

## Analysis of Spermatogenesis and CREM Gene Expression in Testis of Rat Treated with *Matricaria recutita*

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**Abstract:** Camp-Responsive Element Modulator (CREM) is a key factor in the regulation of the expression of number of post-meiotic genes during spermatogenesis. *Matricaria recutita* shows different pharmacological activities like anti-inflammatory, anti-cancer, treatment of stress and depression, anti-allergic and etc. The objectives of this study are to investigate effects of *Matricaria recutita* on spermatogenesis and CREM expression at mRNA level. Animals were divided into 3 groups with 7 rats in each. The extract administered orally in the doses 100 and 150 mg/kg/day to rats for 56 consecutive days. Sperm analysis and RT-PCR were carried out to investigate rat reproductive function. Results indicated significant decrease in the epididymal sperm counts and sperm motility of experimental groups compared to control group. Reverse Transcription Polymerase Chain Reaction (RT-PCR) showed a decrease in the expression of CREM in the testes of experimental groups. From the data it can be concluded that *Matricaria recutita* has anti-spermatogenic properties.

**Keywords:** CREM gene expression, *Matricaria recutita*, spermatogenesis

### INTRODUCTION

Camp-Responsive Element Modulator (CREM) belongs to the CAMP-Responsive Element Binding Protein (CREB) family, multiple isoforms of CREM that act either as repressors or activators (Foulkes and Sassone-Corsi, 1992). CREB family play important roles in regulating transcription in response to various stresses, metabolic and developmental signals (Sassone-Corsi, 1995; Hummler *et al.*, 1994). CREM transcription factors have been known to function in many physiological systems, including cardiac function (Müller *et al.*, 2003; Isoda *et al.*, 2003), circadian rhythms (Sassone-Corsi, 2000), pituitary function and memory and long-term potentiation, locomotion and spermatogenesis (Sassone-Corsi, 1998). During puberty, activator isoforms of CREM (CREM $\tau$ ) accumulated in pachytene spermatocytes and spermatids, while the CREM $\tau$  protein can only be detected in haploid spermatids (Delmas *et al.*, 1993). CREM deficient mouse was sterile due to post-meiotic arrest at the first step of spermiogenesis (Weinbauer *et al.*, 1998; Peri and Serio, 2000). *Matricaria recutita*, once it has been called as *Marticaria chamomilla*, *Chamomilla recutita* and *Chamomilum nobile* family Asteraceae and commonly it is known as German chamomile, Roman chamomile, English chamomile, Camomilla and Flos Chamomile (WHO), (Micromedex Healthcare Series, 2003). It mainly grows indigenously

in Europe, NW. Asia and N. Africa and cultivated in N. America and in many parts of the world (Wald and Brendler, 1998). This herb has been used as herbal remedies for thousands of years. One of the most commonly consumed single ingredient herbal tea is chamomile, prepared with dried flowers from *Matricaria recutita*. The composite flower is white in color with a yellowish orange center (WHO). Infusions and essential oils from fresh or dried flower heads have aromatic, flavoring and coloring properties. Both are used in a number of commercial products including soaps, detergents, perfumes, lotions, ointments, hair products, baked goods, confections, alcoholic beverages and herbal teas. Chamomile flowers contain 0.24-2.0% volatile oil that is blue in color (Wald and Brendler, 1998). *Matricaria recutita* is well known for its pharmaceutical properties including; anti-inflammatory (Shipochliev *et al.*, 1981; Al-Hindawi *et al.*, 1989), immunomodulatory activity (Uteshev *et al.*, 1999), arcarcicadal property (Macchioni *et al.*, 2004), anti-cancer activity (Srivastava and Gupta, 2007), antipruritic effect (Kobayashi *et al.*, 2005), wound healing property (Nayak *et al.*, 2007; Jarrahi, 2008; Jarrahi *et al.*, 2008; Martins *et al.*, 2009), treatment of oral mucositis (Mazokopakis *et al.*, 2005), intracanal irrigant (Sadr Lahijani *et al.*, 2006), Treatment of infant botulism (Bianco *et al.*, 2008), treatment of gastrointestinal disorders (Mahady *et al.*, 2005), antimicrobial activity (Nogueira *et al.*, 2008), antiulcer

Table 1: The effects of *Matricaria recutita* extract on sperm count and motility of adult male rat

| Parameters                              | Control          | Experimental 1    | Experimental 2    |
|---|------------------|-------------------|-------------------|
| Sperm count ( $\times 10^3/\text{mL}$ ) | 48.29 $\pm$ 1.53 | 42.71 $\pm$ 1.14* | 42.14 $\pm$ 0.73* |
| Sperm motility (%)                      | 74.23 $\pm$ 0.43 | 65.67 $\pm$ 0.36* | 64.97 $\pm$ 0.47* |

Values are mean $\pm$ SEM (n = 7); \*: p<0.05 (significantly different) vs. control

activity (Khayyal *et al.*, 2001), treatment of stress and depression (Pinto *et al.*, 2008), anti-allergic activity (Kobayashi *et al.*, 2003), antisolar agent (Ramos *et al.*, 1996), inhibition of poliovirus replication (Vilaginès *et al.*, 1985), anxiolytic agent (Amsterdam *et al.*, 2009), Prevent osteoporosis (Kassi *et al.*, 2004). With regard to these properties, this study was undertaken to evaluate the effects of ethanolic extract of *Matricaria recutita* on spermatogenesis and CREM gene expression, since up to now no pharmacological evaluation has been done on *Matricaria recutita* for these effects.

## MATERIALS AND METHODS

**Plant material:** *Matricaria recutita* collected from Agricultural Research institute at University of Zabol, Zabol, Iran, in March 2011 and authenticated at Department of Biology, University of Zabol. Its leaves, fruits and flowers were dried, under shade and powdered. The extract was prepared by maceration method (80% ethanol in 300 g/L for 48 h), filtered with filter paper. Ethanol was removed by rotary evaporator. The extract was dissolved in normal saline and administered orally into rats.

**Animals and treatments:** Twenty one Healthy adult male wistar rats (10-12 weeks old, 200-300 g), purchased from Razi Institute (Karaj, Iran) and were housed in animal house, at ambient room temperature with a controlled light and dark period of 12 h. The animals were fed with a standard laboratory food (pellets) and provided ad libitum. They were weighted before and after the study. After 7 days for adapting to the new environment, the rats were randomly divided into 3 groups of control (treated with normal saline for the period of 56 days, n = 7), experimental 1 (treated with 100 mg/kg/day of *Matricaria recutita* extract for 56 days, n = 7) and experimental2 (treated with 100 mg/kg/day of *Matricaria recutita* extract for 56 days, n = 7) groups.

**Tissue preparation:** At the end of the treatment period, the pentobarbital sodium (40 mg/kg i.p.) was administered for anesthesia. Testes were removed, cleaned of adhering tissue and weighed. The epididymis was removed, used for sperm analysis and testes samples were frozen before RT-PCR.

**Sperm analysis:** Epididymis was exposed by scrotal incision and sperms were expressed out by cutting distal end of the caudal epididymidal tubule. Sperms

with epididymal fluid were diluted with physiological saline and sperm motility and morphology were studied. Sperm suspension was placed on both sides of Neubauer's hemocytometer and allowed to settle in a humid chamber for 1 h. The number of sperms in the appropriate squares of the hemocytometer was counted under the microscope of 100 magnifications.

**RNA isolation and RT-PCR:** Total RNA from the testes was extracted by RNeasy Mini Kit (QIAGEN, USA.) Total RNA samples were analyzed using gel electrophoresis. The final amount of RNA was estimated by determining the optical density at 260 nm. First strand cDNA synthesis with total RNA was performed using reverse transcriptase. Subsequently, PCR-amplification was performed. The sequence of CREM primers were 5'-GATTGAAGAAGAAAA ATCAGA-3' (forward primers, exon B) and 5'-TTGACATATTCTTTCTTCTT-3' (reverse primer, exon H), while for rat GAPDH, 5'-GGTGGTAGCAGT GAATGA-3' and 5'-CCA CGTTTCCTTTGTATCT-3' was used. The PCR products were separated on 1.5% agarose gels visualized under UV light and analyzed using NTSYS software.

**Statistical analysis:** Data were expressed as mean $\pm$ standard deviation. Student's t-test was used to compare means. A level of p<0.05 was considered as statistically significant.

## RESULTS

Our data indicated that administration of *Matricaria recutita* extract (100 and 150 mg/kg/day) for 56 consecutive days, has significantly reduced sperm counts and motility in experimental groups in comparison with control group (p<0.05) (Table 1). Reverse transcription polymerase chain reaction (RT-PCR) was performed to evaluate the effect of *Matricaria recutita* extract (100 and 150 mg/kg/day) on CREM gene expression in rat testes. The CREM fragment was detected at 520 bp. CREM mRNA levels has not significantly difference in testes from the experimental groups in compared to control group (data not shown).

## DISCUSSION

Whereas no attempt appears to have been made, so far, to determine the effects of *Matricaria recutita* on male rat fertility with emphasis on CREM gene expression, we were interested in doing this research.

*Matricaria recutita* has a large history of herbal use. Our results indicated that administration of *Matricaria recutita* extract creates marked reduction in the sperm counts and motilities compared with control group. A significant reduction in the number and motility of sperm ( $p < 0.05$ ) was observed in experimental groups, which could be due to the influence of the extract on the cell cycle, cell division and expression of genes necessary for the spermatogenesis. It is also possible that these changes might be result of changes in the microenvironment of epididymis and creation of a toxic microenvironment presence in *Matricaria recutita* extract, thus influencing sperm count and motility. CREM is essential for spermatogenesis and males lacking functional CREM gene are sterile due to their round spermatid maturation arrest (Foulkes and Sassone-Corsi, 1992; Sassone-Corsi, 1995). The CREM gene encodes the transcription activator CREM, which is highly expressed in male germ cells (7-9) and regulates the expression of many important post-meiotic genes (3, 4). In the present study, testes of rats treated with *Matricaria recutita* exhibited no significant difference in CREM mRNA level, in comparison with control group. Given the results, it is concluded that *Matricaria recutita* has specific effects on sperm parameters and reproductive functions of male rats however the level of CREM expression is equal between 3 groups of our study. The decreasing effect of *Matricaria recutita* on spermatogenesis may lead to the manufacturing of new drugs for regulation of spermatogenesis and fertility in males.

## REFERENCES

- Al-Hindawi, M.K., I.H. I-Deen, M.H. Nabi and M.A. Ismail, 1989. Anti-inflammatory activity of some Iraqi plants using intact rats. *J. Ethnopharmacol.*, 26(2): 163-8.
- Amsterdam, J.D., Y. Li, I. Soeller, K. Rockwell, J.J. Mao and J. Shults, 2009. A randomized, double-blind, placebo-controlled trial of oral *Matricaria recutita* (chamomile) extract therapy for generalized anxiety disorder. *J. Clin. Psychopharmacol.*, 29(4): 378-82.
- Bianco, M.I., C. Lúquez, L.I. De Jong and R.A. Fernández, 2008. Presence of clostridium botulinum spores in *Matricaria chamomilla* (chamomile) and its relationship with infant botulism. *Int. J. Food Microbio.*, 121(3): 357-60.
- Delmas, V., F. Van der Hoorn, B. Mellström, B. Jégou and P. Sassone-Corsi, 1993. Induction of CREM activator proteins in spermatids: Downstream targets and implications for haploid germ cell differentiation. *Mol. Endocrinol.*, 17: 1502-1514.
- Foulkes, N.S. and P. Sassone-Corsi, 1992. More is better: Activators and repressors from the same gene. *Cell*, 68(3): 411-414.
- Hummler, E., T.J. Cole, J.A. Blendy, R. Ganss, A. Aguzzi, W. Schmid, F. Beermann and G. Schutz, 1994. Targeted mutation of the CREB gene: Compensation within the CREB/ATF family of transcription factors. *Proc. Natl. Acad. Sci. USA*, 91(12): 5647-5651.
- Isoda, T., N. Paolucci, C. Haghghi, Y. Wang, D. Wang, G. Georgakopoulos, M.A. Servillo, E.G. Della Fazi, A.A. Kranias, P. Depaoli-Roach and P. Sassone-Corsi, 2003. Novel regulation of cardiac force-frequency relation by CREM (cAMP response element modulator). *FASEB J.*, 17: 144-151.
- Jarrahi, M., 2008. An experimental study of the effects of *Matricaria chamomilla* extract on cutaneous burn wound healing in albino rats. *Nat. Prod. Res.*, 22(5): 422-427.
- Jarrahi, M., A.A. Vafaei, A.A. Taherian, H. Miladi and A. Rashidi Pour, 2008. Evaluation of topical *Matricaria chamomilla* extracts activity on linear incisional wound healing in albino rats. *Nat. Prod. Res.*, 22(14): 1197-202.
- Kassi, E., Z. Papoutsi, N. Fokialakis, I. Messari, S. Mitakou and P. Moutsatsou, 2004. Greek plant extracts exhibit Selective Estrogen Receptor Modulator (SERM)-like properties. *J. Agric. Food Chem.*, 52(23): 6956-6961.
- Khayyal, M.T., M.A. El-Ghazaly, S.A. Kenawy, M. Seif-el-Nasr, L.G. Mahran, Y.A. Kafafi and S.N. Okpanyi, 2001. Antiulcerogenic effect of some gastrointestinally acting plant extracts and their combination. *Arzneimittelforschung*, 51(7): 545-53.
- Kobayashi, Y., Y. Nakano, K. Inayama, A. Sakai and T. Kamiya, 2003. Dietary intake of the flower extracts of German chamomile (*Matricaria recutita* L.) inhibited compound 48/80-induced itch-scratch responses in mice. *Phytomedicine*, 10(8):657-64.
- Kobayashi, Y., R. Takahashi and F. Ogino, 2005. Antipruritic effect of the single oral administration of German chamomile flower extract and its combined effect with antiallergic agents in ddY mice. *J. Ethnopharmacol.*, 101(1-3): 308-312.
- Macchioni, F., S. Perrucci, F. Cecchi, P.L. Cioni, I. Morelli and S. Pampiglione, 2004. Acaricidal activity of aqueous extracts of camomile flowers, *Matricaria chamomilla*, against the mite *Psoroptes cuniculi*. *Med. Vet. Entomol.*, 18(2): 205-207.
- Mahady, G.B., S.L. Pendland, A. Stoia, F.A. Hamill, D. Fabricant, B.M. Dietz and L.R. Chadwick, 2005. In vitro susceptibility of helicobacter pylori to botanical extracts used traditionally for the treatment of gastrointestinal disorders. *Phytother Res.*, 19(11): 988-991.

- Martins, M.D., M.M. Marques, S.K. Bussadori, M.A. Martins, V.C. Pavesi, R.A. Mesquita-Ferrari and K.P. Fernandes, 2009. Comparative analysis between *Chamomilla recutita* and corticosteroids on wound healing. An in vitro and in vivo study. *Phytother Res.*, 23(2): 274-278.
- Mazokopakis, E.E., G.E. Vrentzos, J.A. Papadakis, D.E. Babalis and E.S. Ganotakis, 2005. Wild chamomile (*Matricaria recutita* L.) mouthwashes in methotrexate-induced oral mucositis. *Phytomedicine*, 12(1-2): 25-27.
- Micromedex Healthcare Series, 2003. Micromedex, Inc., Englewood, Colorado, Vol. 115, Expires 3.
- Müller, F.U., G. Lewin, G. Matus, J. Neumann, B. Riemann, J. Wistuba, G. Schütz and W. Schmitz, 2003. Impaired cardiac contraction and relaxation and decreased expression of sarcoplasmic Ca<sup>2+</sup>/ATPase in mice lacking the CREM gene. *FASEB J.*, 17(1): 103-105.
- Nayak, B.S., S.S Raju and A.V. Rao, 2007. Wound healing activity of *Matricaria recutita* L. extract. *J. Wound Care*, 16(7): 298-302.
- Nogueira, J.C., F. Diniz Mde and E.O. Lima, 2008. In vitro antimicrobial activity of plants in acute otitis externa. *Braz. J. Otorhinolaryngol.*, 74(1): 118-124.
- Peri, A. and M. Serio, 2000. The CREM system in human spermatogenesis. *J. Endocrinol. Invest.*, 23: 578-583.
- Pinto, S.A., E. Bohland, P. Coelho Cde, M.S. Morgulis and L.V. Bonamin, 2008. An animal model for the study of *Chamomilla* in stress and depression: Pilot study. *Homeopathy*, 97(3): 141-144.
- Ramos, M.F., E.P. Santos, C.H. Bizarri, H.A. Mattos, M.R. Padilha and H.M. Duarte, 1996. Preliminary studies towards utilization of various plant extracts as antisolar agents. *Int. J. Cosmet. Sci.*, 18(3): 87-101.
- Sadr Lahijani, M.S., H.R. Raof Kateb, R. Heady and D. Yazdani, 2006. The effect of German chamomile (*Matricaria recutita* L.) extract and tea tree (*Melaleuca alternifolia* L.) oil used as irrigants on removal of smear layer: A scanning electron microscopy study. *Int. Endod. J.*, 39(3): 190-195.
- Sassone-Corsi, P., 1995. Transcription factors responsive to cAMP. *Annu. Rev. Cell Dev. Biol.*, 11: 355-377.2.
- Sassone-Corsi, P., 1998. CREM: A master-switch governing male germ cell differentiation and apoptosis. *Semin. Cell Dev. Biol.*, 9: 475-482.
- Sassone-Corsi, P., 2000. CREM: A master-switch regulating the balance between differentiation and apoptosis in male germ cells. *Mol. Reprod. Dev.*, 56: 228-229.
- Shipochliev, T., A. Dimitrov and E. Aleksandrova, 1981. Anti-inflammatory action of a group of plant extracts. *Vet. Med. Nauki.*, 18(6): 87-94.
- Srivastava, J.K. and S. Gupta, 2007. Antiproliferative and apoptotic effects of chamomile extract in various human cancer cells. *J. Agric. Food Chem.*, 1455(23): 9470-9478.
- Uteshev, B.S., I.L. Laskova and V.A. Afanasev, 1999. The immunomodulating activity of the heteropolysaccharides from grape seed during air and immersion cooling. *Eksp. Klin. Farmakol.*, 62(6): 52-55.
- Vilagines, P., P. Delaveau and R. Vilagines, 1985. Inhibition of poliovirus replication by an extract of *Matricaria chamomilla*. *C R Acad. Sci. III.*, 301(6): 289-294.
- Wald, G. and T. Brendler, 1998. PDR for Herbal Medicines. 1st Edn., Lyle Medical Economics Company Publishers, Montville, NJ., pp: 1742.
- Weinbauer, G.F., R. Behr, M. Bergmann and E. Nieschlag, 1998. Testicular CAMP Responsive Element Modulator (CREM) protein is expressed in round spermatids but is absent or reduced in men with round spermatid maturation arrest. *Mol. Hum. Reprod.*, 4: 9-15.