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ARAŞTIRMA MAKALESİ **RESEARCH PAPER**

Investigation of *Momordica Charantia* Effects on Foot Inflammation Model in Rats and Behavioral Changes Due to This Inflammation; Experimental Model. Investigation of the Effects of Momordica Charantia[*]

Aybike TÜRKMEN[1] Ali TAGHIZADEHGHALEHJOUGHI[2,4]* Maryam MOHAMADZADEH AKIN[3] Sidika GENC[4] Ahmet HACIMUFTUOGLU[4]

[1]*Adnan Menderes University, Faculty of Veterinary Medicine, Department Pharmacology and Toxicology, 09016 Aydın, Turkey*
[2]*Ataturk University, Faculty of Veterinary Medicine, Department of Pharmacology and Toxicology, 25240 Erzurum, Turkey*
[3]*Departman of Pharmacy, Faculty of Pharmacy, Cyprus international university, 99258 North Nicosia, Northern Cyprus*
[4]*Ataturk University, Faculty of Medicine, Department of Medical Pharmacology, 25240 Erzurum, Turkey*

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: https://orcid.org/0000-0002-4119-7490
*: https://orcid.org/0000-0002-3506-0324
: https://orcid.org/0000-0003-1220-1697
: https://orcid.org/0000-0003-0000-5103
: https://orcid.org/0000-0002-9658-3313

***Corresponding author's:**
Ali TAGHIZADEHGHALEHJOUGHI
Faculty of Veterinary Medicine, Department of Pharmacology and Toxicology, Ataturk University, 25240 Erzurum, Turkey.
✉: ali.tgzd@atauni.edu.tr

Abstract: Pain is an unpleasant sensation originating from any part of the body, associated with actual or possible tissue damage, and linked to past human experiences. *Momordica Charantia* (MC) or bitter melon mainly is used all around the world for the treatment of diabetes, cancer and inflammation- associated conditions due to the existence of many bioactive ingredients which have vigorous biologic effect. The aim of our study is to investigate how low and high doses of MC plant affect foot inflammation and animal's behavior. Sixteen male rats randomly divided into 4 experimental groups. Formaldehyde was (hind-paw formalin injection) administered for inducing pain model. After 24 hours MC (50 mg/kg, 200 mg/kg) was administered every day for five days. During the experiment, the pain thresholds, locomotor activity measurements and inflamed foot volume, Locomotor Activity Test and Water Overflow Method in sequence.
According to our result, when we consider the foot volume, pain threshold and locomotor activity data of our control group, there is no significant difference between the beginning and the last day, on the 7th day, the pain threshold continues to decrease as the foot volume of our animals in our positive control group does not decrease. 200 mg concentration of MC has provided a great treatment by reducing the increasing foot volume at the same time pain threshold and motor activity was in highest level at 4th day.
Our experiment shows that MC (200 mg/ml MC) has high analgesic activity provides significant pain reduction and improvement in symptoms

Keywords: Artificial Surface, Classification, Heat Island.

Momordica Charantia'nın Ratlarda Oluşturulan Ayak İnflamasyon Modeli ve Buna Bağlı Olarak Gelişen Davranış Değişiklikleri Üzerine Etkilerinin İncelenmesi

Öz: Ağrı, vücudun herhangi bir yerinden kaynaklanan, gerçek veya olası doku hasarı ile ilişkilendirilen ve insanın geçmiş deneyimleriyle bağlantılı hoş olmayan bir histir. *Momordica charantia* (MC), güçlü biyolojik etkiye sahip birçok biyoaktif bileşenin varlığından dolayı, tüm dünyada başlıca diyabet, kanser ve iltihap ile ilişkili durumların tedavisinde kullanılmaktadır. Çalışmamızın amacı, MC bitkisinin düşük ve yüksek dozlarının ayak iltihabını ve hayvanın davranışını nasıl etkilediğini araştırmaktır.

***Sorumlu yazar:**
Ali TAGHIZADEHGHALEHJOUGHI
Veteriner Fakültesi, Atatürk Üniversitesi, Farmakoloji ve Toksikoloji Anabilim Dalı, 25240 Erzurum, Türkiye.
✉: ali.tgzd@atauni.edu.tr

On altı erkek sıçan rastgele seçilerek 4 deney grubuna ayrılmıştır. Ağrı modelini indüklemek için formaldehit (arka pençe formalin enjeksiyonu) uygulanmış ve Formaldehit uygulanmasından 24 saat sonra beş gün boyunca her gün MC (50 mg / kg, 200 mg / kg)uygulanmıştır. Deney sırasında ağrı eşikleri, lokomotor aktivite ölçümleri ve iltihaplı ayak hacmi, Lokomotor Aktivite Testi ve Su Taşma Yöntemi testleri çalışılmıştır.
Sonucumuza göre, kontrol grubumuzun ayak hacmi, ağrı eşiği ve lokomotor aktivite verilerini göz önüne aldığımızda başlangıç ile son gün arasında anlamlı bir farklılık gözlenmemiştir.7. günde ağrı eşiği düşmeye devam ederken Pozitif kontrol grubumuzdaki hayvanlarımızın ayak hacminde azalma görülmemiştir. 200 mg MC konsantrasyonu artan ayak hacmini azaltmasının yanı sıra ağrı eşiğini de azaltarak harika bir tedavi sağlamıştır. Bunların yanı sıra lokomotor aktivitenin ise 4. günde en yüksek seviyede olduğu tespit edilmiştir. Deneyimizin sonucuna göre MC'nin (200 mg / ml MC) yüksek analjezik aktiviteye sahip olduğunu, belirgin ağrı azalması ve semptomlarda iyileşme sağladığını göstermektedir.

Anahtar kelimeler: Formaldehite bağlı ağrı modeli, İnflamasyon, *Momordica charantia*.

INTRODUCTION

Pain is a pathetic feeling due to intense or damaging stimulus. The International Association for the Study of Pain's greatly used description, defines pain as "An unpleasant sensory and emotional experience related, or look alike that associated with, actual or potential tissue damage (Raja et al., 2020). Nociceptive and neuropathic pain are two main types of physical pain. Nociceptive pain can be considering as pain related to tissue injury or even potential damage: nociceptors are sensory endings on nerves that can be excited or sensitized and signal potential tissue damage. When there has been tissue injury, it is natural to have an inflammatory response and this is a good thing (Williams et al., 2012).

Inflammation is arranged body system response that is designed to help recover the tissue damage (Schug & Goddard, 2014). The inflammatory response is well organized and include blood-borne chemicals, immune system chemicals and some chemicals secreted from specialized nerve fibers (Moseley & Flor, 2012). Sprained ankle, nonspecific low back pain or neck pain, broken bones, or pulled muscles are clear example for nociceptive pain. The symptom of tissue injury such as swelling, redness and later purple or yellowing of the skin, a limb that seems damaged, increased susceptibility, to touch and movement (Smolen et al., 2010).

In the medical, FA is used in Anatomy, Histology and Pathology laboratories as a solution for embalming and fixation by employees. Formaldehyde, which is inescapably taken up exogenously to organisms, usually occurs through the skin and digestive system, and normally through the respiratory system (Cheney & Collins, 1995; Nilsson et al., 1998; Ozen et al., 2005; Restani & Galli, 1991; Songur et al., 2010; Thrasher & Kilburn, 2001; Zararsiz et al., 2007).

FA, which causes an increase in cytotoxic effects by compromising the intracellular balance, can combine with proteins, nucleic acids (DNA and RNA) and unsaturated fatty acids by non-enzymatic processes. These combinations cause inflammatory reactions, allergic reactions, cytotoxicity, necrosis and mutagenic and carcinogenic effects by developing denaturation in proteins. Also, free oxygen radicals are increased in the tissues affected by FA, and this increase speeds up the processes (apoptosis or necrosis) that induce cell death (Songur et al., 2008; Zararsiz et al., 2006).

There are available data in the literature that FA has toxic effects on the skin, respiratory system, nervous system, gastrointestinal system and urinary system, as well as allergic effects (Kim et al., 1999; Nilsson et al., 1998; Ozen et al., 2005; Songur et al., 2010; Thrasher & Kilburn, 2001; Zararsiz et al., 2007).

When Formalin test compared to other nociceptive stimulus (e.g. thermal, electrical, or mechanical stimulation), the properties of this test (i.e. a progressive pain response, which has a relatively long duration and is inevitable) are thought to most nearly imitate clinical pain (Clavelou et al., 1995; Coderre & Melzack, 1992).

The formalin test in mice or rat is a valid and reliable model of nociception and is sensitive for various classes of analgesic drugs. Hind paw injection of formalin is a model used to assess intense, short-lasting (minutes to tens of minutes) persistent pain and evaluate analgesic drugs in laboratory animals. Injection of formalin (1%-10%) to glabrous part of the hind paw creates behavioral nociceptive responses that are described by three phases (Clavelou et al., 1995; Coderre & Melzack, 1992).

The early phase of activity (0–7 min) starts instantly after injection and reflects a direct activation of peripheral nociceptors. Following this time, the interphase begins, which is defined by weakening of nociceptive behaviors. There are many information about using formalin test as an acute and tonic inflammatory pain model, containing inflammatory and chronic pain condition. The second phase (15–90 min) starts nearly 15 minutes after the injection and reflects ongoing peripheral activity and central sensitization (Azhdari-Zarmehri et al., 2013; Coderre & Melzack, 1992; Erami et al., 2012; Gheibi

et al., 2013). Also, beside of biphasic painful behaviors electrophysiological responses from dorsal horn neurons of the spinal cord, can be recorded for longer than one hour after formalin injection (Clavelou et al., 1995; Coderre & Melzack, 1992; 2012; Hunskaar, 1987).

However, the different nociceptive behaviors induced by formalin injection into the glabrous vs hairy skin are not well examined until now. Analyzing the differences in these formalin-induced nociceptive behaviors can help us to get the mechanisms underlying the nociceptive responses that happen in various phases of the formalin test.

On the other hand, *Momordica charantia* (MC), belongs to the family Cucurbitaceae and generally identify as bitter gourd or melon (Zhang et al., 2016). MC grows in tropical and subtropical zone and has been properly used in folk medicine in Pakistan, India, China and Sri Lanka for a long period of time (Polito et al., 2016). This plant has been used against of different illnesses inclusive diabetes, cancer, obesity, viral and bacterial infection , hypertension, and AIDS (Grover & Yadav, 2004), and these medicinal properties are refer to the fruits, specially under ripe fruits , seeds, leaves, and roots of the plant (Scartezzini, 2000).

The way in which a person lives and dietary habits effect to a chronic state of low grade inflammation, which can switch immune status and bowel flora. a lot of dietary constituent have the potential to lighten tendency to chronic inflammatory and can be helpful in their remedy (Çelik et al., 2019). Even so, the correlation among most of these dietary ingredients and their anti-inflammatory mechanisms is vague (Minihane et al., 2015).

MC dietary supplementation has been greatly studied to treat many diseases, such as type 2 diabetes, dyslipidemia, obesity and cancer, so showing that MC extracts have hypoglycemic and lipid-lowering features, even if clinical trials conducted until now gave inadequate results (Alam et al., 2015).

Different evidences point out that oxidative stress plays a role in chronic inflammatory diseases. Thus, oxidative stress and inflammation are nearly related pathophysiological processes that able to activate each other. MC advantageous traits dependent on its anti-oxidant and inflammatory activities (Biswas, 2016; Chao et al., 2014).

Various MC extracts were found to construct inflammation largely through NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) signaling pathway inhibition: in RAW 264.7 cells, bitter melon reduced TNF-α (tumor necrosis factor-alpha) production, induced by LPS, decreasing the expression of LPS (lipopolysaccharide) induced inflammatory genes, including those for IL1α (interleukin), IL-1β, and TNF-α.

The MC extracts also reduced NF-κB DNA binding activity and phosphorylation of p38, ERKs (extracellular signal-regulated kinases), JNKs (c-Jun N-terminal Kinases) as well as MAPKs (mitogen-activated protein kinases) (Kobori et al., 2008a; Kobori et al., 2008b). In addition, MC demonstrated reduction of LPS-induced NO and prostaglandin E2 production together with decrease of inducible NO synthase and IL-1β expression (Lii et al., 2009). Some evidences noticed that MC extracts reduced expression levels of inducible NO synthase and cyclooxygenase-2, prohibit NF-κB, and activator protein-1 (AP-1) activity by down regulation of ERKs and Akt (Hsu et al., 2013; Yang et al., 2018).

The effects of a triterpene purified from bitter melon was studied against TNF-α-induced inflammation by AMP-activated protein kinase in FL83B cells. This composite prevented the TNF-α-induced expression of inflammatory markers, containing inducible NO synthase, p65

Subunit of NF-κB, IL-1β, and TNF-α (Cheng et al., 2012; Xu et al., 2014). According to study that conducted in 2014, high-fat diet supplemented with MC is given to C57BL/6 mice, a reducing in serum C reactive protein and IL-6 concentrations together with a loss of and hyperlipidemia hyperglycemia was reported. The MC-containing diet also standardized serum levels of the cytokines proposing its duty in decreasing inflammation, obesity and insulin resistance in obese mice (Bao et al., 2013).

MATERIAL AND METHOD

Chemicals: In the study, FA (37% solution) was purchased from biokim, (Turkey), MC (1000 mg film-coated tablets), was obtained from (Izmir, Turkey)

Animals: In this trial, we used 16 male Albino Sprague Dawley rats weight 350-400 g. The experimental animals were housed and fed in plastic cages with sawdust in a natural day and night at normal room temperature (22 °C) in the laboratory. The principle supports the well-being of animals used for scientific targets, and aim to reduce their experience of suffering. This study was conducted by obtaining ethical approval from Atatürk University Experimental Animal Ethics Committee dated 16.12.2020 and numbered 36643897-000-E.2000313486.

Inflammation model in rat: Our study consists of four groups, with four animals in each group. On the first day of our experiment, basal levels of behavior, pain, foot volumes and weight of our animals were measured. After basal measurement, 0.5 ml 37% Formaldehyde injection was applied to produce mechanical hyperalgesia and allodynia. To reduce pain in animals, 400 mg of paracetamol was added to the animals' water as a painkiller.

After 24 hours of formaldehyde injection, the animals' basic behavior, pain, foot volume and weight were measured**.**

Pain assessment: Pain thresholds were measured with the aid of analgesia meter (Ugo Basile, Italy). The Randall-Selitto paw pressure test (Randall & Selitto, 1957) is a susceptible test, is used for evaluation of the pain response in animals (Hacimuftuoglu et al., 2020). This test is ground on detection of the animal threshold response to pain stimulated by the application of a continuously increasing pressure on the paws (Hacimuftuoglu et al., 2020; Randall & Selitto, 1957).

All measurements were made from the left feet of the animals until the end of the experiment.

Behavior (Locomotor activity function determination) Test: In our study, the locomotor activityactivities of animals were measured with a biotech device. Locomotor Activity Test gives an idea about the stereotypical movements and aggressiveness of the animal. The basis of the locomotor activity measurement system is a square-shaped quadrilateral with infrared light sources on each side. A square shaped plexiglass cage that is large enough to fit into this rectangle is placed. When the experimental animal makes a movement in the cage, it interrupts the communication between the mutual infrared sensors and this experiment is recorded by a recorder connected to the device according to the shape of the movement of the animal. The horizontal, vertical and ambulatory activities of animals can be recorded with the help of this system (Sturman et al., 2018).

The animals were placed in the device in such a way that they could not see each other, and their rest periods and distance traveled were measured for 15 minutes.

Evaluation of foot volumes: The measurement of foot volumes was made using the water overflow method. For this, a falcon tube was filled with water until the water reached the overflow stage and water was carried over by immersing the feet of the animals. It was brought back to the same volume by adding as much liquid as the water in the ground, and the added water was measured and used for analysis.

Statistical analysis: The analysis of the data of our study was evaluated with SPSS 21.0 program and One Way Anova method and $P<0.05$ was considered significant.

RESULTS

In our study, animals were divided into four groups (n=4). On the first day of our experiment (day 0), Pain threshold, basal levels of behavior and foot volumes were measured by Randall–Selitto analgesiometer, Locomotors Activity Test and water overflow method respectively. The animals' weights were measured before and after the experiment. Three groups of rats were (hind-paw formalin injection) injected (day 0) with 0.5 ml of 37% FA to create mechanical hyperalgesia and allodynia. Also 400 mg of paracetamol was added to the animals' water as a pain reliever to reduce pain and relieve animals. After 24 hours of formaldehyde injection, the animals' basic behavior, pain, foot volume and weight were measured. As a result of painful swelling in the foot after 24 hours, 50, 200 mg/kg concentration of MC were administered consecutively every day for five days to two groups by gavage. 1 ml distilled water IP were given to control group.

Pain assessment results: The results of our experiment are shown in Figure 1. and 2. In our results, the data obtained from the pain threshold measured on the first day and the pain thresholds on the last day were evaluated. In our control group, the pain threshold was measured as 48.75 on the 1st day, while the result on the 7th day was measured as 46.5 and there was no significant difference between the data. However, in our positive control group (FA (37% solution)), there is a 17.5 difference between the initial and last day results. This difference shows that the level of pain increases. In our treatment groups, although the threshold increased in the 50 mg/ml MC group, there was no significant difference with the basal level. At 200 mg/ml MC group results, the pain threshold increased from 43.5 to 56.25 and the pain sensation disappeared completely.

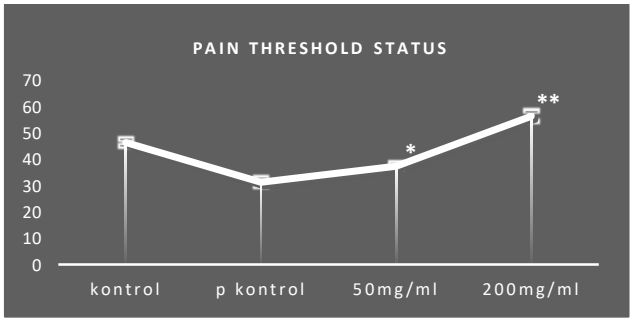

Figure 1. The results of pain threshold status.
* P<0.05, ** P<0.001 compared to P control group.

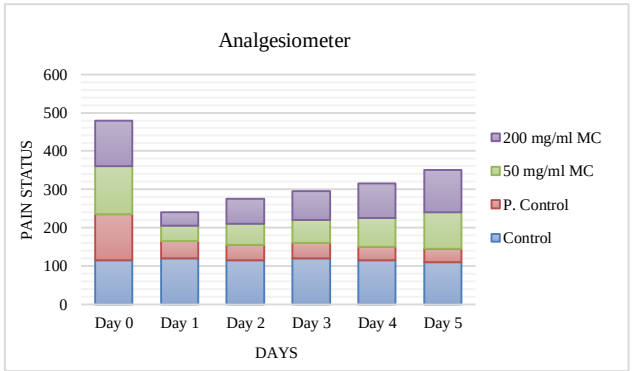

Figure 2. The results of analgesiometer.

Behavior (function determination) test results: Our data includes travel distance (Figure. 3) and resting time (Figure. 4). According to our results, mobility showed a great decrease after formaldehyde injection in all groups except control group. However, in our treatment groups, the movement started to improve from the 2nd day and the distance covered increases from the 4th day. In addition, in the 7th Day results, the range of motion became almost equal to its basal level in the 200 mg/ml MC group. However, the ability to move decreases even on the 7th day, as the inflammatory condition continues in the positive control group. Similarly, resting periods correlate with movement data. At the beginning, while resting time is longer in painful animals, with the initiation of the treatment process, the rest decreases at 50 and 200 mg/ml MC doses.

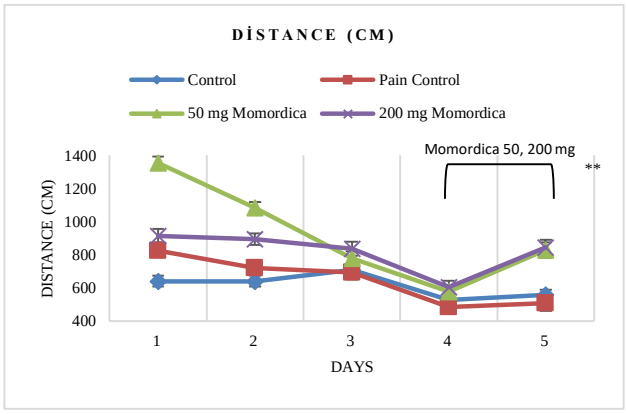

Figure 3. Locomotor activity function test (distance traveled cm)**.**
* P<0.05, ** P<0.001 compared to P control group.

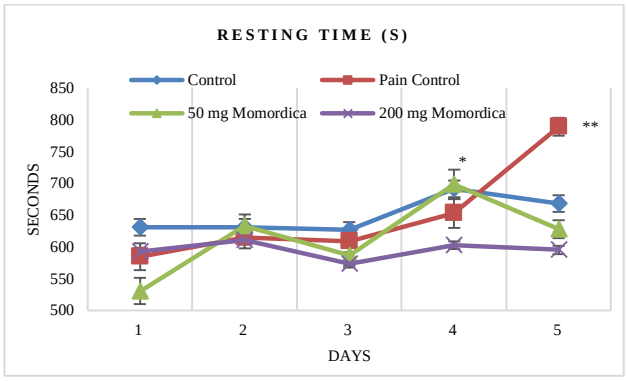

Figure 4. Locomotor activity function test (Resting time in second)**.**
* P<0.05, ** P<0.001 compared to control group.

The inflammation result: As a result of the administration of normal saline to the control group and FA to the other groups, an increase in foot volumes was observed. Although the foot volume in our control group decreased rapidly from the 2nd day to 7th day, the foot volume in the positive control group gradually increased and swelling continued to be seen at the end of the 7th day (Fig. 5). In our treatment groups, as a result of the treatment applied for 5 days after FA application, a decrease in foot volume occurred from the 4th day. However, at the end of the 7th day in high concentration, it was found that the swelling was greatly reduced (Fig. 5).

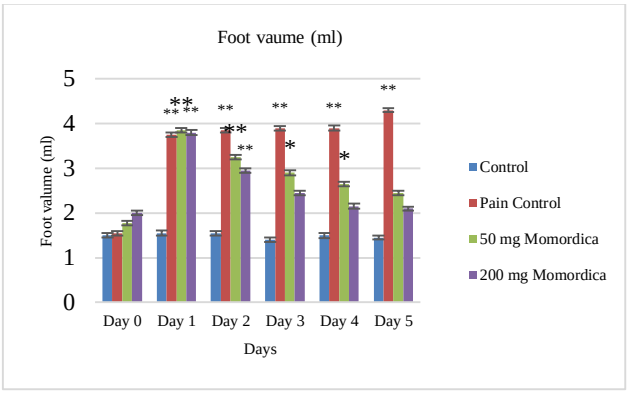

Figure 5. Inflammation test Foot total volume (ml).
* P<0.05, ** P<0.001 compared to P control group.

DISCUSSION

Hind paw injection of formaldehyde is a model used to assess persistent pain and evaluate analgesic drugs in laboratory animals. A solution of formaldehyde is injected subcutaneously into hind paw. Formaldehyde causes cell death when it comes into contact with tissues. MC is known to be a plant with antioxidant, analgesic and anti-inflammatory effects. The aim of our study is to investigate how low and high doses of MC plant affect foot inflammation and what effect they have on the animal's behavior.

In a study conducted by Raish M. et al, the mechanism of action of MC against gastric ulcer caused by ethanol in rats was investigated. Macroscopic changes, lipid peroxidation, antioxidant activity, apoptosis (caspase 3, Bax, and Bcl-2) and inflammation (tumor necrosis factor-α [TNF-α], interleukin-6 [IL-6], and myeloperoxidase [MPO]) markers were examined to determine the effect of *M. charantia*. The study showed that the prophylactic administration of MC reduced ethanol-induced gastric damage in rats through suppression of gastroenteritis and oxidative stress (Raish et al., 2018). In our study, it was observed that MC removed the inflammation in the feet in a short time. The effect of MC by reducing inflammation markers may be the mechanism that explains the elimination of inflammation in the feet in our study.

Jain V. et al. (2014) investigated the anti-nociceptive and anti-allodynic effects of MCL. In neuropathic pain caused by tibial and sural nerve transection (TST) in rats. As a result of the study, it was observed that the administration of MC (200, 400 and 800 mg / kg) for 24 days significantly reduced TST-induced behavioral changes such as cold, mechanical and heat hyperalgesia, dynamic mechanical allodynia and cold

allodynia. MC also significantly weakened TFI (tibial function index). In addition, MC treatment also prevented TST-induced increase in nerve tissue TNF-alpha and TBARS levels (Jain et al., 2014). This study correlates with our study. According to our results, mobility decreased significantly after formaldehyde injection in all groups except the control group. However, movement started to improve in our treatment groups from the 2nd day. In the 200 mg/ml MC group, his mobility became almost equal to his basal level. TNF-alpha is a prototype pro-inflammatory mediator (Kang et al., 2006). The role of TNF-alpha has been well documented in peripheral as well as central sensitization in neuropathic pain (Leung & Cahill, 2010). In addition, the decrease in TNF alpha level may be one of the possible mechanisms of our study.

It was reported in previous studies that MC alleviates diabetic neuropathy. These results show that MC has potent neuro-protective activity against global cerebral ischemia-reperfusion-induced neuronal damage and its neurological deficits in diabetic mice (Malik et al., 2011). MC has also been shown to exhibit anti-inflammatory activity in acid-induced inflammatory visceral pain in mice (Choi et al., 2002). Additionally, the effects of MC in reducing pain in patients with primary knee osteoarthritis have been studied. Thirty-eight and thirty-seven primary knee osteoarthritis patients underwent MC and placebo supplements for 3 months, respectively. It has been determined that MC supplementation provides significant pain reduction, improvement in symptoms, daily activities and quality of life. Additionally, it reduced analgesia intake and had no significant adverse effects (Soo May et al., 2018). Similarly, in our study, the pain threshold of control group was measured as 48.75 on the 1st day, while the result on the 7^{th} day was measured as 46.5 However, in our positive control group (FA (37% solution)), there is a 17.5 difference between the initial and last day results. In our treatment group (200 mg/ml MC group results), the pain threshold increased from 43.5 to 56.25 and the pain sensation disappeared completely. This shows that MC is a substance with high analgesic activity.

CONCLUSION

Injection of formaldehyde subcutaneously into hind paw cause persistent pain and local inflammation. It has been determined that MC supplementation provides significant pain reduction, improvement in symptoms, daily activities and quality of life. Our experiment shows that MC has high analgesic activity but for detection exact mechanism of action we need to future experiment and in vivo research.

REFERENCES

Alam, M.A., Uddin, R., Subhan, N., Rahman, M.M., Jain, P. & Reza, H.M. (2015). Beneficial role of bitter melon supplementation in obesity and related complications in metabolic syndrome. *Journal of Lipids*, 496169.

Azhdari-Zarmehri, H., Esmaeili, M.H., Sofiabadi, M. & Haghdoost-Yazdi, H. (2013). Orexin receptor type-1 antagonist SB-334867 decreases morphine-induced antinociceptive effect in formalin test. *Pharmacology Biochemistry Behavior*, ***112***, 64-70.

Bao, B., Chen, Y.G., Zhang, L., Na Xu, Y.L., Wang, X., Liu, J. & Qu, W. (2013). Momordica charantia (Bitter Melon) reduces obesity-associated macrophage and mast cell infiltration as well as inflammatory cytokine expression in adipose tissues. *Plos One* , ***8***, e84075.

Biswas, S.K. (2016). Does the Interdependence between Oxidative Stress and Inflammation Explain the Antioxidant Paradox? *Oxidative Medicine and Cellular Longevity*, 5698931.

Chao, C.Y., Sung, P.J., Wang, W.H. & Kuo, Y.H. (2014). Anti-inflammatory effect of Momordica charantia in sepsis mice. *Molecules*, ***19***, 12777-12788.

Cheney, J.E. & Collins, C.H. (1995). Formaldehyde disinfection in laboratories: limitations and hazards. *British Journal of Biomedical Science*, ***52***, 195-201.

Cheng, H.L., Kuo, C.Y., Liao, Y.W. & Lin, C.C. (2012). EMCD, a hypoglycemic triterpene isolated from Momordica charantia wild variant, attenuates TNF-alpha-induced inflammation in FL83B cells in an AMP-activated protein kinase-independent manner. *European Journal of Pharmacology*, ***689***, 241-248.

Choi, J., Lee, K.T., Jung, H., Park, H.S. & Park, H.J. (2002). Anti-rheumatoid arthritis effect of the Kochia scoparia fruits and activity comparison of momordin lc, its prosapogenin and sapogenin. *Archives of Pharmacal Research*, ***25***, 336-342.

Coderre, T.J. & Melzack, R. (1992). The role of NMDA receptor-operated calcium channels in persistent nociception after formalin-induced tissue injury. *Journal of Neuroscience*, ***12***, 3671-3675.

Erami, E., Azhdari-Zarmehri, H., Ghasemi-Dashkhasan, E., Esmaeili, M.H. & Semnanian, S. (2012). Intra-paragigantocellularis lateralis injection of orexin-A has an antinociceptive effect on hot plate and formalin tests in rat. *Brain Research*, ***1478***, 16-23.

Çelik, A.D., Gümüşkaya, E., Kırcı, H. & Erişir, E. (2019). The Oxidative Pretreatments of Cellulose for Cellulosic Superabsorbents. Anatolian Env. and Anim. Sciences, 4(4), 657-661. DOI: 10.35229/jaes.641313

Gheibi, N., Saroukhani, M. & Azhdari-Zarmehri, H. (2013). The effect of food deprivation on nociception in formalin test and plasma levels of

noradrenaline and corticosterone in rats. *Basic and Clinical Neuroscience*, ***4***, 341-347.

Grover, J.K. & Yadav, S.P. (2004). Pharmacological actions and potential uses of Momordica charantia: a review. *Journal of* Ethnopharmacology, ***93***, 123-132.

Hacimuftuoglu, A., Mohammadzadeh, M., Taghizadehghalehjoughi, A., Taspinar, N., Togar, B., Nalci, K.A., Okkay, U. & Gundogdu, B. (2020). The analgesic effect of metformin on paclitaxel-induced neuropathic pain model in rats: By considering pathological results. *Journal of Cancer Research and Therapeutics*, ***16***, 34-39.

Hsu, C., Fang, S., Lju, C. & Chen, Y. (2013). Inhibitory effects of new varieties of bitter melon on lipopolysaccharide-stimulated inflammatory response in RAW 264.7 cells. *Journal of Functional Foods*, ***5***, 1829-1837.

Hunskaar, S. & Hole, K. (1987). The formalin test in mice: dissociation between inflammatory and non-inflammatory pain. *Pain*, ***30***, 103-114.

Jain, V., Pareek, A., Paliwal, N., Ratan, Y., Jaggi, A.S. & Singh, N. (2014). Antinociceptive and antiallodynic effects of Momordica charantia L. in tibial and sural nerve transection-induced neuropathic pain in rats. *Nutritional Neuroscience*, ***17***, 88-96.

Kang, Y.M., Zhang, Z.H., Johnson, R.F., Yu, Y., Beltz, T., Johnson, A.K., Weiss, R.M. & Felder, R.B. (2006). Novel effect of mineralocorticoid receptor antagonism to reduce proinflammatory cytokines and hypothalamic activation in rats with ischemia-induced heart failure. *Circulation Research*, ***99***, 758-766.

Kobori, M., Nakayama, H., Fukushima, K., Ohnishi-Kameyama, M., Ono, H., Fukushima, T., Akimoto, Y., Masumoto, S., Yukizaki, C., Hoshi, Y., Deguchi, T. & Yoshida, M., (2008a). Bitter gourd suppresses lipopolysaccharide-induced inflammatory responses. *Journal of Agricultural and Food Chemistry*, ***56***, 4004-4011.

Kobori, M., Ohnishi-Kameyama, M., Akimoto, Y., Yukizaki, C. & Yoshida, M. (2008b). Alpha-eleostearic acid and its dihydroxy derivative are major apoptosis-inducing components of bitter gourd. *Journal of Agricultural and Food Chemistry* ***56***, 10515-10520.

Leung, L. & Cahill, C.M. (2010). TNF-alpha and neuropathic pain--a review. *Journal of Neuroinflammation*, ***7***, 27.

Lii, C.K., Chen, H.W., Yun, W.T. & Liu, K.L. (2009). Suppressive effects of wild bitter gourd (Momordica charantia Linn. var. abbreviata ser.) fruit extracts on inflammatory responses in RAW264.7 macrophages. *Journal of Ethnopharmacol*, ***122***, 227-233.

Malik, Z.A., Singh, M. & Sharma, P.L. (2011). Neuroprotective effect of Momordica charantia in global cerebral ischemia and reperfusion induced neuronal damage in diabetic mice. *Journal of Ethnopharmacol*, ***133***, 729-734.

Minihane, A.M., Vinoy, S., Russell, W.R., Baka, A., Roche, H.M., Tuohy, K.M., Teeling, J.L., Blaak, E.E., Fenech, M., Vauzour, D., McArdle, H.J., Kremer, B.H., Sterkman, L., Vafeiadou, K., Benedetti, M.M., Williams, C.M. & Calder, P.C. (2015). Low-grade inflammation, diet composition and health: current research evidence and its translation. *British Journal of Nutrition*, ***114***, 999-1012.

Moseley, G.L. & Flor, H. (2012). Targeting cortical representations in the treatment of chronic pain: a review. *Neurorehabil Neural Repair*, ***26***, 646-652.

Nilsson, J.A., Zheng, X., Sundqvist, K., Liu, Y., Atzori, L., Elfwing, A., Arvidson, K. & Grafstrom, R.C. (1998). Toxicity of formaldehyde to human oral fibroblasts and epithelial cells: influences of culture conditions and role of thiol status. *Journal of Dental Research*, ***77***, 1896-1903.

Ozen, O.A., Akpolat, N., Songur, A., Kus, I., Zararsiz, I., Ozacmak, V.H. & Sarsilmaz, M., (2005). Effect of formaldehyde inhalation on Hsp70 in seminiferous tubules of rat testes: an immunohistochemical study. *Toxicology and Industrial Health*, ***21***, 249-254.

Polito, L., Bortolotti, M., Maiello, S., Battelli, M.G. & Bolognesi, A. (2016). Plants Producing Ribosome-Inactivating Proteins in Traditional Medicine. *Molecules*, ***21***, 1560, 1-27.

Raish, M., Ahmad, A., Ansari, M.A., Alkharfy, K.M., Aljenoobi, F.I., Jan, B.L., Al-Mohizea, A.M., Khan, A. & Ali, N. (2018). Momordica charantia polysaccharides ameliorate oxidative stress, inflammation, and apoptosis in ethanol-induced gastritis in mucosa through NF-kB signaling pathway inhibition. *International Journal of Biological Macromolecules*, ***111***, 193-199.

Raja, S.N., Carr, D.B., Cohen, M., Finnerup, N.B., Flor, H., Gibson, S., Keefe, F.J., Mogil, J.S., Ringkamp, M., Sluka, K.A., Song, X.J., Stevens, B., Sullivan, M.D., Tutelman, P.R., Ushida, T. & Vader, K. (2020). The revised International Association for the Study of Pain definition of pain: concepts, challenges, and compromises. *Pain*, ***161***(9), 1976-1982.

Randall, L.O. & Selitto, J.J. (1957). A method for measurement of analgesic activity on inflamed tissue. *Archives Internationales de Pharmacodynamie et de Therapie*, ***111***, 409-419.

Restani, P. & Galli, C.L. (1991). Oral toxicity of formaldehyde and its derivatives. *Critical Reviews in Toxicology*, ***21***, 315-328.

Scartezzini, P. & Speroni, E. (2000). Review on some plants of Indian traditional medicine with antioxidant activity. *Journal of Ethnopharmacology*, ***71***, 23-43.

Schug, S.A. & Goddard, C. (2014). Recent advances in the pharmacological management of acute and

chronic pain. *Annals of Palliative Medicine*, ***3***, 263-275.

Smolen, J.S., Landewe, R., Breedveld, F.C., Dougados, M., Emery, P., Gaujoux-Viala, C., Gorter, S., Knevel, R., Nam, J., Schoels, M., Aletaha, D., Buch, M., Gossec, L., Huizinga, T., Bijlsma, J.W., Burmester, G., Combe, B., Cutolo, M., Gabay, C., Gomez-Reino, J., Kouloumas, M., Kvien, T.K., Martin-Mola, E., McInnes, I., Pavelka, K., van Riel, P., Scholte, M., Scott, D.L., Sokka, T., Valesini, G., van Vollenhoven, R., Winthrop, K.L., Wong, J., Zink, A. & van der Heijde, D. (2010). EULAR recommendations for the management of rheumatoid arthritis with synthetic and biological disease-modifying antirheumatic drugs. *Annals of the Rheumatic Diseases*, ***69***, 964-975.

Songur, A., Ozen, O.A. & Sarsilmaz, M. (2010). The toxic effects of formaldehyde on the nervous system. *Reviews of Environmental Contamination and Toxicology*, ***203***, 105-118.

Songur, A., Sarsilmaz, M., Ozen, O., Sahin, S., Koken, R., Zararsiz, I. & Ilhan, N. (2008). The effects of inhaled formaldehyde on oxidant and antioxidant systems of rat cerebellum during the postnatal development process. *Toxicology Mechanisms and Methods*, ***18***, 569-574.

Soo May, L., Sanip, Z., Ahmed Shokri, A., Abdul Kadir, A. & Md Lazin, M.R. (2018). The effects of Momordica charantia (bitter melon) supplementation in patients with primary knee osteoarthritis: A single-blinded, randomized controlled trial. *Complementary Therapies in Clinical Practice*, ***32***, 181-186.

Sturman, O., Germain, P.L. & Bohacek, J. (2018). Exploratory rearing: a context- and stress-sensitive behavior recorded in the open-field test. *Stress*, ***21***, 443-452.

Thrasher, J.D. & Kilburn, K.H. (2001). Embryo toxicity and teratogenicity of formaldehyde. *Archives of Environmental & Occupational Health*, ***56***, 300-311.

Williams, A.C., Eccleston, C. & Morley, S. (2012). Psychological therapies for the management of chronic pain (excluding headache) in adults. *Cochrane Library: Cochrane Reviews*, ***11***, CD007407.

Xu, J., Cao, K., Li, Y., Zou, X., Chen, C., Szeto, I.M., Dong, Z., Zhao, Y., Shi, Y., Wang, J., Liu, J. & Feng, Z. (2014). Bitter gourd inhibits the development of obesity-associated fatty liver in C57BL/6 mice fed a high-fat diet. *Journal of Nutrition*, ***144***, 475-483.

Yang, W.S., Yang, E., Kim, M.J., Jeong, D., Yoon, D.H., Sung, G.H., Lee, S., Yoo, B.C., Yeo, S.G. & Cho, J.Y. (2018). Momordica charantia Inhibits ınflammatory responses in murine macrophages via suppression of TAK1. *The American Journal of Chinese Medicine*, ***46***, 435-452.

Zararsiz, I., Kus, I., Akpolat, N., Songur, A., Ogeturk, M. & Sarsilmaz, M. (2006). Protective effects of omega-3 essential fatty acids against formaldehyde-induced neuronal damage in prefrontal cortex of rats. *Cell Biochemistry and Function*, ***24***, 237-244.

Zararsiz, I., Kus, I., Ogeturk, M., Akpolat, N., Kose, E., Meydan, S. & Sarsilmaz, M. (2007). Melatonin prevents formaldehyde-induced neurotoxicity in prefrontal cortex of rats: an immunohistochemical and biochemical study. *Cell Biochemistry and Function*, ***25***, 413-418.

Zhang, F., Lin, L. & Xie, J. (2016). A mini-review of chemical and biological properties of polysaccharides from Momordica charantia. *International Journal of Biological Macromolecules*, ***92***, 246-253.