

## The Effect of Diabetes on Induced Pain of Formalin and Baclofen Analgesia in Rats

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### ABSTRACT

One of the side effects of diabetes epidemic today in the world is painful neuropathy, the reasons and treatments of which are un- known. Due to the importance of the problem of pain treatment as one of the harmful phenomena in life, this research studies the effect of continued diabetes on the formalin induced pain and Baclofen analgesia in rats. Moreover, the effect of Baclofen as a non-opiate, analgesic drug on the increased pains in the quiescent phase as the model of diabetic pain is investigated. The method is experimental, evaluating the pain level through conducting the formalin test in 3 groups of rats. The first group was divided to control (injection normal saline) and diabetic (injection aloxan 100 mg/kg) which were tested, after one to four weeks from the beginning of diabetes, the second one was divided to a new control and diabetic group, and before performing formalin test, the Baclofen (10 mg/kg) was injected to them. And the third one was divided to two diabetic groups that received Baclofen and normal saline and then the pain of the quiescent phase was compared in them. The results indicate that diabetes increases formalin induced pain ( $P<0.05$ ) and remained with continued diabetes. It also indicates that diabetes establishes increased pain in the quiescent phase ( $P<0.05$ ), yet, it has had no influence on the Baclofen analgesic effect on the first phase of formalin test but increased it on the second phase. Moreover, Baclofen can quiet the increased pain in quiescent phase ( $P<0.05$ ) very well. Due to the results of this study it seems that diabetes, with the changes in the central and peripheral pathways of the pain and also pain control, increases the pain. More studies are required for determining its mechanisms. These changes are accompanied with weakening the internal anti pain systems such as Gaba ergic, which can be treated with Baclofen. Diabetes has no interaction with the Baclofen's analgesic effect, so, Baclofen may be recommended as an effective drug to comfort painful diabetic Neuropathy.

**Keywords:** Diabetic Painful Neuropathy; Baclofen; Formalin Test

### INTRODUCTION

While conventional medical treatment of diabetes mellitus markedly prolongs life-span, serious medical complications affect a large proportion of more than 10 million people believed to have diabetes in the USA. Peripheral neuropathy is the most common complication, affecting one third of newly diagnosed cases. The frequency of neuropathy increases with duration of disease to affect over half of all diabetics. Nerve dysfunction usually progresses to become a distal symmetrical poly-neuropathy with morphologic evidence of paranodal

widening, segmental demyelination and remyelination, axonal atrophy and ultimate fiber loss. This pathology is accompanied by loss of sensory function that, when coupled with impaired healing processes and vascular disease, can lead to gangrene and limb amputation [1]. Pain as a phenomenon is the subject of study in many different researches. On the other hand; relieving pain is an essential problem which causes to use many different drugs. Diabetes, one of the epidemic human diseases in recent years, has major vicinity with pain of which our knowledge about their relationship is little to be

beneficial [2].

Somatic neuropathies can have various causes, e.g. severing of the nerve due to amputation or accident, blood circulation disorder upon manifestation of arterial occlusive disease or diabetes mellitus [3]. Diabetes causes painful neuropathy; a disgusting clinically unsolved problem and scientists suggest variety of drugs to appease it such as 3 cycle antidepressant [4], yet, neuropathic pain caused by nerve degeneration resists to opiate or non steroid drugs.

The mechanism of neuropathic pain and other physiologic normal pains are different [5].

Gabaergic System, a pain relief mechanism, produces inhibitory neurotransmitter in CNS. Baclofen, has excitatory synaptic effect on GABA receptors so used as an anti-nociceptive drug in the treatment of trigeminal neuralgia [6]. Baclofen essentially manages pain by central mechanism compared to diabetic neuropathy which acts peripherally [7]. It is also used as an anti-inflammatory drug [8]. Baclofen and GABA drugs affect pancreas in vivo to increase insulin of plasma to reduce blood glucose of diabetic rats [9]. Exogenic administration of GABA agonist reverses spinal nerve ligation-induced hyperalgesia [10]. Baclofen is a GABA.sub.B receptor agonist that has been used in the United States since 1977 for alleviating the signs and symptoms of spasticity resulting from multiple sclerosis or spinal cord injury. The mechanism of action of Baclofen in spasticity appears to involve agonism at GABA.sub.B receptors of the spinal cord. Thermal hyperalgesia showed a reduced sensitivity to the anti-nociceptive effect of Baclofen (4 mg/kg i.p) [11], Different dosages of Baclofen show its antinociceptive controlling effect [12], the lower the dosage, the higher effect specially in locomotive disorders [13]. The analgesic effect of Baclofen is similar to morphine; with no addictive effect [14, 27]. Low dose usage of Baclofen and Gabapentin dose not affect motor performance, but produced dose-dependent inhibition of both phases in Formalin test in mice [15]. Baclofen injection to VB complex of thalamus reduces transmission and modulation of noxious information [16]. GABAPENTIN and Baclofen inhibit both phases in FT in mice [17]. The inhibitory effects of gaba and Baclofen in trachea is greater than other tissues in diabetic and control rats [18].

Recently, Gallop et al. have developed new

prodrugs of (R)-Baclofen and Baclofen analogs that are well absorbed in the large intestine/colon [19]. A formalin assessment test is performed according to the procedure described in literature [20] Formalin Test is a well validated model and serves as a model of neuropathic pain behavior in rats. It is an effective prescreening test of molecules and may facilitate drug targeting [21]. Alternative animal models of neuropathic pain conditions may involve selection of an animal that naturally possesses a painful disease condition providing neuropathic pain and its symptoms such as HIV or Herpes or cancer or diabetes. Animals may be modified to possess a pain condition due to a disease in a variety of ways for example by administration of Streptozocin to induce a diabetic neuropathy [22]. The effect of FT (Formalin Test) in diabetic and normal rats as a pain inducer and the anti-analgesic function of Baclofen as a pain reliever is studied in this article to find out Baclofen behavioral and functional effects in different phases of FT in diabetic rats.

## MATERIALS AND METHODS

The method is experimental by evaluating the pain level through conducting the formalin test in 3 groups of rats (70 rats, NMRI). The first group was divided to control (injection normal saline) and diabetic (injection aloxan 100mg/kg) subgroups which following the observation of the routine signals of diabetes, after one to four weeks from the beginning of diabetes were tested. The second group was divided to the control and diabetic subgroups, which before performing formalin test; the Baclofen (10 mg/kg) was injected to the previous subgroup. And the third group was divided to two diabetic subgroups that received Baclofen and normal saline, after which the pain of the quiescent phase was compared between them.

Formalin Test: After the injection of 50 $\mu$ lit formalin %2.5 (IC) into the right claw foot of the rat, the animal was put into a plaxi glass room (29, 29.30 cm) on a glass with a mirror under it. The animal's pain behavior was divided into 4 positions and we scored them between 0 to 3; then the pain observed in 60 minutes, the time between (0–5) as phase one, the time between (6–15) as quiescent phase and the time between (16–60) as phase two, were determined [20].

Diabetes creation and blood glucose

concentration measurement: Diabetes creation was done with injection of alloxan monohydrate (100 mg/kg), (IC) over the neck and 7 days after that, the blood glucose was measured with orthotoluiden method, after one to four weeks from the beginning of diabetes, and the formalin test was conducted.

Analgesia creation: Baclofen (10 mg/kg) injection (IP) was performed 15 minutes before the formalin injection.

Drugs: Alloxan (sigma Co)-Baclofen (sigma Co).

### Statistical analysis

For comparing pain due to formalin in the 5 groups at each of the three phases, 3 one-way ANOVA tests, and for multiple comparisons Tukey HSD method were used. To compare the rate of pain decrease due to Baclofen in the 5 groups at each of the three phases, 3 one-way ANOVA tests, and for multiple comparisons Tukey HSD method were utilized. For comparing quiescent phase pain in each of saline and Baclofen groups at week 1 and 4, two independent sample t-tests were used.

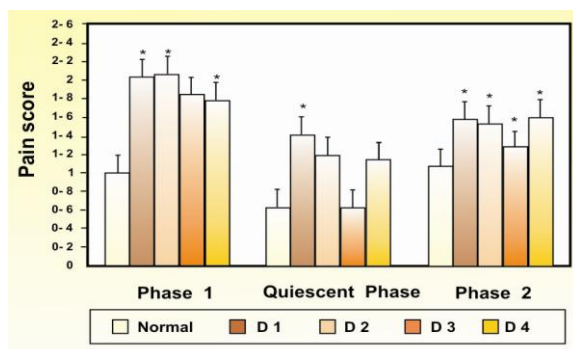
### RESULTS

1- Diabetes effects on the formalin induced pain: it significantly increases the formalin induced pain ( $P < 0.05$ ) (figure 1). With diabetes continued (1-4 weeks), the formalin induced pain continues and there are no significant differences in the same period. Diabetes also, created formalin induced pain in quiescent phase (6-15 min) that was significant in the first week of diabetes (figure 1).

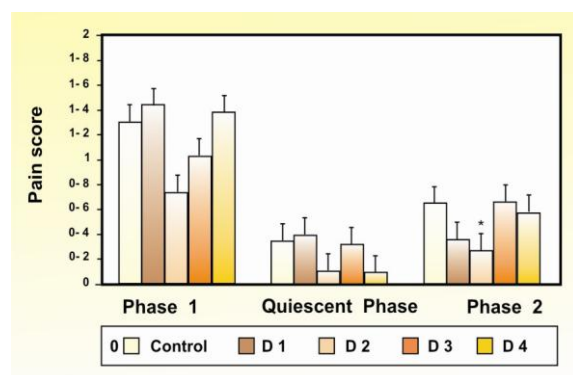
2- The effect of diabetes on Baclofen analgesia: diabetes didn't create significant differences in Baclofen analgesia in the first phase of formalin test (figure 2). Also, diabetes increased Baclofen analgesia in the second phase and in 2 weeks it was significant ( $P < 0.05$ ) (figure 2).

3- The effect of Baclofen on the created pain in quiescent phase of formalin test in diabetes: injection of Baclofen before injection of formalin significantly reduces the created pain in quiescent phase in one week following diabetes (figure 3) ( $P < 0.05$ ).

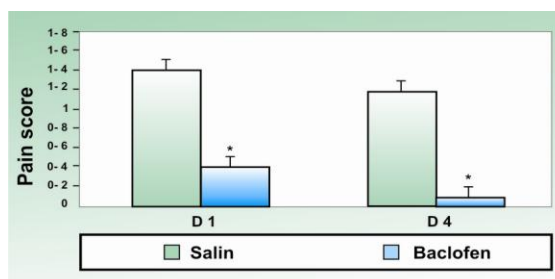
In addition, in 4 weeks after diabetes, injection of Baclofen significantly ( $P < 0.05$ ) reduces the quiescent phase's pain (figure 3).



**Figure 1.** Formalin induced pain increases significantly in phases 1, quiescent, and 2 in diabetic rats



**Figure 2.** In phase 2, the effect of Baclofen analgesia on diabetic rats increases significantly



**Figure 3.** Baclofen significantly decreases the increased pain in the quiescent phase in diabetic rats

## DISCUSSION

This study shows that diabetes increased the formalin induced pain; insufficient studies about painful diabetic neuropathy prohibit its treatment. Anatomical studies couldn't differentiate between painful diabetic neuropathy, so the treatment of such patients has limited the use of usual anti pain drugs. It seems that the creation of experimental diabetes in rodents is a good way to test some drug effects in the lack of main neuropathy.

There are several methods to detect the pain in the lab animals [8]. We have applied the formalin method that Faster recommended as the point score in the damaged tissue [2]; in this method we can also recognize the target site of the anti pain drugs that work centrally or peripherally or both [22]. The formalin test is easier to perform, and a well validated model [20]. The formalin test has two major phases (acute and chronic) and a quiescent phase between them [19].

In our research we studied the effect of diabetes on formalin induced pain and on the Baclofen analgesia. We also studied the effect of Baclofen on the created pain in quiescent phase. Baclofen is a potent GABA receptor agonist used in the treatment of trigeminal neuralgia [16]. Exogenic administration of GABA agonist reverses spinal nerve ligation-induced hyperalgesia [10]. In this research we found out that diabetes could increase pain in our model animals. There are several reasons that indicated the effect of diabetes on formalin induced pain. In hyperglycemic patients with STZ, protein kinas C is increased, that creates pain after mechanical stimulation. Protein kinas C acts as intermediary to increase the ability of C fiber stimulation that in this painful neuropathy model creates pain [23]. The researchers indicated that in hyperglycemia, aldoseducats was the first enzyme produced in CNS and peripheral nerves and with the effect of tolerated (the enzyme's inhibitor) the pain decrease [24].

As we know pain stimulation reaches to the spinal cord, descending pain control systems and also some of the local mechanisms act on it; it then reaches to the thalamus and cortex.

Disease, inflammation and hurt to the nerve system create important changes in pain pathways such as stimulation, gene adjustment and will express new molecules such as neurotransmitter enzymes and receptors [12, 13]. An increased release of glutamate and activation

of the NMDA [N-methyl-D-aspartate) receptor would maintain the hyperalgesia state. Data suggest that diabetes-induced hyperalgesia may be the consequence of increased excitatory tone within the spinal cord [11]. Also due to the fact that there is an increase in some metabolite such as sorbitol and Fructose in nerve and spinal cord in diabetic animals with STZ (streptozotocin) [24], the summation of these materials can be one of the factors in the creation of pain in diabetes. Thermal hyperalgesia showed a reduced sensitivity to the anti-nociceptive effect of Baclofen (4 mg/kg i.p.) [11].

In this study diabetes increased pain in quiescent phase in addition of the first and second phases of formalin test. The pharmacological change of quiescent phase is unknown, it seems that, there is a network for decreasing pain in the quiescent phase, in the creation of this phase; both descending pain control system and endogmic anti pain system are involved. Also it was indicated that the quiescent phase maybe because of inhibitory cabaergic system or endogmic opiate system that with the effect of GABA antagonist or opiate antagonist, it creates a pain activity in this phase [13], so the creation of pain in quiescent phase indicated that in diabetes the endogmic pain control system is inhibited and it seems that diabetes can inhibit or decrease the pain control system, and this indicated that diabetes is effective in both pain transmissions pathways and also the pain control systems. Another point is that it seems that diabetes could speed up the spinal cord activity in the responses to the main stimulation and the quiescent phase pain; moreover, it can be the reaction of second phase pain velocity. It means that diabetes can increase the velocity of inflammatory reaction, and so it speeded up the beginning of chronic pain. Previous studies indicated that in the 4 weeks after the beginning of diabetes, there is an increased pain in quiescent phase [24].

In this study we indicated that the creation of the pain is before 4 weeks of the onset of diabetes (it begins from the first week). It is suggested in a short time of experimental diabetes, the content of substance in the peripheral nerves and the ganglion of the posterior roots of the spinal cord may be different. Therefore, it doesn't seem in such short time of diabetic period, the axons degenerate. Another subject in this study was the effect of diabetes on the Baclofen analgesia.

The former reports indicated that the hyperglycemia didn't decrease AGF44 analgesia [23, 26] but it decreases morphine analgesia [7], so because of the different effects of the hyperglycemia on the anti pain drug effect, the effect of diabetes on the Baclofen analgesia as an anti pain drug was studied and indicated that hyperglycemia didn't decrease the Baclofen analgesia, so it didn't interfere with it.

Former studies indicated that the effect of an anti pain drug in the first phase of formalin test is because of the central effects and in the second phase it is because of the peripheral effects of drug [25]. Although researchers indicated that the effect of Baclofen in the treatment of central pain is more effective than peripheral pain such as diabetic painful neuropathy [7], the literature indicated that Baclofen in the first phase didn't work as an anti pain drug, but in the second phase it was effective, so it might be that Baclofen in addition to its central effects has peripheral effects as well [5].

This research indicated that in diabetes the Baclofen analgesic effect increased in the second phase, so we can say in hyperglycemia, Baclofen is effective in peripheral pain. In spite of the fact that indicated diabetes was not effective on Baclofen analgesia in first phase of formalin test (because of increasing pain in diabetic models), Baclofen decreased it, so the result of these two factors creates no difference in the first phase and this recommends that Baclofen in hyperglycemia has both central and peripheral effects. The inhibitory effects of both GABA and Baclofen were found to be significantly greater in trachea from control rats compared with tissues from diabetic rats [17]. The results of this study showed that diabetes didn't decrease the Baclofen analgesic effect, so this drug can be useful as an anti pain drug in diabetes.

Moreover, it is useful for quieting increased pain in quiescent phase, as the third result of this study supported it. In a study Gomez and his colleagues demonstrate that Baclofen and other GABA drugs act the endocrine pancreas in vivo, ultimately increasing plasma insulin and decreasing high blood glucose levels of diabetic rats [9]. Many researchers suggest that Baclofen may be effective in delaying diabetes onset in mice by stimulating GABA activity, as this neurotransmitter, localized in the islets, may modulate insulin secretion and the antigen expression associated with it.

Another point is that unlike morphine,

diabetes didn't have any interference with Baclofen analgesia, former study indicated that hyperglycemia decreased morphine analgesic effect, so because of the interference between hyperglycemia and morphine analgesia, and also because morphine, as an opiate drug creating tolerance and addiction, is not a perfect drug, this study suggested Baclofen as a non opiate drug for quieting diabetic pain. It seems that Gabaergic system is effective in creating the quiescent phase, that was weakened with diabetes, so in addition to the hyperglycemia effect and also creation of some difficulties in the opiate endogenous system for the increase of pain in quiescent phase, we have to pay more attention to another factor such as weakness of Gabaergic system with diabetes, that after injection of Baclofen as agonist of GABAB receptors, the increased pain in quiescent phase decreased, so this, supported the probability of Gabaergic system effect on the creation of the quiescent phase.

The dosage of Baclofen in this study was 10 mg/kg; Baclofen with this dosage has an anti pain effect [5]. Although it was indicated that Baclofen analgesic effect works in a lower dose than it needs to create a disorder movement [12], due to the decrease in muscle activity in diabetes, and possibility of creating a disorder in their movement activity suggested that we have to pay more attention to the subject of the Baclofen resonance effect. The authors suggest more studies about the dosage of Baclofen.

## CONCLUSION

Diabetes creates a difference in transmission pathways and also in the pain control mechanisms, it can increase formalin induced pain that needs more study to understand the mechanism.

Diabetes creates an increased pain period that can be prevented with Baclofen and it can be used as a chronic pain model and be common with some of experimental pains in diabetes, and be suggested as a diabetic pain model.

The created pain in the quiescent phase in diabetes may also be the indication of the early second phase of pain in the formalin test.

Diabetes didn't decrease the Baclofen analgesic effect, so Baclofen as an effective drug is useful for quieting the created pain in quiescent phase as a model of diabetic pain; therefore, in the treatment of the diabetic painful neuropathy Baclofen can be a drug of choice which needs more attention.

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