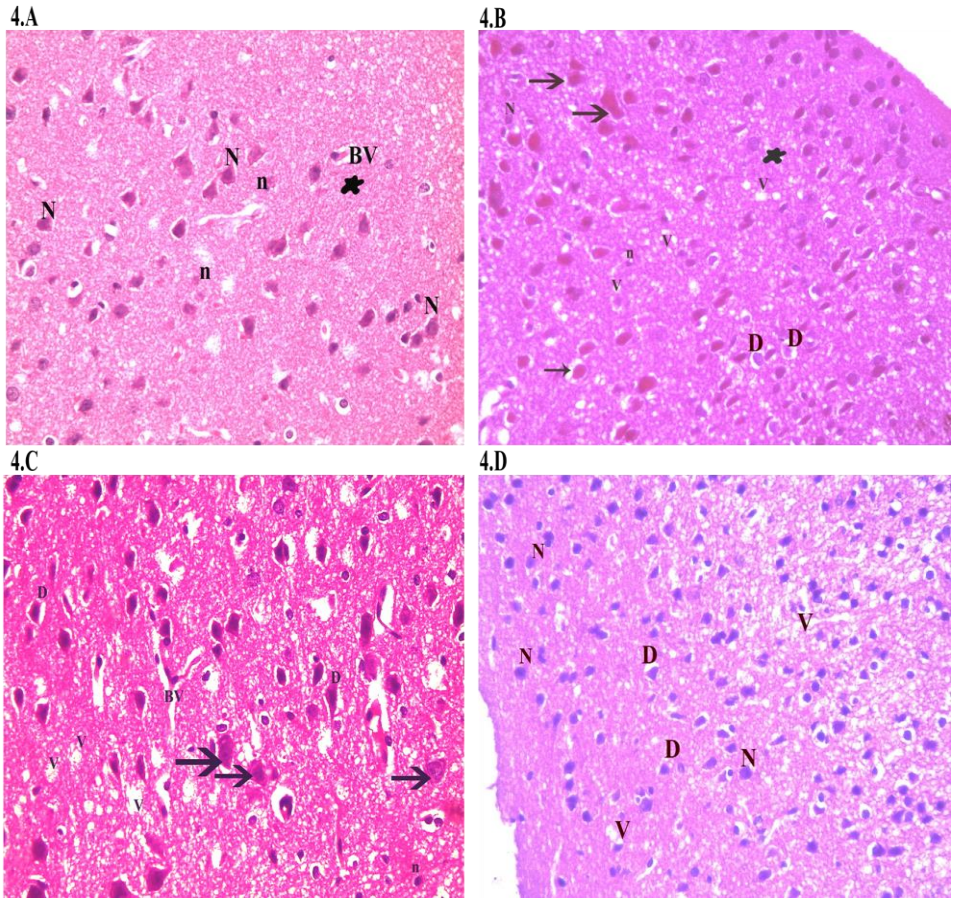

Fig. 4: A photomicrograph for a cerebral cortex section **(A):** The control mice showing pyramidal nerve cells with vesicular nuclei (N), glial cells (n), blood vessels (BV), neuropil (*).
(B): The NLB-treated mice showed many apoptotic cells (thick arrow), degenerated cells (thin arrow), vacuolated neuropil (v), normal nerve cells (N), and normal glial cells (n). **(C):** The stevia extract and NLB-treated mice showed normal nerve cells (N), some degenerated cells **(D):** a few apoptotic cells (arrow), and blood vessels (BV). **(D):** The stevia extract, MK-801, and NLB-treated mice showed normal nerve cells (N), degenerated cells (D), and vacuoles (v).

DISCUSSION

Opioid tolerance and dependence are major issues in chronic pain management, which restricts the therapeutic use of these drugs. We aimed to evaluate, for the first time, the potential role of stevia extract alone and in combination with MK-801 on NLB-induced tolerance and dependence in mice, and the potential contribution of GLU, NO, and oxidative stress in this effect.

Stevia rebaudiana (*Bertoni*) is a natural, noncaloric sweetener of beverages and foods for long history and has many health benefits[21].

It is widely known that MK-801, non-competitive NMDA receptors antagonist, decrease opioid tolerance and dependence development[20].

In current investigation, we observed that repeated NLB administration to mice twice daily for 14 days produced a progressive analgesic latency decrease in hot plate test till reaching control values indicated tolerance development to nalbuphine analgesia. But, pretreatment of mice with stevia extract 30 minutes prior every NLB injection, for 14 days produced increase in analgesic latency in hot plate test indicated inhibition of the tolerance development to NLB analgesia.

Also, we found that NX administration to NLB-treated mice on fourteenth day of treatment precipitated withdrawal manifestations including rearing, teeth chattering, tremor, and jumping, indicated dependence development on nalbuphine. However, Stevia extract, administration to NLB-dependent mice at a dose of 300 mg/kg twice daily for 14 days, reduced all undesirable withdrawal symptoms, indicating that it may be possible to use stevia extract to avoid the hyperexcitability that is brought on by NLB withdrawal.

These previous findings agreed with[3] who reported that Alpha lipoic acid inhibited morphine dependence and tolerance development in mice. However, the current study concern with effect of stevia extract on nalbuphine-induced tolerance and dependence.

The inhibitory effect on nalbuphine-induced tolerance and dependence produced by pretreatment with stevia extract can't be attributed to analgesic effect of stevia extract as stevia extract has no analgesic response on hot plate test and the reason for this inhibition may be counter regulatory effect of stevia extract on nalbuphine-induced neuroadaptive change that happened during this process. So, we examined the effect of stevia extract on nalbuphine-induced change in glutamate, nitrite, MDA, GSH, and GSH-PX.

GLU is a significant excitatory neurotransmitter in the brain, was previously approved to play an undisputable integral role in opioid dependence and tolerance[5]. A previous finding reported that co-administering MK-801 significantly reduced morphine dependence and tolerance provided more evidence for the significance of GLU in opioid dependence and tolerance[4]. Furthermore, GLU activation of the NMDA receptor was reported to stimulate NO synthase and NO production[22]. Over the past few years evidence that strongly suggests that NMDA receptors and NO are the main players in the emergence of opioid dependence and tolerance has developed[23].

High extracellular GLU concentrations have been shown to inhibit cystine transport, the primary building block of intracellular GSH, by interfering with it and as result reducing intracellular GSH and consequently reduced the GSH-Px activity[24]. As a result, reduced GSH depletion decreases the cell's capacity to act as an antioxidant, resulting in accumulation of reactive oxygen species (ROS), and ultimately causing apoptotic cell death[25]. Similarly, administering NLB was reported to reduce intracellular GSH level in rat brain[26]. Moreover, elevated extracellular GLU concentrations were reported to increase MDA production and lipid peroxidation in rat brain cortex[27].

The current study showed that NX administration to NLB-dependent mice on 14th day of treatment produced increase in brain GLU and serum nitrite levels significantly. Also, this treatment resulted in oxidative stress establishment in mice brain tissues (significant decrease in brain intracellular GSH level and in GSH-Px activity and a consequent elevation in the level of brain MDA). This finding came in agreement with[28]. However, there is no previous study indicated the effect of nalbuphine on brain glutamate level, nitric oxide production, and oxidative stress biomarkers.

These finding confirmed by histopathological results that showed many apoptotic cells, degenerated cells, vacuolated neuropil, few normal nerve cells, and few normal glial cells as consequence of oxidative stress caused by NLB. This results agreed with Bekheet *et al.*[29] who studied histological changes caused by morphine administration to rat.

In the present trial, we found that stevia extract's administration before each NLB injection twice daily for 14 days in mice caused inhibition of NLB dependence and tolerance with restoring GLU level near to control level. Similarly, Hamdy *et al.*[4] findings revealed that bupropion inhibition of morphine dependence and tolerance was related with reducing GLU level to normal level. However, the current study is the first study concerned with the effect of stevia extract on nalbuphine- induced tolerance and dependence and glutamate level.

Additionally, Stevia extract inhibition of NLB dependence and tolerance was related with decrease in nitrite level. This finding in accordance with[30] as they revealed that NO inhibition prevent morphine dependence and tolerance.

Regarding this finding, stevia extract was previously reported to exhibit NO inhibitory and anti-inflammatory effect[31]. Stevia extract was reported to hinder the production of NO and inducible NO synthase also, it reduces the release of inflammatory mediators, TNF-α, mast cell protease 1, IL-6, and IL-1β, in lipopolysaccharide-stimulated macrophages in rats[14].

Finally, stevia extract's inhibition of NLB dependence and tolerance was mediated by lowering oxidative stress incidence in mice brains. This in agreement with previous study of Lauro *et al.*[32] as they revealed that oxidative stress mitigation prevent morphine dependence and tolerance. It has previously been discovered that stevia extract has antioxidant activity via increasing the mRNA levels of the protective enzyme superoxide dismutase[33&34]. Also, stevia extract was found to have excellent ROS scavenging activity and exhibit a significant protective role against H_2O_2-induced damage in multiple tissues and delayed the oxidation process. Furthermore, stevia extract was found to attenuate malondialdehyde accumulation after carbon-tetrachloride injection in rats and to have a hepatoprotective effect against the toxic effect of carbon-tetrachloride[16]. These findings further confirmed by histopathological study that showed several normal nerve cells, some degenerated cells, and a few apoptotic cells compared to NLB-treated group.

In this trial, stevia extract attenuating effect on NLB dependence and tolerance development in mice was boosted by the concurrent administration of MK-801. Additionally, co-administration of MK-801 increased the inhibitory effect of stevia extract on NX-induced elevations in brain GLU, nitrite level, and malondialdehyde level. Also, enhance the NX-induced reduction of brain intracellular reduced GSH level, and GSH-Px activity in NLB-dependent mice. These results came in accordance with Abdel-Zaher *et al.*[28].

The improvements seen in the histological examination, which were evidenced by an increase in nerve cell integrity and appearance as well as a decrease in apoptosis and neuropil vacuolation, were likewise supportive of the biochemical data.

Conclusion

Our outcomes indicate that even in the presence of NLB dependence and tolerance, the combination of stevia extract and NLB may have therapeutic relevance for the management of pain. Furthermore, these findings show that stevia extract prevents NLB dependence and tolerance by acting on NMDA/NO pathway and oxidative stress mitigation.

Recommendation

More research is needed for clarification of the exact mechanisms by which stevia extract inhibit NLB-induced dependence and tolerance.

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Conflict of interest

There is none to be declared.

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Presentations: Nil.

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تاثير المستخلص المائي لنيات ستيفيا منفرد ومع MK-801 علي حدوث الاطاقة والتعود لعقار نالبيوفين في الفئران: تاثير جلوتاميت ، نيتريك أوكسيد والاجهاد التاكسيدي

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أستهدف هذا البحث بيان تاثير المستخلص المائي لنبات ستيفيا منفرد ومع إضافة مادة ديزوسلبين علي حدوث الاطاقة والتعود لعقار نالبيوفين في الفئران وذلك لأول مرة.

وقد لوحظ من خلال هذا البحث ان معالجة الفئران ب ١٠ مجم/كجم من مادة نالبيوفين تحت الجلد تعطي تاثيرا مسكنا للالم في اختبار السطح الساخن. كما لوحظ ان إعطاء جرعة مادة نالبيوفين هذه مرتين يوميا لمدة ١٤ يوم متوالية يؤدي الي حدوث الاطاقه.

ولقد لوحظ أيضا ان المعالجة المسبقة للفئران بمستخلص ستيفيا بجرعة مقدارها ٣٠٠ مجم/كجم عن طريق الفم لمدة نصف ساعة قبل إعطاء كل جرعة من جرعات مادة نالبيوفين تؤدي الي تقليل من حدوث هذه الاطاقة. وان هذا التاثير المحدث بمستخلص ستيفيا علي حدوث الاطاقة لمادة نالبيوفين يزيد بالحقن المتزامن في التجويف البريتوني لمادة ديزوسلبين في جرعة مقدارها ٠.٢٥ مجم/كجم.

وكذلك اثبتت نتائج هذا البحث ان الفئران المعالجة بمادة نالبيوفين لمدة ١٤ يوم عند حقنها في التجويف البريتوني بمادة نالوكسون في جرعة مقدارها ٥ مجم/كجم بعد الجرعة الاولي من اليوم ١٥ للمعالجة لمادة نالبيوفين بساعتين ظهرت عليها اعراض انسحاب مادة نالبيوفين.

ولقد أوضحت نتائج هذا البحث أيضا ان المعالجة المسبقة بمستخلص ستيفيا في جرعة مقدارها ٣٠٠ مجم/كجم عن طريق الفم لمدة نصف ساعة قبل حقن كل جرعة من جرعات مادة نالبيوفين يقلل من حدوث هذه الاعراض وان هذا التاثير لمستخلص ستيفيا علي حدوث اعراض السحب لمادة نالبيوفين يزيد بالحقن في التجويف البريتوني مادة ديزوسلبين في جرعة مقدارها ٠.٢٥ مجم/كجم.

وكذلك اثبتت هذه الدراسة ان الفئران المعالجة مرتين بمادة نالبيوفين في جرعة مقـــدارها ١٠ مجم/كجم تحت الجلد بفارق ١٢ ساعة لمدة ١٤ يوم عند حقنها في التجويف البريتوني في اليوم التـــالي بمادة النالكسون في جرعة مقدارها ٥ مجم/كجم بعد ساعتين من الجرعه الاولي يؤدي الي زيـــادة فـــي مستوي مادة جلوتامات ومالون داي الدهيد في مخ الفئران ومادة نيتريت في دم الفئران. والـــي نقـــص مستوي جلوتاثيون ونشاط انزيم جلوتاثيون بيراوكسيدز في مخ الفئران.

ولقد اثبتت هذه الدراسة ان المعالجة المسبقة بمستخلص ســـتيفيا فـــي جرعـــة مقـــدارها٣٠٠ مجم/كجم أو ستيفيوزيد في جرعة مقدارها ٢٠٠ مجم/كجم قبل كل جرعة من جرعات نالبيوفين تقلـــل من حدوث هذه التغيرات.

ولقد اثبتت هذه الدراسة ان هذا التأثير لمستخلص ستيفيا او ستيفيوزيد على التغيرات الحادث في الفئران المعالجة بمادة نالبيوفين يزيد بالحقن في التجويف البريتوني لمادة الديزوســـلبين فـــي جرعـــة مقدارها ٠.٢٥ مجم/كجم.

ولقد أوضح الفحص النسيجي ان المعالجة بمادة نالبيوفين أدت الي تغيرات انتكاسية في الخلايا العصبية والتي تحسنت بالمعالجة المسبقة بالمستخلص المائي لنبات ستيفيا.