ANALGESIC EFFECT OF VANADYLE SULFATE AND METHYLENE BLUE ON EXPERIMENTALLY INDUCED PAIN IN MICE

Khudhair Abd Al-Rahman[1] **Muhmood Abed Al-Ani**

Mustafa Ahmed Jasim **Muna Muhammed Ismael**

Physiology and Pharmacology Dept., College of Vet. Med., Diyala University- Iraq

[1]Corresponding author: dr.khudhairalani@gmail.com

ABSTRACT

The present study was designed to determine the potential analgesic effects of both Vanadyl sulfate and Methylene blue simultaneously and in combination using isobolographic analysis, to determine the sort of interaction of Vanadyl sulfate and Methylene blue by up and down method and investigate the analgesic activity of Vanadyl sulfate and Methylene blue in mice. The calculated median lethal dose (LD_{50}) was (456.08 mg Kg^{-1} B.W) of vanadyl sulfate and (1878 mg Kg^{-1} B.W) of methylene blue. The analgesic activity of Vanadyl sulfate at the dose (152 mg kg^{-1}) and Methylene blue at the dose (626 mg kg^{-1}) by using acetic acid induces writhing and hot plate methods to induced pain in albino male mice. The test and standard drugs (Ibuprofen 100 mg kg^{-1}) significantly ($p<0.05$) reduced the number of abdominal constriction and stretching of hind limb induced by acetic acid injection. The analgesic activity results by acetic acid showed a significant decrease in the numbers of writhing in (30) minutes of test-1 (Vanadyl Sulfate) and test-2 (Methylene blue) at a level of ($P<0.05$) in comparison with control group while analgesic activity results by hot plate method that showed a significant increase in reaction time at (30, 60,120 and 180) minutes of: Test -1 (Vanadyl Sulfate) and: Test -2 (Methylene blue) at a level of ($P<0.05$) in comparison with control group.

Key words: Pain, Analgesic, Ibuprofen, Methylene blue, Vanadyl sulfate, Acetic acid.

INTRODUCTION

Pain is that the commonest reason for physician consultation in most developed countries (Debono *et al.* 2013). It's a serious symptom in several medical conditions and can interfere with a human quality of life and general functioning (Moore *et al.* 2015). Hypnotic suggestion, social support, distraction or excitement its psychological factors may be considerable affect pain's unpleasantness or intensity (Weyers, 2006). In some arguments place fourth in assisted killing or suicide debates, has been used the pain as an argument to permit people who are terminally sick to lives end (IASP, 1979). As a result of it's a complex, subjective phenomenon, process pain has been a

challenge. The International Association for the study of Pain's widely used definition states: "Pain is an emotional experience and unpleasant sensory associated with potential or actual tissues harm, or described in terms of such harm" (Bonica, 1979).

Methylene blue, is known as methylthioninium chloride, is a dye and medication. It is used mainly in treatment methemoglobinemia (BNF, 2015). Specifically, used to treatment methemoglobinemia levels when are more than 30% and in which there are symptoms despite oxygen therapy (Alan, 1984). Methylene blue, previously been used for treating the urinary tract infections and cyanide poisoning but this use is no longer recommended. It's given by injection into a vein (BNF, 2015). Use often turns the sweat, stool and urine blue to green in color. The uses of methylene blue during the pregnancy maybe harm the baby and not given in methemoglobinemia is likely very dangerous (Alan, 1984). Methylene blue is a thiazine dye (BNF, 2015). It works by converting the ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}) in hemoglobin (Alan, 1984).

Vanadyl (IV) sulfate, $VOSO_4$, is an inorganic compound of vanadium. It's one of the most common sources of vanadium in the laboratory and it's very hygroscopic blue solid, reflective its high stability. The vanadyl ion features, it (VO^{2+}), that has been referred to as "most stable diatomic ion" (Bauer *et al.* 2005). Vanadyl sulfate is an intermediate during extraction of vanadium from petroleum residues, the vanadium is a major commercial source (Crans *et al.* 2011). Some experimental drugs and food supplements are components from vanadyl sulfate. Vanadyl sulfate work as insulin-like effects (Krivovichev *et al.* 2007). The most water-soluble sulfates, the vanadyl sulfate are rare in nature. Pauflerite are an anhydrous form (Hawthorne *et al.* 2001), a mineral of fumarolic origin. Hydrated forms, also rare, include trihydrate, hexahydrate, and pentahydrates (minasragrite, orthominasragrite, (Schindler *et al.* 2003) and anorthominasragrite (Berneth and Azine, 2008).

The main aim of present study was to investigate the analgesic effects of both vanadyl sulfate and methylene blue on pain induced by acetic acid and hot plate methods in albino mice.

MATERIALS AND METHODS

Experimental Animals

Twenty- four Wister albino male mice, the body weight ranged between 25-30 g and about 3 months of age were used in this experiment. Placed the mice in a special housing room in College of Veterinary Medicine/ Department of Physiology and Pharmacology for 2 weeks for acclimation. Standard rodent diet

and tap water were freely available. Housing conditions were maintained at 20-25 ºC in the air-conditioned room, the light/ dark cycle was 14/10 in housing place, while the air of the room was changed continuously by using ventilation vacuum.

LD_{50} measurement of Vanadyl sulfate and Methylene blue

Determination of acute toxic median lethal dose (LD_{50}) of both Vanadyl sulfate and Methylene blue in albino male mice was done by up and down method (Dixon, 1980), and it was calculated according to the following equation:

LD_{50} = xf + kd

LD_{50} = Median lethal dose

xf = Last dose used in the experiment

d = Difference between doses

k = Factor of change from

Acetic acid induced writhing

The analgesic activity evaluation by writhing method induced by acetic acid. The acetic acid solution in concentration (1% v/v) prepared with distilled water. Ibuprofen solution used as (Reference drug) (dose-100 mg kg^{-1} 10 ml^{-1}) was prepared with normal saline. Each of test -1: A solution of vanadyl sulfate (152 mg kg^{-1}) according to LD_{50} result, and test -2: A solution methylene blue (626 mg kg^{-1}) according to LD_{50} result, was prepared in (10) ml of normal saline. The mice were divided into (4) groups (6) mice in each group. Food was withdrawn before about (12) hours of administration of Ibuprofen, vanadyl sulfate and methylene blue. The Ibuprofen, vanadyl sulfate and methylene blue were given orally and after (60) minutes writhing were induced by (ip) injection of (1%) of acetic acid as (0.1 ml 10 g^{-1}) of body weight. The writhing were recorded for (30) minutes (Shanmugasundaram and Venkataraman, 2005).

Hot plate method

The hot plate method applies by used hot plate apparatus, consists of an electrically heated surface. The temperature is between 55-56 °C. The mice are placed on the hot plate and the time recorded by a stop-watch until either jumping or licking occurs. Each of test -1: A solution of vanadyl sulfate (152 mg kg^{-1}) according to LD_{50} result, and test -2: A solution methylene blue (626 mg kg^{-1}) according to LD_{50} result was prepared. The mice were divided into (4) groups (6) mice in each group, the mice were marked individually. Food was withdrawn before about 12 hours of administration of Ibuprofen, vanadyl sulfate and methylene blue. The Ibuprofen, vanadyl sulfate and methylene blue were

given orally and after 60 minutes placed the animals on the hot plate and record the observations at the time (0, 30, 60, 120 and 180) minutes (Oz *et al.*, 2011).

Statistical Analysis

The values are expressed as the mean ± the standard error (M±SEM). The values were analyzed by using one-way analysis of variance ANOVA, and then the test of the least significant difference LSD applied to find the significant differences (Steel and Torrie, 1984). The significant level of the test was $P<0.05$.

RESULTS

Acute Toxicity Study (LD_{50}) of Methylene Blue

The value results of oral LD_{50} of Methylene blue were 1878 mg kg^{-1} B. W. as in table 1.

Table 1. Calculate of LD_{50} for Methylene blue by up and down method

Range of doses	3200-1600 mg Kg^{-1} BW
Decrease or increase in dose	400 mg Kg^{-1} BW
Death or survival of animal after 24 hours.	XXXXOOXO
Value of (K) table	-0.305
Last used dose (xf)	2000 mg Kg^{-1} BW
Value of LD_{50}	1878 mg Kg^{-1} BW

O: survival, X: death

Acute Toxicity Study (LD_{50}) of Vanadyl sulfate

The value results of oral LD_{50} of Vanadyl sulfate were 456.08 mg kg^{-1} B.W. as in table 2.

Table 2. Calculate of LD_{50} for Vanadyl sulfate by up and down method

Range of doses	160 – 400 mg Kg^{-1} BW
Decrease or increase in dose	80 mg Kg^{-1} BW
Death or survival of animal after 24 hours.	OOOOXOXO
Value of (K) table	0.701
Last used dose (xf)	400 mg Kg^{-1} BW
Value of LD_{50}	456.08 mg Kg^{-1} BW

O: survival, X: death

Study the interaction between methylene blue & vanadyl sulfate toxic doses

The result of isobolographic analysis by using the LD_{50} dose of both methylene blue and vanadyl sulfate alone and in combination are listed in table 3 and figure 1.

Table 3. Isobolograph analysis of both methylene blue &vanadyl sulfate LD_{50} alone and in combination

Parameters Groups	Range of doses mg kg^{-1} B.W.	Initial dose mg kg^{-1} B.W.	Final dose mg kg^{-1} B.W	No. of animal	Result after 24 hours	LD50 mg kg^{-1}
Methylene blue	3200-1600	3200	2000	8	XXXXOOXO	1878
Vanadyl sulfate	160 – 400	160	400	8	OOOOXOXO	456.08
Methylene blue + Vanadyl sulfate	2441.4-1502.4 + 594.2-364.8	2441.4 + 594.2	1502.4 + 364.8	8+8	XXXXOXXX	1472.91 + 357.49

O: survival, X: death

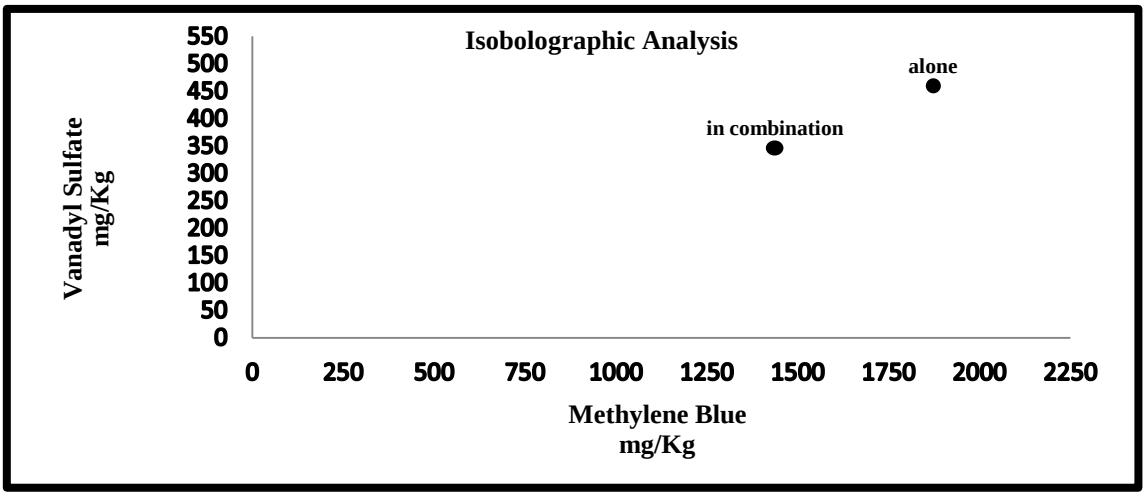

Figure 1. Isobolograph analysis of both methylene blue and vanadyl sulfate LD_{50} alone and in combination

Writhing Test

The results of writhing test induced by acetic acid in mice showed a significant decrease in the numbers of writhing in (30) minutes of both test -1 (Vanadyl Sulfate) and test -2 (Methylene blue) at a level of ($P<0.05$) in comparison with control group, as in table 4.

Table 4. Analgesic Activity of Writhing Test Induced by Acetic Acid

Group	Treatment	Dose (mg kg^{-1})	Writhes numbers in 30 min. (mean ± sem)	Inhibition (%)
Control	Distal Water	---------	57.4 ± 4.645	---------
Standard	Ibuprofen	100	34.2 ± 2.753*	40.4
Test - 1	Vanadyl Sulfate	152	37.2 ± 3.965*	35.1
Test - 2	Methylene blue	626	46.4 ± 1.725*	19.1

The values are expressed in Mean ± SE; * Significance at $P< 0.05$.

Hot Plate Test

The results of analgesic activity by hot plate method in mice that showed a significant increase in the reaction time at (30, 60, 120 and 180) minutes of both test -1 (Vanadyl Sulfate) and test-2 (Methylene blue) at a level of ($P<0.05$) in comparison with control group, as in table 5.

Table 5. Analgesic Activity by Hot Plate Method

Group	Treatment	Dose (mg kg^{-1})	Reaction time in seconds at the time (min.) (mean ± sem)				
			0	30	60	120	180
Control	Distal Water	-----	5±0.317	5.4±0.245	5±0.317	5.2±0.375	5.8±0.375
Standard	Ibuprofen	100	6±0.317	9.8±0.584*	10. ±0.511*	12.1±1.051*	15.2±0.584*
Test - 1	Vanadyl Sulfate	152	5.6±0.245	8.2±0.862*	9.2±0.375*	10.2±0.665*	14±0.838*
Test - 2	Methylene blue	626	5.8±0.375	8.6±0.601*	9.8±0.919*	11.2±0.736*	15.4±1.169*

The values are expressed in Mean ± SE; * Significance at $P < 0.05$

DISCUSSION

The study indicated that administration of vanadyl sulfate and methylene blue can be suppressed the pain that induced by writhing and hot plate methods in male mice. Local injection of methylene blue has been evaluated in treatments for various pain diseases and has shown a remarkable long-acting analgesic effect (Peng *et al.* 2007). Methylene blue is a nontoxic dye agent that inhibits the soluble guanylate cyclase and nitric oxide synthase. Nitric oxide regulates physiological functions such as pain and analgesia by activating soluble guanylate cyclase to increase intracellular cyclic guanosine monophosphate (Bhattacharyya and Tracey, 2001). Methylene blue, as an oxidizing-reducing agent, demonstrates a strong affinity to nerve tissues when applied locally, which can directly block the electrical conductivity of nerve fibers, thereby affecting the neural excitability and impulse conductivity. Recent studies have shown that a low dose (0.5% or 1%) of methylene blue may block peripheral nerve fibers at the incision (Kaneria *et al.* 2007).

Hyperglycemia leads to the toxicity of neurons due to increased glucose oxidation, lead to increased reactive oxygen species that may be controlled by the treatment with antioxidant (Andrade *et al.* 2012). Various studies have suggested the capacity of vanadium to enhance the blood glucose control of diabetics and also to enhance the negative side effects associated with diabetes. Therefore, the vanadyl sulfate may be given the chance to decrease the glucose in blood that leads to decrease in the pain.

CONCLUSION

From the previous result, it is concluded that vanadyl sulfate and methylene blue have a good analgesic effect due to directly block the electrical conductivity of nerve fibers, thereby affecting the neural excitability and impulse conductivity.

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التأثير المسكن لكل من كبريتات الفناديل والمثيلين الأزرق في الألم المستحدث تجريبياً في الفئران

خضير عبد الرحمن[1] محمود عبد العاني مصطفى أحمد جاسم منى محمد اسماعيل

قسم الفسلجة والفارماكولوجي، كلية الطب البيطري، جامعة ديالى، العراق

[1] المسؤول عن النشر: dr.khudhairalani@gmail.com

المستخلص

صممت الدراسة الحالية لغرض تقييم قوة التأثيرات المسكنة لكل من الفينداىل سلفيت والمثيلين بلو بشكل منفرد ومزدوج بوساطة استخدام طريقة التحليل بالأيزوبولكراف من اجل تقييم قوة التداخل للفينداىل سلفيت والمثيلين بلو عن طريق استخدام طريقة الصعود والنزول، والكشف عن الفعالية المسكنة للفينداىل سلفيت والمثيلين بلو في الفئران، وكانت الجرعة النصف قاتلة (LD_{50}) للفينداىل سلفيت 456.08 ملغم كغم$^{-1}$ من وزن الجسم و1878 ملغم كغم$^{-1}$ من وزن الجسم للمثيلين بلو. اما التجربة الثانية فاجريت لغرض تقييم التأثيرات المسكنة للفينداىل سلفيت بجرعة 152 ملغم كغم$^{-1}$، والمثيلين بلو بجرعة 626 ملغم كغم$^{-1}$ بوساطة اعطاء حامض الخليك وطريقة الصفيحة الساخنة لاستحداث الالم في ذكور الفئران البيضاء. أظهرت النتائج انخفاض في عدد الانقباضات البطنية وتمدد الأطراف الخلفية المستحدثة بوساطة حامض الخليك لكل من الفينداىل سلفيت والمثيلين بلو ودواء الأيبوبروفين بجرعة 100 ملغم كغم$^{-1}$ بمستوى معنوية ($p<0.05$). نتائج الفعالية المسكنة المستحدثة بوساطة حامض الخليك أظهرت نقصان في عدد الالتواءات لمدة 30 دقيقة لكل من الفينداىل سلفيت والمثيلين بلو عند مستوى معنوية ($P<0.05$) بالمقارنة مع مجموعة السيطرة، بينما أظهرت النتائج بوساطة استخدام طريقة الصحيفة الساخنة زيادة معنوية في فترة الوقت عند (180, 120, 60, 30) دقيقة لكل من الفينداىل سلفيت والمثيلين بلو عند مستوى معنوية ($P<0.05$) بالمقارنة مع مجموعة السيطرة.

الكلمات المفتاحية: ألم، مسكن، أيبوبروفين، مثيلين بلو، فينداىل سلفيت، حامض الخليك.