Original Research Article

The Action of Adrenergic Agonists and Antagonists in the Present of Bupropion in the Vta Nucleus on Morphine Withdrawal Syndrome on Episodic Behaviors in Rats

ABSTRACT.

Bupropion is a unique and non-nicotine smoke cessation agent, and it is using smoke cessation drugs under FDA approval despite its antidepressant effect. In the current study, we considered the short-term interaction of bupropion, adrenoceptors proprietary agonists and antagonists (phenylephrine, prazosin, clonidine and yohimbine) in intra VTA injection on naloxone precipitated withdrawal symptoms. The injection guide cannulae were implanted into VTA nuclei according to stereotaxic surgery under sterile conditions. The sham group was received saline as a drug vehicle, but treated groups received 2.5 mg/µl/min of bupropion alone and with doses of phenylephrine (0.2 µg/µl/minute), prazosin (1 µg/µl/minute), clonidine and yohimbine (2 µg/µl/minute) 20 before bupropion. Dependent and non-dependent control groups had surgery, but they had no intra-VTA injection. In each treatment group, 8 rats were used. Withdrawal signs were precipitated by naloxone (1.2 mg/Kg, s.c.), observed continuously and registered online each minute. The behaviors captured by 3 digital cameras indifferent view (30 min) for offline analysis. Signs were counted and analyzed within and between groups. The results showed that intra-VTA bupropion can alter the occurrence of withdrawal symptoms and decrease more of them. Adrenoceptor agonists decrease some of the symptoms. This decrease was significant statistically in comparison with sham and control groups and between treatment groups. Occurrence extents of withdrawal symptoms have no significance statistically in naive control and sham groups toward the witness group. Results showed adrenergic system has a role in the opioid system and drug dependence; also between influences extent of adrenoceptor's species is a difference in accession of diverse withdrawal signs.

***Keywords*:** Bupropion, Clonidine, Morphine dependence, Naloxone, Phenylephrine, prazosin, Withdrawal syndrome, Yohimbine, Ventral tegmental area.

1. INTRODUCTION

In the present century, rarely can a country be found which is not associated with the problem of drug abuse and subsequent drug addiction. Also, there are reports of financial losses and, on the other hand, the ineffectiveness of treatment methods in the treatment of addiction [2]. The lack of definitive treatment for addiction requires research and study in this category. Two of treat addiction withdrawal, human behavior, reward and punishment, or satisfaction and hatred play an important role. Human learns and experiences are accustomed to rewards and satisfaction [18].

Therefore, if the stimulus creates a reward for man, it can create a strong reminiscence in his memory. After this, the human makes behaviors for seeking behavior. Drug abuse is a delightful stimulus that leads to human search behavior to re-discover the drug. This behavior is a sign of psychological dependence on a substance that stimulates the rewards of the brain [1]. The so-called "physical attachment" to the misuse of drugs results in a compromise that occurs in brain circuits. After that, it controls the visible physical function, such as heart rate or blood pressure [24]. Different research has shown that in the onset of drug allergy, the dopamine route, the ventral tegmental area (VTA) to the nucleus accumbens (NAc: nucleus accumbens) and the prefrontal cortex are the main roles [17]. From the cellular-molecular point of view, the most important changes should be in the abdominal tegmentum region and the combined core [17, 21]. The pathways of the brain reward system are vast that these areas include different brain regions such as the hypothalamus, abdominal tangential area, occipital nucleus, anterior central cortex, amygdala, hippocampus, foreskin, Lucus serratus, and premolars [24]. The extensive studies of electrophysiology and neuroscience support this theory which in the creation of reward behaviors, many neurotransmitter systems are involved, including dopaminergic, glutamatergic, serotonergic, adrenergic and endogenous opioid peptides [14]. Among the numerous neurotransmitter systems involved in the reward and reinforcement process, it appears that the mesolimbic dopamine system plays an important role [25]. Many researchers have introduced the mesolimbic dopamine system due to its unique interference in the regulation of reward-related behavior as a reward neurochemical substrate [10]. VTA has dopaminergic neurons (group A10) and many passers-by send to limbal areas that are involved in positive motivation and enhancement [21]. Less than 60% of the VTA neurons in the rat are dopaminergic and there

are neurons of gababergy and glutamatergic [12]. In general, neurons in this core contribute to the reward system, motivational behaviors, cognitive actions, substance dependence, and several types of psychological performance [18]. Many studies have shown that alpha-adrenergic and opioids systems can have a kind of interactive interaction complex [5]. There are also reports that opioids increase the Change and turn (turn) of norepinephrine [3].

Bupropion is an abnormal anti-depressant that acts as a dopamine and norepinephrine reuptake inhibitor and nicotine antagonist [19]. Bupropion is the only newly developed anti-depressant drug available in the United States without serotonergic activity [20].

Bupropion has beneficial effects on cigarette smoking [23]. Therefore, in recent years, its effects have been studied on some of the drug dependencies such as dependence on methamphetamine. These studies have shown that bupropion is capable of effectively affecting symptoms associated with deprivation of methamphetamine and can be appropriate for the treatment of methamphetamine dependence. And also can be a good drug for the treatment of methamphetamine dependence [16]. Based on the similarity of drug dependence mechanisms, it is expected that this drug can also reduce the symptoms of morphine withdrawal syndrome. The purpose of the present study was to determine the extent and mode of action of adrenergic receptors in the VTA nucleus in the presence of bupropion in the incidence and prevalence of dependence symptoms in the morphine-dependent rat. Also, in order to determine the extent and mode of action of the recipients, morphine deprivation behaviors caused by naloxone injection were investigated. These behaviors are divided into episodic and non-episodic behaviors in this study we examined the incidence of three episodic behaviors: chewing wet-Dog shake and teeth chattering.

2. MATERIALS AND METHODS

2.1 Place of experiments and studied animals

The present study was done in order to the action of adrenergic agonists and antagonists in the presence of bupropion in the VTA nucleus on morphine-free syndrome on episodic behaviors in Rats in the Faculty of Science, Urmia University, Urmia, Iran during 2010–2012. Experiments were performed on 96 Wistar male rats (200-290 gr), which were purchased from the Institute of Pasteur Institute of Iran. Animals were kept at the appropriate temperature (22 ± 3) and lighting - darkness 12 hours (start of lighting at 8 o'clock in the morning). The animals had enough water

and food, and each rat was tested only once. In this study, animals were divided into 8 groups as follows: the control group (Nive) (n = 12), the surgical control group (n = 7), the sham group (n = 6), the bupropion treatment group (2.5 mg / µl / minute) (n = 8), adrenoceptor agonist α1, phenylephrine (0.2 µg / µl / minute), bupropion (N = 9), adrenoceptor antagonist α1 treatment group, prazosin (1 µg / µl / minute), bupropion (N = 11), adrenoceptor agonist α2, clonidine (2 µg / µl / minute), bupropion (N = 8), adrenoceptor antagonist α2, yohimbine (2 µg / µl / minute), bupropion (N = 7). Injections were taken into VTA. In the control and sham groups, saline was injected as a drug carrier. The intact naive group did not receive any injections and surgery in the VTA.

2.2. Surgery with stereotaxic device

In the present study, the rats were anesthetized with a mixture of ketamine (20 mg/kg) and azpromazine (10 mg/kg) and placed in a stereotactic apparatus (Narishige, Japan). Scarves with a score of 23 were placed as guide cannula in the VTA core based on the atlas of Paxinos and Watson [15]. The cannulas embedded in the skull bone were fixed with the aid of a cold dental acrylic. The injection electrode was connected to the polyethylene tube and the other one was attached to a suitable Hamilton syringe (1 µl). Surgery and injections were performed in sterile conditions. After surgery, animals were recovered for at least a week and then tested. All experiments and procedures were designed and implemented based on animal protection codes and Helsinki treaties on laboratory animals were considered.

2.3.The drugs and chemicals used in this study

In the current study, morphine sulfate (Candida Co., Iran), naloxone hydrochloride (Sigma Aldrich, USA), bupropion hydrochloride (Dipharma Italy, donated Dr. Abidi Pharmacy, Iran), phenylephrine, prazosin, clonidine and yohimbine (Sigma-Aldrich, USA) were used. Drugs were dissolved and used in the physiologic sterile serum (Razi, Iran). To anesthesia of rats, ketamine, Parke-Davies, Freiburg, Germany, 20 mg / kg, and aspromazine (Bayer, Leverkusen, Germany, 10 mg / kg) were used. The salts used to prepare the phosphate buffer, the formalin used for tissue stabilization and the colors required for coloring the sections of the tissue from the company of Merck Germany were prepared.

2.4. How to induce morphine dependence

The morphine dependency test was performed under the standard protocol. Morphine was injected intraperitoneally 3 times a day (8:00, 14:00 and 20:00), and this was repeated for 4 days. Doses were incremental and were as follows: The first day (15, 15 and 20), the second day (20, 20 and 25), the third day (25, 25 and 30), and the fourth day (30, 30 and 35). The dose of the fourth day was maintained until the test was completed. The control groups received the serum of physiology with the same program. On day 5, morphine withdrawal symptoms from subcutaneous injection of naloxone (1.2 mg / Kg) were recorded half an hour after the last administration of morphine [2]. To detect the acute effect of bupropion, adrenocortical agonists and antagonists on naloxone-induced morphine deprivation behaviors, drug treatments were injected 30 minutes in the VTA core 30 minutes before naloxone. Signs of deprivation are constantly observed and recorded regularly every minute. The rats were placed in a test box for 10 minutes before the test. Every minute, episodic symptoms of withdrawal morphine including behaviors of chewing, Wet-Dog shake and teeth chattering were examined. The behaviors were recorded with three digital cameras from different angles for later analysis.

2.5. Determine injection position and tissue confirmation

At the end of the drug injection testing, the animals were anesthetized to find the location of the cannula and then using the same injection cannula, 1 μL solution of 1% of the vital methylene blue color was injected within one minute. The animals were immediately subjected to perfusion of the phosphate buffer solution by opening the chest through the heart. The brain of the animal was cut with a microtome, and the location of the injection and deposition of the cannula was determined. In cases where the location of the cannula was not suitable for injection, the data from that animal was removed from the dataset. In Fig. 1, an example of coloring the site of the VTA core after the completion of the experiments with the corresponding plot of the atlas Paxinos and Watson [15] is shown.

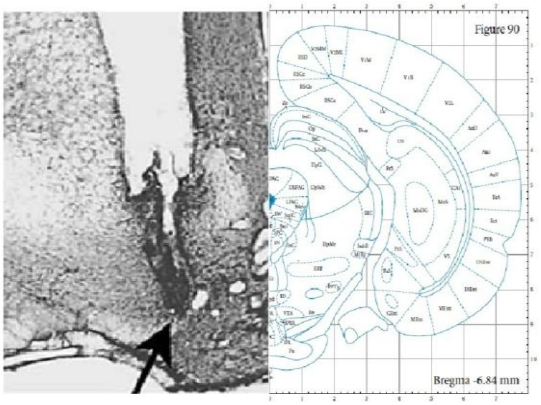

Fig. 1. an image of the tissue sections from the VTA core location on the left (tip of the cannula with the arrow shown). On the right, the plot is from atlas Paxinos and Watson [15]

2.6. Data analysis

The data of recorded behaviors were collected and analyzed for each group of rats in each minute. The data from the scattering point were analyzed by the Kolmogorov-Smirnov test. The difference between the mean of observed behaviors within groups and between groups was analyzed by one-way ANOVA with posthoc Tukey HSD Test.

3. RESULTS

3.1 Behaviors of withdrawal morphine in control groups

In this study, there were two control groups. The first control group was a group of rats that had no surgery and no morphine dependence. In this rat, naloxone injection did not show any symptoms of withdrawal morphine. The second control group was rats that were treated with stereotaxic surgery, but no morphine dependence was induced in them. In this rat, naloxone injection did not cause any sign of dependence. The results showed that surgery and cannulation within the core of VTA did not affect animal behavior.

3.2. Behaviors of withdrawal morphine in control group and dependent control group

The control group in this study was the animals on which stereotactic surgery was first performed on them, and after the recovery period, they were subjected to a morphine-dependent protocol. In this group of rats, the physiologic serum was injected into the VTA core and injected into the process for the treatment groups of naloxone. After the injection, the resulting behaviors were evaluated. Since these rats were dependent on morphine, and also in the control rat no

morphine exclusion mark was registered. Therefore, in the control rat, the observed deprivation behaviors were used as a statistical comparison criterion for data from treatment groups.

3.3. Morphine deprivation behaviors in control and treatment groups

3.4. Chewing behavior

Chewing behavior is a motor behavior that appears in deprivation of morphine. The results showed that no significant difference was observed between treatments in different groups to reduce this sign ($p \leq 0.05$). On the other hand, treatment groups 2 and 6 have increased this behavior. Also, treatment group 6 (bupropion and yohimbine) significantly increased this behavior compared to the control group. Treatment group 5 (bupropion and clonidine) had the greatest effect on decreasing the symptoms, but the change was not significant (Fig. 2).

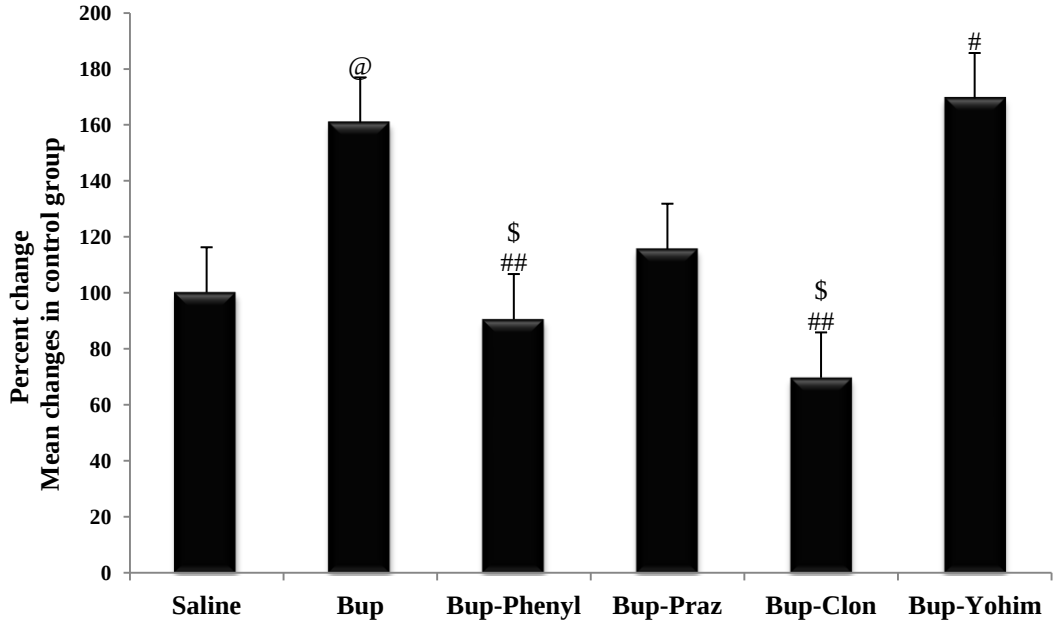

Fig. 2. Comparison of the average chewing behavior in rats of the control group and treatment with different drugs by one-way ANOVA. (#: $p<0.05$ was higher than the control group, @: $p <0.01$ and $p <0.001$, compared to group 2 (bupropion treatment), $: $p <0.01$ and $p <0.001$, compared to group 2 (bupropion treatment), ##: $p <0.01$ and $p <0.001$ Compared to group 6 (bupropion and yohimbine)).

3.5. Wet-Dog shake behavior

This behavior is visible in the deprivation syndrome of morphine. Since this behavior is separable from other behaviors, therefore it can easily be counted. The results showed that the changes were significant only in treatment group 5 ($p<0.05$), and this treatment had the greatest effect on decreasing this sign. Other drug treatments did not have a significant effect on this behavior (Fig. 3).

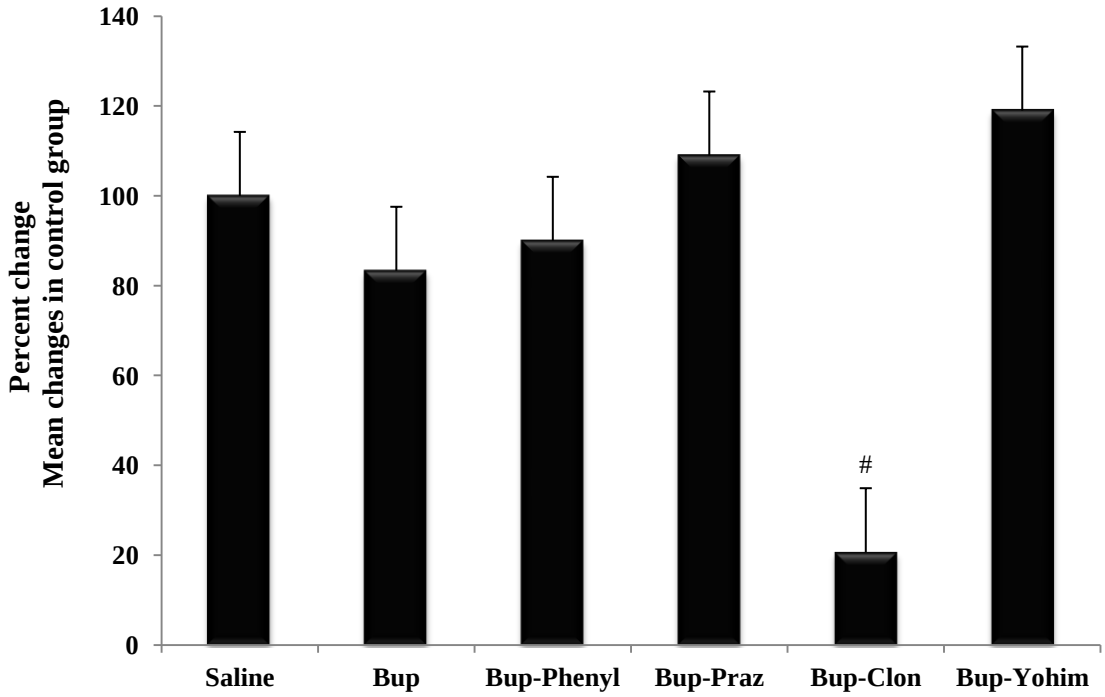

Fig. 3. Comparison of the average behavior of shake-like dogs in different groups within 30 minutes after deprivation of morphine by one-way ANOVA. (#: $p<0.05$ compared to the control group and the rest of the groups).

3.6. Teeth chattering behavior

The behavior of teeth chattering is a motor behavior that appears in the deprivation syndrome of morphine. The results showed that the difference between the effects of different treatment groups is significant. Treatment groups 3 and 5 showed a significant difference ($p<0.05$), but the other groups did not have any significant difference with the control group ($p\leq0.05$). The results showed that treatment 5 had the most effect on reducing symptoms of teeth chattering behavior (Fig 4).

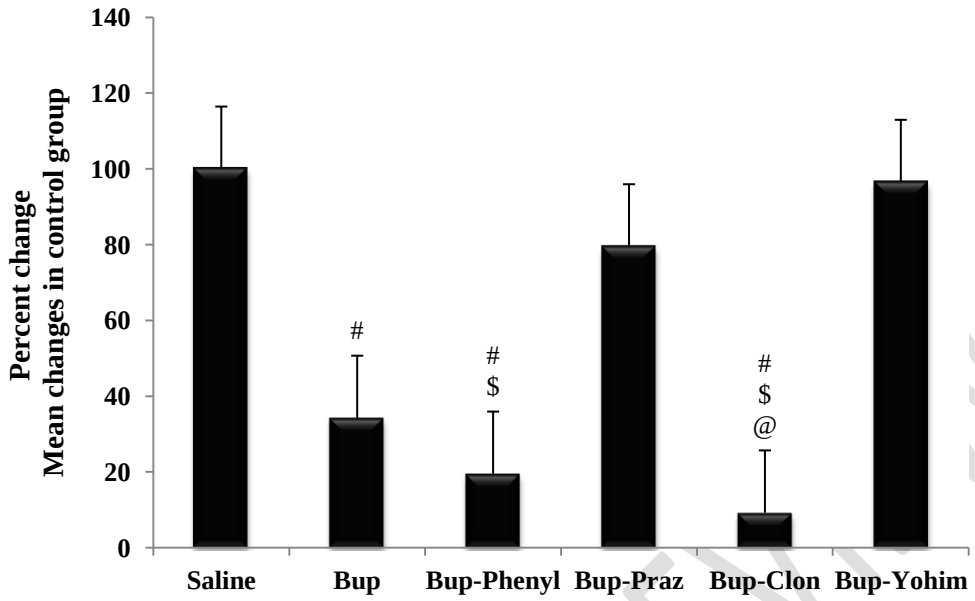

Fig. 4. Comparison of the average behavior of shake-like dogs in different groups within 30 minutes after deprivation of morphine by one-way ANOVA (#: $p = 0.01$, $p < 0.01$ and $p < 0.01$ compared to the control group, $: $p > 0.05$ compared to group 2 (bupropion treatment), @: $p < 0.01$ compared to the control, control groups 4 and 6)

4. DISCUSSION

Physical dependence is an effective factor in enhancing the effect of psychological dependence on the drug and the tendency toward it after the withdrawal of addiction. In physical dependence, repeated use of a drug changes the physiological state of the body. So that taking the drug is necessary to prevent the emergence of a specific syndrome, and discontinuation of the drug causes severe physical discomfort, which these disorders call a withdrawal syndrome [4]. Studies have shown bupropion can effectively be an antagonist to nicotinic acetylcholine (nAChRs) receptors, as well as synaptic reuptake inhibitors of dopamine and noradrenaline [13]. The inhibition of these carriers and neurotransmitter receptors may be a reason for bupropion to be a good drug for cessation and depression [5]. Also, studies have shown bupropion can reduce brain reward activity in smokers, craving, cracks, cracking effects and rewards effects [6, 22]. Given the similarity of drug dependence mechanisms, it is expected that this drug can also reduce the symptoms of morphine withdrawal syndrome. The results showed that bupropion reduces some of the symptoms and increases some. And also by disrupting the pattern of occurrence of some

behaviors, it can be an interesting drug for cases of drug abuse. Bupropion affects several neurotransmitter receptors, so it can be concluded that this drug is important and appropriate among other drugs.

According to the results of this study, bupropion seems to be effective in reducing some morphine withdrawal symptoms in cases of morphine dependence. Also, due to its effects on other neurotransmitter receptors and the results of this study, it is useful in controlling the behaviors of withdrawing morphine. A study by Joshi and coauthors [9] showed that chronic and acute treatment with bupropion-independent rats can reduce the incidence of craving independent rats. The results of Joshi and coauthors [9] were consistent with the results of our study. In the present study, the rate of craving behaviors in bupropion injected decreased and seems to confirm the involvement of D-receptor dopamine receptors and adrenergic receptors in these morphine-related behaviors. Lu and coauthors [11] have shown that the Velnafaxine drug as a serotonin and norepinephrine reuptake inhibitor can significantly reduce symptoms such as jumping or escape from the cage in deprivation of morphine. The results of the current study were consistent with the results of Lu et al. [11]. The results of this study also showed that bupropion was effective on adrenergic and serotonergic receptors and significantly decreased the trait symptoms of independent rats when deprived of morphine. The change in receptors in dopamine receptor treatments in the Ikeda and coauthor study showed that the role of D1-dopamine receptors in the behavior of chewing is more than the other receptors [7]. Based on the results of the present study, chewing behavior in the rat of morphine-dependent was increased by the effect of bupropion in the VTA nucleus, and this increase was probably due to D1-dopamine receptors. The results of Joshi and coauthors [8] showed that bupropion reversed morphine-induced tolerance and dependence in rats.

The interaction between morphine and bupropion observed in this study may also result from the effects of bupropion on DA reuptake, norepinephrine and nAChRs, but the precise mechanism is still unknown. Our study confirmed the effects of interactions between morphine and bupropion in the pattern of symptoms of withdrawal addiction. However, our research has developed new insight into the interactions between bupropion and opioid systems. Also, the present study showed that bupropion is the first anti-depressant to improve morphine withdrawal symptoms.

5. CONCLUSIONS

The results suggest that adrenergic agonists and antagonists are effective in the amount of morphine withdrawal symptoms, which confirms that the adrenergic system has an interactive opioid system. The results showed that bupropion treatment reduced some symptoms of drug addiction, and also according to previous findings and this study, clonidine treatment reduced some symptoms of drug addiction. According to the results, combined treatment of bupropion and clonidine greatly reduced the symptoms of drug addiction. Therefore, the combination of bupropion and clonidine is a good treatment to prevent the return to the material. Finally, this study opened a new insight into the effects of various neurotransmitter systems and in particular the adrenergic system on the addiction and symptoms of opioid withdrawal.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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