Available online at www.derpharmachemica.com

ISSN 0975-413X
CODEN (USA): PCHHAX

Der Pharma Chemica, 2016, 8(14):216-222
(http://derpharmachemica.com/archive.html)

Ethnobotanical study and Pharmacological properties of *Chelidonium Majus*

Sepideh Miraj

Infertility Fellowship, Medicinal Plants Research Center, Shahrekord University of Medical Sciences, Shahrekord, Iran

---

ABSTRACT

Chelidonium Majusto the plant family of Asteraceae , native to temperate Asia, but naturalized in many countries including scattered parts of North America. The aim of this study was to overview its Anti-Malarial properties, Immunosuppressive properties, Anti-inflammatory properties, Anti-cancer properties. This review article was carried out by searching studies in PubMed, Medline, Web of Science, and IranMedex databases .The initial search strategy identified about 98 references. In this study,46 studies was accepted for further screening and met all our inclusion criteria [in English, full text, therapeutic effects of Chelidonium majusand dated mainly from the year up to 2016.The search terms were "Artemisia annua", "therapeutic properties", "pharmacological effects". It was said to be good for cancer treatment. Anti-tumor effect, Anti-microbial effect, Hepato-toxicity, Antioxidant effect, Hepatocarcinogenesis effect , Apoptogenic activity effect, Proapoptotic activity effect, Immunomodulatory activity effect, The antimitotic effects, Chemical integrity effect, Anti-inflammatory and analgesic activities effect, Oxidative stress effect, Inflammation effect, Proinflammatory effect, Skin problem effect, Liver effect, Antigenicity and ant ovalbumin effect ,Inhibition of the serotoninergic system effect. Chelidonium majususedfor the treatment of various diseases and possess lots of effects. In this study, Antioxidant effect, Anti-inflammatory and anti-cancer properties of this plant are presented using published articles in scientific sites. Besides, it was said to be good for skin problem treatment.

Keywords: Chelidonium Majus, Phytochemicals, Therapeutic effects, Pharmacognosy, Alternative and complementarymedicine

---

INTRODUCTION

It is proved that herbal medicine is effective in the treatment of many diseases[1-10].*Chelidonium majus*, belongs to the buttercup family, is a herbaceous perennial plant, the only species in the genus Chelidonium. It is native to Europe and western Asia and introduced widely in North America . Greater celandine is a perennial herb with an erect habit, and reaches 30 to 120 cm high. The leaves are pinnate with lobed and wavy-edged margins, up to 30 cm long. When injured, the plant exudes a yellow to orange latex [2].

The flowers consist of four yellow petals, each about 1 cm long, with two sepals. A double-flowered variety occurs naturally. The flowers appear from late spring to summer inumbelliform cymes of about 4 flowers [3]. The seeds are small and black, borne in a long, cylindrical capsule. Each has anelaiosome, which attracts ants to disperse the seeds .It is considered an aggressive invasive plant in natural areas. Control is obtained mainly via pulling or spraying the plant before seed dispersal. The whole plant is toxic in moderate doses as it contains a range of is oquinolinealkaloids but there are numerous therapeutic uses when used at the correct dosage. The main alkaloid

present in the herb and root is coptisine [4]. Other alkaloids present include methyl 2'-[7, 8-dihydrosanguinarine-8-yl] acetate, allocryptopine, stylopine, protopine, norchelidonine, berberine, chelidonine, sanguinarine, chelerythrine, and 8-hydroxydihydrosanguinarine. Some alkaloids have shown potential activity against methicillin-resistant Staphylococcus aureus [5].

The effect of the fresh herb is of a mild analgesic, cholagogic, antimicrobial, oncostatic and central nervous system sedative in in vitro experiments on animal cells celandine is shown to be cytostatic. An immune stimulating effect has also been noted. Some studies show that the alkaloid extraction can have the same effects. The alkaloids are known to cause immobilization in mice after being ingested orally or injected. The alkaloids cause limpness and tone reduction of smooth muscle in rabbits. The alkaloids are also noted to stimulate the heart and lungs of frogs, cats and dogs, raising the blood pressure and widening the arteries [6].

The latex could be employed for cauterizing small open wounds. Early studies of celandine showed that it causes contact dermatitis and eye irritation, particularly from contact with the poisonous red to yellow latex of the stem. This effect has not been observed in animal studies; no inflammation was observed in rabbit eye tests. The latex can leave a non-permanent stain. Stains on skin of the fingers are sometimes reported to cause eye irritation after rubbing the eyes or handling contact lenses. The latex is also known to stain clothes [7, 8].

The characteristic latex also contains proteolytic enzymes and the phytocystatin chelidostatin, a protease inhibitor. These co-constituents could explain the topical use of greater celandine against warts and moles [9].

"Ukrain" [Ukrainian: Укpаїн] is an alternative medicine promoted to treat cancer based on Chelidonium. The drug was created in 1978 by the Ukrainian chemist VasylNovytskyi. The drug is named in honor of the nation of Ukraine and is produced by an Austrian company Nowicky Pharma. Although the drug was never approved by any regulators, Novytskyi claimed it to be a complete cure for all cancers, radiation-induced diseases and AIDS and was arrested in Vienna for aggravated fraud on September 4, 2012. C. majus has shown analgesic activity at 200 mg/kg dose in mice [10].

Anti-cancer

The effects of naturally occurring homochelidonine in comparison to chelidonine on cell cycle progression and cell death in leukemic T-cells with different p53 status were reported. The data indicate that chelidonine and homochelidonine are potent inducers of cell death in cancer cell lines, highlighting their potential relevance in leukemic cells [11].

The blockage effect of GU and CM obviously depends on the doses used. GU and CM could also impede the occurrence of stomach cancer induced by MNNG, and the impeding rate is about sixty percent [12].

Only three different cases treated with preparation Ukrain. Result showed that Ukrain can be helpful in improving the general condition and prolonging life by reduction of the tumour progression and its immunomodulation effect on the organism [13].

In vitro possibility that Ukrain may influence the phenotype of lymphocyte subpopulations isolated from healthy donors was evaluated[14].

The preliminary results of two independent clinical trials conducted with the preparation "Ukrain", containing thiophosphoric acid alkaloid derivatives from the plant *Chelidonium majusL*. [greater celandine] was summarizedin order to investigate whether it has immunopotentiating properties in cancer patients. Result shows that some therapeutic benefit from the use of *Chelidoniummajus* ["Ukrain"] as an immunostimulant in cancer patients can be achieved [15].

The biological effect of two nucleases isolated from *Ch. majus* milk sap, CMN1 of 20 kDa and CMN2 of 36 kDa, on HeLa and CHO tumour cell lines was evaluated. Results of this study show that purified nucleases CMN1 and CMN2 isolated from *Ch. Majus* milky sap exhibit apoptotic activity in HeLa tumour cell line, but not in CHO cells, without inflammatory reaction [16].

The effect of lectin from *Chelidonium majusL* on normal and cancer cells in culture in vitro was described. Evident apoptotic lesions were observed in CHO cells and less well marked apoptotic lesions in R2C cells. In contrast, only insignificant numbers of fibroblasts reacted to the applied lectin [17].

The synergistic biological action of five celandine alkaloids in normal and cancer cells was investigated. The results showed a differential ability of celandine alkaloids to penetrate into the normal and cancer cell interior, which was inversely proportional to their cytotoxic activity. Cytotoxicity tests demonstrated selective and profound apoptotic effects of a five-alkaloid combination in the mouse melanoma B16F10 cell line[18].

Anti-cancer effect of chelidonine and an alkaloid extract from *Chelidonium majus*on overcoming MDR of different cell lines was demonstrated. *Chelidonine* and the alkaloid extract inhibited P-gp/MDR1 activity in a concentration-dependent manner in Caco-2 and CEM/ADR5000 and reversed their doxorubicin resistance. In addition, chelidonine and the alkaloid extract inhibited the activity of the drug modifying enzymes CYP3A4 and GST in a dose-dependent manner. [19].

The effects of C. majus L. extract on human epidermoid carcinoma A431 cells through multiple mechanisms was investigated. C. majus L. extract not only inhibited NF-κB activation, but it also activated p38 MAPK and MEK/ERK signaling. Taken together, these results demonstrate that C. majus L. extract inhibits the proliferation of human epidermoid carcinoma A431 cells by inducing apoptosis through caspase activation and NF-κB inhibition via MAPK-independent pathway [20].

One new alkaloid, together with 10 known compounds were isolated from the aerial parts of *Chelidonium majus*L. by repeated silica gel column chromatography. These compounds were screened for cytotoxicity against human non-small lung carcinoma, breast cancer and liver cancer. In a series of cytotoxic tests, compounds 9 and 10 displayed potent cytotoxic activity against H1299, MCF-7 and SMMC-7721, with the IC50 values of 8.16-35.25 μg/mL [21].

Anti-tumor effect

The effects of the drug on cell survival, alteration of the cell cycle and induction of apoptosis were examined without and in combination with ionizing radiation (IR). The TP53 status of the cell lines used was also investigated. Differential effects of Ukrain in modulating radiation toxicity of human cancer cell lines and its protective effect in normal human fibroblasts suggest that this alkaloid may have potential properties for clinical radio chemotherapy (22).no evidence was found for the selective cytotoxicity previously reported for Ukrain(TM) [23].

The antitumour activity of a C. majus extract in vitro and in vivo was investigated. Low to almost no cytotoxic effect was observed on primary endometrium cancer cells PC-EM005, PC-EM002 and on normal fibroblast cells 3T3, when treated with CM2B. Besides, less metastases were counted in mice treated with 1.2 mg/kg CM2B, but not with 3.6 mg/kg Ukrain(TM), compared to the control group. The extract did not affect the weight of the primary tumours [24].

Direct cytotoxic effects, as well as indirect antitumor effects of *Chelidonium majus*ethanolic extract against different tumor cell lines was investigated. The results indicate possible usefulness of *Chelidonium majus*crude extract in antitumor therapy, whether through its direct cytotoxic effect, by prevention of metastasis, or as adjuvant therapy [25].

Anti-microbial

A shotgun proteomic approach combined with label-free protein quantitation according to the exponentially modified protein abundance index (emPAI) was examined. Result revealed a similar stress and defense-related protein composition of pharmacologically active plant species and showed the presence of different pathogenesis-related and low molecular inducible antimicrobial peptides. These findings could form the basis for further elucidation of the mechanism of the strong pharmacological activities of these medicinal plant extracts [26].

Hepato-toxicity

Both the potencies of Chel exhibited anti-tumor and anti-oxidative stress potential against artificially induced hepatic tumors and hepato-toxicity in rats. More studies are warranted [27].

Antioxidant
In an in vitro study, antioxidant effect of a methanol extract isolated from the greater celandine *Chelidonium majus* L. (CME) was investigated.it was concluded that the extract of C. majus L. had a strong antioxidant potential and exerted the antiproliferative activity via apoptosis on leukemia cells. CME due to the presence of the isoquinoline alkaloids and the flavonoid components may play an important role in both cancer chemoprevention through its antioxidant activity and modern cancer chemotherapy as cytotoxic and apoptosis-inducing agent [28].

Hepatocarcinogenesis
the ability of *Chelidonium majus*to potentiate the hepatic effect of a sub-toxic dose of acetaminophen, in rats wasassessed. C. majus does not modify the hepatic effects of acetaminophen in our in vivo experimental model(29). Several cytogenetical and enzymatic protocols were used to test if two microdoses of Chelidoniummajus, namely Chelidonium-30 (Ch-30) and Chelidonium-200 (Ch-200), used as homeopathic drugs, showed anti-tumor activity and also favorably modulated genotoxic damages produced by an azo dye in mice at several intervals of fixation [30].

Anti-tumor, hepato-protective and anti-genotoxic effects of Ethanolic whole plant extract of *Chelidonium majushas* been examined. The results suggest anti-tumor, anti-genotoxic and hepato-protective effects of the plant extract, showing potentials for use in cancer therapy [31].

Apoptogenic activity
The involvement of mitochondria in apoptosis induction by both alkaloids was supported by cytochrome C elevation in cytosol, with an accompanying decrease in cytochrome C content in the mitochondrial fraction. It was shown earlier, that CHE, in contrast to SAN, does not interact directly with DNA. This fact is in line with DNA damaging effects of the alkaloids detected in the COMET assay. Nevertheless, apoptosis-inducing activity of CHE even slightly exceeded that of SAN [32].

Proapoptotic activity
The importance of apoptosis induction for the antineoplastic activity of Ukrain was examined. Apart from sanguinarine and chelerythrine, chelidonine turned out to be a potent inducer of apoptosis triggering cell death at concentrations of 0.001 mM, while protopine and allocryptopine were less effective. Similar to Ukrain, apoptosis signalling of chelidonine involved Bcl-2 controlled mitochondrial alterations and caspase-activation [33].

Immunomodulatory activity
The immunostimulatory characteristics have been investigated in several experiments such as generation of activated killer (AK) cells, proliferation of splenocytes, activation of macrophages and granulocyte macrophage-colony forming cell (GM-CFC) assay. It showed mitogenic activity on both spleen cells and bone marrow cells. CM-Ala induced proliferation of splenocytes by 84 fold and increased GM-CFC numbers by 1.48 fold over than the non-treated. On the contrary, CM-Ala had cytotoxic activity to a diverse group of tumor cells. [34].

The antimitotic effects
The antimitotic actions of Ukrainwas found to be reversible in low doses in vitro, as shown by flow cytometry and concurrent haematoxylin and eosin stains. We hypothesize that the lack of side-effects found in vivo may be due to the lack of therapeutically effective dosages being administered, therefore enabling cells to overcome the metaphase arrest and survive [35].

Chemical integrity
Ukrain has been described as a semi-synthetic *Chelidonium majus*alkaloid derivative, consisting of three chelidonine alkaloids combined to triaziridide. We found the actions of Ukrain to be similar to the Chelidonium alkaloids it is prepared from, and therefore became concerned about its chemical integrity. Chemical analyses of Ukrain by thin layer chromatography, high-performance liquid chromatography and liquid chromatography-mass spectrometry was inconsistent with the proposed trimeric structure and demonstrated that at least some commercial preparations of Ukrain consist of a mixture of C. majus alkaloids (including chelidonine)[36].

Anti-inflammatory and analgesic activities
Analgesic activity ("hot plate" test), anti-inflammatory activity (carrageenan-induced paw edema) and locomotor activity was evaluated in rats under the influence of three fractions of *Chelidonium majus*herb extract. The precise

mechanisms involved in the production of anti-nociceptive and anti-inflammatory responses of studied fractions are not completely understood, but they may be caused rather by the presence of protein more than alkaloids-enriched fraction. This fraction of the extract could be used as an alternative therapy for the prevention of inflammatory-related diseases in the future, but further studies are needed [37].

Oxidative stress

The possible protective potentials of chelidonine and its poly lactide-co-glycolide (PLGA) encapsulated nano-form against cadmium chloride ($CdCl_2$) induced oxidative stress and hepatotoxicity in mice, ex vivo and in vivo was evaluated. Result showed that Expression pattern of certain inflammatory and apoptotic signal proteins also indicated better hepato-protective abilities of nano-chelidonine, making it a more suitable protective drug than chelidonine against cadmium toxicity in mice [38].

Anti-Inflammatory effect

Chelidonine effect and mechanism in airway inflammation in a mouse model of allergic asthma was investigated. Chelidonine has profound inhibitory effects on airway inflammation and this effect is caused by suppression of IL-4, eotaxin-2, and OVA-specific IgE production through the STAT6 and Foxp3 pathways. So chelidonine can improve allergic asthma in mice and be a novel anti-asthma therapeutic [39].

The levels of inducible nitric oxide synthase (iNOS) and COX-2 protein expressions were markedly suppressed by stylopine in a concentration dependent manner. These results suggest that stylopine suppress the NO and PGE2 production in macrophages by inhibiting the iNOS and COX-2 expressions. These biological activities of stylopine may contribute to the anti-inflammatory activity of *Chelidonium majus* [40].

Immune-stimulatory effect of 6-acetonyl-5,6-dihydrosanguinarine (ADS) from C. majus was investigated its. The results suggest that ADS from C. majus, as a positive immune modulator, induces inflammatory cytokines that might improve immunity, via the ROS-ERK/JNK-NFκ Bpathway [41].

Skin problem

Effect of *Chelidonium majus*(CM) on atopic dermatitis was investigated using NC/Nga mice as an AD model. The results suggest that CM may be a potential therapeutic modality for AD [42].

Liver

In sub mitochondrial particles, berberine and coptisine had a marked inhibitory effect on NADH dehydrogenase activity but practically no effect on succinate dehydrogenase activity, whereas chelerythrine and sanguinarine inhibited more strongly succinate dehydrogenase than NADH dehydrogenase, which is in agreement with the results found for mitochondrial respiration. Protopine and allocryptopine, which did not inhibit mitochondrial respiration, strongly inhibited NADH dehydrogenase in submitochondrial particles, but had no effect on succinate dehydrogenase activity [43].

The total ethanolic extract, the phenolic and the alkaloidal fraction of the herb of *Chelidonium majus*L. (Papaveraceae) were tested for their choleretic activity using the isolated perfused rat liver. The total extract significantly caused chloresis by increasing the bile acid independent flow (BAIF); the observed weak activity of both fractions, tested each and as combination, however, was not significant [44].

Antigenicity and ant ovalbumin

The ability of the *Chelidonium majus* L. alkaloid derivative Ukrain (UK) to inhibit ovalbumin-induced sensitization was tested in BALB/c and F1(BALB/c x C57BL/6J) mice. The results suggest that UK pretreatment of OA may affect its antigenic property and the ability to react with anti-OA IgE antibodies raised against the native IgE molecules [45].

Inhibition of the serotoninergic system

The effects of thiophosphoric acid alkaloid derivatives from *Chelidonium majus*L. on the central nervous system (CNS) of mice and rats was studied. It had no protective effect against electroshock or pentetrazol-induced seizures. In rats, ip administration of Ukrain in dose of 14 and 28 mg/kg potentiated the action of amphetamine and apomorphine but had no effect on catalepsy induced by haloperidol. These findings demonstrate that the central

action of Ukrain involves the stimulation of the dopaminergic system and the inhibition of the serotoninergic system [46].

REFERENCES

[1] Miraj S, Azizi N, Kiani S. *Der Pharm Lett*, **2016**, 8 (6):229-237.
[2] Miraj S , Kiani S. *Der Pharm Lett*, **2016**, 8 (9):276-280.
[3] Miraj S , Kiani S. *Der Pharm Lett*, **2016**, 8 (6):59-65.
[4] Miraj S ,Kiani S. *Der Pharm Lett*. **2016**;8 (6):59-65.
[5] Miraj S ,Kiani S . *Der Pharm Lett*. **2016**;8 (9):137-140.
[6] Miraj S , Kiani S. *Der Pharm Lett*, **2016**, 8 (6):328-334.
[7] Miraj S .*Environ Monit Assess*. **2016**;188(6):320.
[8] Miraj S , Kiani S.. *Der Pharmacia Lettre*, **2016**, 8 (9):168-173
[9] Baghbahadorani FK, Miraj S. *Electron Physician*. **2016**;8(5):2436.
[10] Masoudi M, Miraj S, Rafieian-Kopaei M. *J Clin Diagn Res*. **2016**;10(3):QC04.
[11] Havelek R, Seifrtova M, Kralovec K, Krocova E, Tejkalova V, Novotny I, et al. *Phytomedicine*. **2016**;23(3):253-66.
[12] Shi G. Zhonghua Yu Fang Yi Xue Za Zhi.**1992**;26(3):165-7.
[13] Lohninger A, Hamler F. Cheudonium Majus L.(Ukrain) In The Treatment Of Cancer Patients. **1993**.
[14] Slesak B, Nowicky J, Harlozinska A.*Drugs Exp Clin Res*. **1993**;18:17-.
[15] Nowicky J, Staniszewski A, Zbroja-Sontag W, Slesak B, Nowicky W, Hiesmayr W. *Drugs Exp Clin Res*. **1990**;17(2):139-43.
[16] Nawrot R, Wolun-Cholewa M, Gozdzicka-Józefiak A. *Folia Histochem Cytobiol*. **2008**;46(1):79-83.
[17] Fik E, Wołuń-Cholewa M, Kistowska M, Warchoł J, Goździcka-Józefiak *AFolia Histochem Cytobiol*. **2000**;39(2):215-6.
[18] Kulp M, Bragina O.*Anal Bioanal Chem*. **2013**;405(10):3391-7.
[19] El-Readi MZ, Eid S, Ashour ML, Tahrani A, Wink M.*Phytomedicine*. **2013**;20(3):282-94.
[20] Park S-W, Kim SR, Kim Y, Lee J-H, Woo H-J, Yoon Y-K, et al. *Chelidonium majus*L.*Oncol Rep*. **2015**;33(1):419-24.
[21] Zhang W-J, You C-X, Wang C-F, Fan L, Wang Y, Su Y, et al. *Nat prod res*. **2014**;28(21):1873-8.
[22] Cordes N, Plasswilm L, Bamberg M, Rodemann H. *Int J Radiat Biol*. **2002**;78(1):17-27.
[23] Panzer A, Hamel E, Joubert A, Bianchi P, Seegers J. Ukrain TM. *Cancer lett*. **2000**;160(2):149-57.
[24] Capistrano R, Wouters A, Lardon F, Gravekamp C, Apers S, Pieters L.*Phytomedicine*. **2015**;22(14):1279-87.
[25] Deljanin M, Nikolic M, Baskic D, Todorovic D, Djurdjevic P, Zaric M, et al.J *Ethnopharmacol*. **2016**;190:362-71.
[26] Nawrot R, Zauber H, Schulze WX. *Fitoterapia*. **2014**;94:77-87.
[27] Banerjee A, Pathak S, Biswas SJ, Roy-Karmakar S, Boujedaini N, Belon P, et al. *Homeopathy*. **2010**;99(3):167-76.
[28] Nadova S, Miadokova E, Alfoldiova L, Kopaskova M, Hasplova K, Hudecova A, et al *Neuro Endocrinol Lett*. **2008**;29(5):649.
[29] Mazzanti G, Di Sotto A, Di Giacomo S, Durazzi F, Mariani P, Nicoletti M, et al.*Exp Toxicol Pathol*. **2013**;65(7):1117-20.
[30] lyoti Biswas S, Khuda-Bukhsh AR.*Indian J Exp Biol*. **2004**;42:698-714.
[31] Biswas S, Bhattacharjee N, Khuda-Bukhsh A.*Food Chem Toxicol*. **2008**;46(5):1474-87.
[32] Philchenkov A, Kaminskyy V, Zavelevich M, Stoika R.*Toxicol In Vitro*. **2008**;22(2):287-95.
[33] Habermehl D, Kammerer B, Handrick R, Eldh T, Gruber C, Cordes N, et al. *BMC cancer*. **2006**;6(1):1.
[34] Song J-Y, Yang H-O, Pyo S-N, Jung I-S, Yi S-Y, Yun Y-S *Arch Pharm Res.* **2002**;25(2):158-64.
[35] Panzer A, Joubert AM, Bianchi PC, Seegers JC. *Cancer lett*. **2000**;150(1):85-92.
[36] Panzer A, Joubert A, Eloff J, Albrecht C, Erasmus E, Seegers *J. Cancer lett*. **2000**;160(2):237-41.
[37] Mikołajczak PŁ, Kędzia B, Ożarowski M, Kujawski R, Bogacz A, Bartkowiak-Wieczorek J, et al *Cent Eur J Immunol*. **2016**;40(4):400.
[38] Paul A, Das J, Das S, Samadder A, Khuda-Bukhsh AR *Environ Toxicol Pharmacol*.**2013**;36(3):937-47.
[39] Kim S-H, Hong J-h, Lee Y-C.*Pharmacol Rep*. **2015**;67(6):1168-77.
[40] Jang S, Kim BH, Lee W-Y, An SJ, Choi HG, Jeon BH, et al.*Arch Pharm Res*. **2004**;27(9):923-9.
[41] Kim DH, Lee JH, Park S, Oh S-s, Kim S, Kim DW, et al.*Food chem toxicol*. **2013**;58:273-9.
[42] Yang G, Lee K, Lee M-H, Kim S-H, Ham I-H, Choi H-Y.*J Ethnopharmacol*. **2011**;138(2):398-403.
[43] Barreto MC, Pinto RE, Arrabaça JD, Pavão ML. *Toxicol lett*. **2003**;146(1):37-47.

[44] Vahlensieck U, Hahn R, Winterhoff H, Gumbinger H, Nahrstedt A, Kemper F. *Planta medica*. **1995**;61(03):267-70.
[45] Wyczólkowska J, Michon T, Nowicky J.*Drugs Exp Clin Res*. **1996**;22:195-200.
[46] Kleinrok Z, Jagiełło-Wójtowicz E, Matuszek B, Chodkowska A.*Pol J Pharmacol Pharm*. **1991**;44(3):227-39.