Anti-inflammatory effects of essential oil in *Echinacea purpurea* L.

Deqiang Yu, Yi Yuan*, Ling Jiang,Yuling Tai, Xiumei Yang, Fang Hu and Zhongwen Xie
College of Life Sciences, Anhui Agricultural University, Botany Laboratory, Hefei 230036, Anhui, China

Abstract: *Echinacea purpurea* L. is a medicinal plant originally from North America. It has become a commonly used herbal medicine worldwide because it contains various biologically active compounds. This study was designed to investigate the anti-inflammatory effects of essential oils from *E. purpurea* in both mice and rats. The extract was obtained from flower of *E. purpurea* by steam distillation. The anti-inflammatory potential was evaluated *in vivo* by using different animal models such as xylene-induced mouse ear edema, egg-white-induced rat paw edema, and cotton-induced granuloma tissue proliferating inflammation in mice. The serial dosages were used *in vivo*: the low dosage, the medium dosage and the high dosage. The low, medium and high dosages of extracts produced inhibitions of 39.24%, 47.22% and 44.79% respectively in the ear edema induced by xylene when compare with the control group. Only the high dosage group showed statistically significant inhibition (48.51%) of paw edema formation induced three hours by egg white compared with the control group ($P<0.01$). Moreover, the granulation formation was also significantly reduced the most by 28.52% in the high dose groups compared with the control group ($P<0.05$). The pro-inflammatory cytokines such as IL-2, IL-6 and TNF-α in the blood were reduced in the treated groups. The essential oils from extracts of *E. purpurea* have anti-inflammatory effects.

Keywords: *Echinacea purpurea* L.; essential oils; extracts; inflammation; animal models.

INTRODUCTION

Echinacea purpurea L. is a plant that belongs to the Family Asteraceae (Cozzolinoa *et al*., 2006). Since its global commercialization several decades ago, *E. purpurea* has been one of the best-selling herbs. In European countries, *E. purpurea* has been used as an immune-enhancing herb (Miller, 2005). Data from chemical analysis showed that *E. purpurea* has seven groups of medically vital components, including polysaccharides, flavonoids, caffeic acid derivatives, essential oils, alkylamides, polyacetylenes, and miscellaneous chemicals (Cozzolinoa *et al*., 2006).

E. purpurea has been reported to act as an immunoregulator, antioxidant, and promoting wound healing. It has an effect against several viral and bacterial infections, and on preventing tumor cell growth (Lee *et al*., 2010). Generally, the immunoregulatory effects of *E. purpurea* are thought to involve stimulation of T-cell production, phagocytosis, lymphocytic activity, cellular respiration, and inhibition of hyaluronidase enzyme secretion. The polysaccharides from *E. purpurea* protect surrounding tissue cells from bacterial and pathogenic invasion as an immunoregulator (Cozzolinoa *et al*., 2006).

Miller reported that *E. purpurea* caused significant increases in natural killer (NK) cell numbers *in vivo* (Miller, 2005). Daily consumption of *E. purpurea* was found to be prophylactic, extended the life span of aging mice, significantly abated the life span of leukemia mice (Miller, 2005). It stimulated specific (adaptive) immunity in the spleen, as well as NK cells and monocytes, both of which are liable for non-specific, spontaneous and non-adaptive immunity.

E. purpurea is also thought to have anti-inflammatory effects (Cech *et al*., 2006). This is only based on anecdotal reports. Based on our best knowledge, there is no report that *E. purpurea* has an anti-inflammatory function *in vivo*. In this study, essential oils obtained from *E. purpurea* were tested in mice and rats, to determine if they possess anti-inflammatory effects. Here, our data show that essential oils from *E. purpurea* did have anti-inflammatory effects. However, the mechanism of the anti-inflammatory effects of *E. purpurea* remain unclear (Gertsch *et al*., 2004; Raduner *et al*., 2006) and further worker need to be done to identify the active constituents functions in the anti-inflammation.

MATERIALS AND METHODS

Plant material

Dried flowers of *E. purpurea* were provided by from Wei Qing Yao Cai Co., Ltd. (Anhui, China). The morphological characters of the flowers were consistent with the description given in The Pharmacopoeia of the People's Republic of China.

Preparation of extraction

The essential oil was extracted from flower of *E. purpurea* by steam distillation. Water was added to powdered flowers (100g) at a ratio of 10:1 (g/mL). The volatile oil extractor was distilled for 8 hour.

**Corresponding author:* e-mail: yuanyizhangqi@126.com

Experimental animals

Male Kunming mice, 18-22 g, and male Wistar rats, 180-220 g, were used in this study. Food and water were supplied ad libitum All the animals were provided by Anhui University of Traditional Chinese Medicine. The animal experiments were performed in accordance with current guidelines for the care of laboratory animals and were permitted by Ethics Committee, Anhui University of Traditional Chinese Medicine.

Preparation and dosage of essential oil

The essential oil was diluted by distilled water before applying to animal studies. The low dose of *E. purpurea* essential oil is a concentration of 2.5 g crude drug/kg (equivalent to 3.75 µl essential oil/kg). The medium dose consisted of 5 g crude drug/kg (equivalent to 7.5 µl essential oil/kg). The high dose concentration is of 10 g crude drug/kg (equivalent to 15 µl essential oil/kg).

Aspirin positive control group

We use 33 mg aspirin per kilogram (body weight) for animal study. Aspirin tablets were ground into a powder and were added to distilled water in a concentration of 0.528% (equivalent to 3.3 mg/ml containing Ah Division aspirin).

The influence of extracts on xylene induced ear edema of mice

The protocol used for xylene induced ear edema was published by Otuki (Otuki *et al*., 2005). Five days after acclimatization, the 60 Wistar rats were divided into six groups randomly: normal control groups, model control groups, and positive controls (aspirin-treated), low, middle and high dose essential oil groups. Each group has 10 rats. Gastric perfusion with essential oil or saline was performed through the mouth once a day for a week. An equivalent volume of distilled water was given to the normal control group and model control group. 40 min after last drug administration, left ear inflammation was induced by applying 0.05 ml xylene. An equal volume of distilled water was applied to the right ear as control. After 4 h, the mice were sacrificed, and 0.5 cm in diameter punches were made in the right and the left ears using an ear-piece hole-puncher. The tissues were weighed on electronic scales. The degree and rate of edema, and inhibition rate were determined according to the formulae below:

The degree of edema (g) = the weight of left ear (g) – the weight of right ear (g)

The rate of edema (%) = the degree of edema/the weight of right ear piece × 100%

The inhibition rate of edema (%) = (degree of edema in the model group–degree of edema in the treated group)/ degree of edema in the model group ×100%

The influence of extracts on egg albumen induced paw edema of rats

We followed the protocol previously described by Oliveira and Kasahara (Oliveira *et al*., 2004; Kasahara *et al.,* 2005). Five days after acclimatization, the 60 Wistar rats were divided into six groups randomly: normal control groups, model control groups, positive controls (aspirin), low, middle and high dose of the oil groups. Each group has ten rats. Gastric perfusion with medicine or saline was performed through the mouth once a day for a week. An equivalent volume of distilled water was given to the normal control group and model control group. The right ankle joints in rats were tagged to measure the capacity in rats with normal foot volume. 40 min after last drug administration, the paws were injected with newly prepared 0.1 mL 10% egg albumen (Xiao *et al*., 2008). After 0.5, 1.0, 1.5, 2.0 and 3.0 h, the volume of injected paws were measured respectively. The degree, rate and the inhibition rate of tested substance on paw edema was calculated according to the formulae below:

The degree of edema (ml) = the foot volume of inflammation (ml) – the foot volume of normal (ml).

The rate of edema (%) = (the foot volume of inflammation – the foot volume of normal)/the foot volume of normal × 100%.

The inhibition rate of edema (%) = (the average degree of edema in the model group – the average degree of edema in the treated group)/the average of degree of edema in the model group ×100%

The influence of extracts on cotton-induced rat granuloma tissue hyperplasia and measurement of the levels of IL-2, IL-6 and TNF-α in the chronic inflammatory exudates

Five days after acclimatization, the 60 Wistar rats were divided into six groups randomly: normal control group, model control group, positive control (aspirin), *E. purpurea* essential oil low, middle and high dose groups. Each group has ten rats. And each rat received sterilized cotton pellets which were weighed and merged with ampicillin (1.0 mg/0.1 ml) before being oven dried at 50ºC and implanted subcutaneously in the groin of the rat (on one side) under ether anesthesia. The normal control group underwent the same procedure but did not have the cotton pellets implanted. Gastric perfusion with medicine or saline was done from the mouth once a day for a week. An equivalent volume of distilled water was given to the normal control group and model control group. 40 min after last drug administration, the rats were anesthetized by the pentobarbital sodium (40 mg/kg) and 5 ml blood was collected. Pleural fluid samples were centrifuged to separate serum after standing, and the contents of IL-2, IL-6 and TNF-a were measured by radioimmunoassay (Lee *et al*., 2010; Otuki *et al*., 2005). Rats were sacrificed, and the granulomatous tissue was carefully taken out. Wet tissues weights were obtained by electric scale, and

hyperplasia assess followed previous description (Xiao *et al*., 2008; Amresha *et al*., 2007). Briefly, the granuloma tissue samples were dried at 90°C for 4 h and dry weights were obtained by scale. The inhibition rate of the test substances on granuloma tissue hyperplasia were calculated according the formula below:

Granulation weight (g) = the dry weight of granulation cotton – the weight of cotton.
The inhibition rate of granulation = (the model group weight of granulation – the drug group weight of granulation) / the model group weight of granulation × 100%

STATISTICAL ANALYSIS

The results are expressed as mean value ± standard deviation. Data were analysed using Windows SPSS. P<0.05 was considered statistically significant.

RESULTS

The influence of extracts on xylene-induced mouse ear edema

The edema model was established successfully using 0.05 ml xylene. Table 1 and fig. 1 show that edema or the weight of the left ear was significantly lower in *E. purpurea*-treated groups, compared with the control (P<0.01). In particular, the medium and high dosage groups showed a greater effect than the control (P<0.05), indicating that the essential oils of *E. purpurea* can inhibit edema or oozing cause by acute inflammation.

The influence of extracts on egg-albumen-induced rat paw edema

Rat paw edema was induced successfully with 0.1 ml egg white. At each time point, there was a statistically significant difference between the *E. purpurea*-treated groups and controls (P<0.01; tables 2, 3; fig. 2). The low dose group showed a statistically significant effect, compared with the control at 2 and 3 h (P<0.05). The medium dosage group showed significant effects, compared with the control at 1.5 h (P<0.05) and 3 h (P>0.01). Meanwhile, the high dosage group showed statistically significant effects, compared with the control at 1.5, 2 and 3h (P<0.01). The result indicates that essential oils from *E. purpurea* can inhibit edema or effusion cause by acute inflammation.

Table 1: Influence of extracts on xylene induced ear edema of mice (n = 10, $\bar{x} \pm s$).

Group	Dose (g/kg)	Weight of left ear (mg)	Weight of right ear (mg)	Degree of edema (mg)	Rate of edema (%)	Inhibition rate (%)
Normal control group	/	3.77±0.47	3.71±0.45	0.06±0.39	1.62	
Model control group	/	6.75±1.52**	3.87±0.45	2.88±1.33**	74.42	
Aspirin group	33mg/kg	5.39±1.17▲	3.98±0.44	1.41±1.07▲	35.43	51.04
Low dosage group	2.5	5.74±1.29	3.99±0.59	1.75±1.15	43.86	39.24
Medium dosage group	5	5.49±0.88▲	3.97±0.38	1.52±1.14▲	38.29	47.22
High dosage group	10	5.26±1.11▲	3.67±0.31	1.59±1.09▲	43.32	44.79

Note: Comparison of model control with normal group: **P<0.01; comparison of dose group with model control: ▲P<0.05.

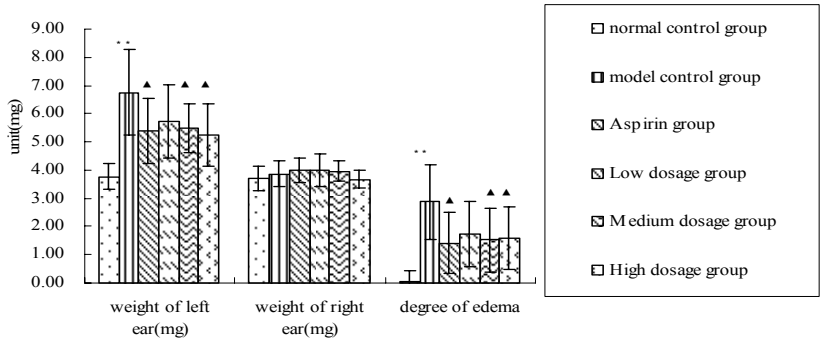


Fig. 1: Influence of extracts on xylene induced ear edema of mice.

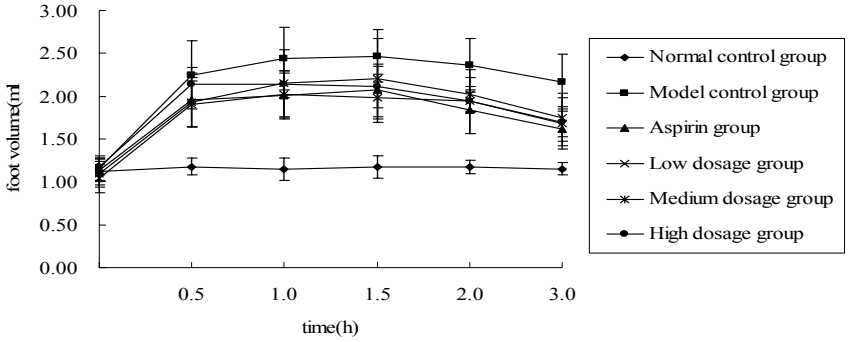
Table 2: Influence of extracts on egg white induced paw edema of rats (n = 10, $\bar{x} \pm s$)

Group	Normal foot volume (ml)	Degree of edema (ml) and rate of edema (%)				
		0.5h	1h	1.5h	2h	3h
Normal control group	1.12±0.15	0.06±0.10 (5.36)	0.03±0.13 (2.68)	0.05±0.13 (4.46)	0.05±0.08 (4.46)	0.03±0.07 (2.68)
Model control group	1.16±0.14	1.09±0.40** (93.97)	1.28±0.37** (110.34)	1.31±0.31** (112.93)	1.20±0.31** (103.45)	1.01±0.32** (87.07)
Aspirin group	1.14±0.14	0.82±0.31 (71.93)	0.87±0.26▲ (76.32)	0.93±0.31▲ (81.58)	0.70±0.27▲▲ (61.40)	0.48±0.20▲▲ (42.11)
Low dosage group	1.09±0.15	0.84±0.29 (77.06)	1.06±0.39 (97.25)	1.12±0.47 (102.75)	0.93±0.20▲ (85.32)	0.66±0.28▲ (60.55)
Medium dosage group	1.04±0.16	0.87±0.27 (83.65)	0.98±0.28 (94.23)	0.94±0.28▲ (90.38)	0.90±0.37 (86.54)	0.60±0.30▲▲ (57.69)
High dosage group	1.18±0.10	0.96±0.19 (81.36)	0.96±0.16▲ (81.36)	0.93±0.24▲▲ (78.81)	0.76±0.13▲▲ (64.41)	0.52±0.18▲▲ (44.07)

Note: compared model control with normal group: *P<0.05 **P<0.01; dose group with model control: ▲P<0.05 , ▲▲P<0.01.

Table 3: Influence of extracts on egg white induced paw edema of rats.

Group	Number of rats (n)	Dose (g /kg)	Inhibition rate (%)				
			0.5h	1h	1.5h	2h	3h
Normal control group	10	/	/	/	/	/	/
Model control group	10	/	/	/	/	/	/
Aspirin group	10	33mg/kg	24.77	32.03	29.01	41.67	52.48
Low dosage group	10	2.5	22.94	17.19	14.50	22.50	34.65
Medium dosage group	10	5	20.18	23.44	28.24	25.00	40.59
High dosage group	10	10	11.93	25.00	29.01	36.67	48.51



The influence of extracts on cotton-induced rat granuloma tissue hyperplasia

Each dose group of essential oils of *E. purpurea* inhibited the granulation to different degrees at all doses tested (table 4; fig. 3). In the medium (P<0.05) and high (P <0.01) dose groups. The wet weights of granulation tissue were lower than the weights in model control. The dry weights of granulation tissue were also significantly reduced both in the medium and high dose groups, compared with the control group (P<0.05).

Measurement of IL-2, IL-6 and TNF-α levels in chronic inflammatory exudates

Essential oils of *E. purpurea* decreased the levels of IL-6 and TNF-**α** in the blood from rats followed by chronic inflammation compared with control rats (table 5). IL-6 levels were significantly reduced in the low dose group (P<0.05). In the high dose group, there was a significant reduction of TNF-α levels, whereas IL-2 levels were increased. These results indicated that the essential oils from *E. purpurea* can reduce blood inflammatory cytokine production.

DISCUSSION

In present study, we first reported that the extracts of essential oil from *E. purpurea* has in *vivo* anti-inflammation effects induced by tumefaction, effusion and hyperplasia. The IL-2, IL-6 and TNF-α levels in the blood of chronic inflammatory also supported the anti-inflammation effect of the extracts. The previous reports have showed that extracts of *E. purpurea* had the significant anti-inflammation *in vitro*. Gertsch *et al* (2004) reported possible molecular mechanisms as efficient immuno-modulators as well as ligands for CB2 receptors in human monocytes. The extracts also induced de novo synthesis of tumor necrosis factor αmRNA in primary human monocytes/macrophages (Cech *et al*., 2006). Raduner *et al.* (2006) reported that alkylamides from *E. purpurea* are new kinds of cannabinomi metics. At nanamolar concentrations, the essential oil was a critical factor for reducing pro-inflammatory cytokines production in human whole blood (Gertsch *et al*., 2004). Taken together, our in *vivo* data, as well as previous *in vitro* studies show the anti-inflammatory effects of essential oil from *E. purpurea*.

According to our research, it would mean that essential oil studied had activity values that were significantly comparable to the positive control. Aspirin is one of the synthetic drugs, and it has the severe side effect on treatment. As a natural medicine, essential oil is easy to extract and has the light side effect. In conclusion, the extract of *E. purpurea* is a complex. Previous publications have revealed the medicinal values of the extracts from *E. purpurea*. There is no doubt that the extracts from *E. purpurea* are potential resources for anti-inflammation medicine. Previous studied showed that it contains

Table 4: Influence of extracts on cotton induced-granuloma tissue hyperplasia of rats (n = 10, $\bar{x} \pm s$).

Group	Dose (g /kg)	Number of rats	Granuloma Wet weight (mg)	Rate (%)	Granuloma dry weight (mg)	Inhibition rate (%)
Model control group	/	10	311.94±82.87	/	95.56±19.13	/
Aspirin group	33mg/kg	10	249.12±42.25▲	20.14	74.26±16.87▲	22.29
Low dosage group	2.5	10	278.28±72.06	10.71	85.89±22.92	10.12
Medium dosage group	5	10	246.46±50.32▲	20.99	76.80±22.36	19.63
High dosage group	10	10	211.65±43.54▲▲	32.15	68.31±30.72▲	28.52

Note: compared model control with normal group: ▲P<0.05, ▲▲P<0.01.

Table 5: Influence of volatile oil on cytokines of chronic inflammation (n = 10, $\bar{x} \pm s$).

Group	Dose (g/kg)	Number of rats	TNF-α (ng/ml)	IL-2 (ng/ml)	IL-6 (pg/ml)
Normal control group			1.01±0.12	2.33±0.97	15.62±11.28
Model control group	/	10	1.50±0.23**	1.43±0.67*	38.04±16.38**
Aspirin group	33mg/kg	10	1.24±0.21▲	1.71±0.93	27.01±7.70
Low dosage group	2.5	10	1.33±0.14	2.00±0.55	25.26±6.22▲
Medium dosage group	5	10	1.29±0.23	2.07±1.21	30.78±13.23
High dosage group	10	10	1.21±0.32▲	2.21±0.74▲	28.94±11.90

Note: compared model control with normal group: ▲P<0.05

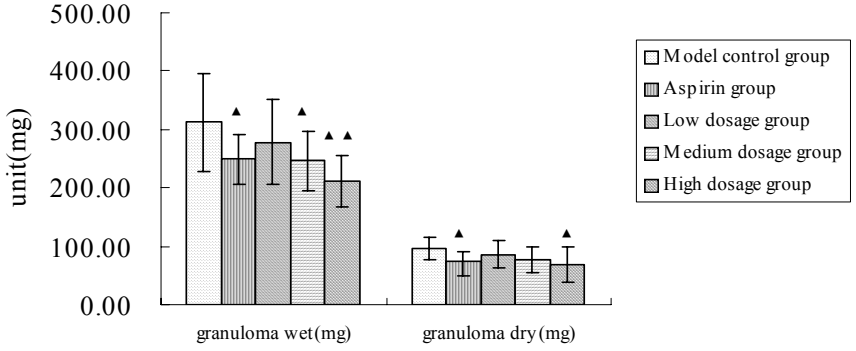


Fig. 3: Influence of extracts on cotton induced-granuloma tissue hyperplasia of rats.

alkylamides (Modarai *et al*., 2011), polysaccharide (Xiao *et al*., 2008) and flavonoids (Yuan *et al*., 2006; Xiao *et al*., 2008). The exactly compound responsible for anti-inflammation function is still unclear. Further studies are needed for dissecting compound's function.

ACKNOWLEDGEMENTS

This work was supported by the Key National Science Foundation of colleges and universities Anhui Province (No. KJ2011A104).

REFERENCES

Amresha G, Reddy GD, Rao Ch. V and Singh PN (2007). Evaluation of anti-inflammatory activity of *Cissampelos pareira* root in rats. *J. Ethnopharmacol.,* **110**: 526-531.

Cech NB, Eleazer MS, Shoffner LT, Crosswhite MR, Davis AC and Mortenson AM (2006). High performance liquid chromatography/electrospray ionization mass spectrometry for simultaneous analysis of alkamides and caffeic acid derivatives from *Echinacea purpurea* extracts. *J. Chromatogr.*, **1103**(2): 219-228.

Cozzolino R, Malvagna P, Spina E, Giori A, Fuzzati N, Anelli A, Garozzo D and Impallomeni G (2006). Structural analysis of the polysaccharides from *Echinacea angustifolia* radix. *Carbohyd. Polym.*, **65**: 263-272.

Gertsch J, Schoop R, Kuenzle U and Suter A (2004). Echinacea alkylamides modulate TNF-a gene expression via cannabinoid receptor CB2 and multiple signal transduction pathways. *FEBS Letters*, **577**: 563-569.

Kasahara Y, Yasukawa K, Kitanaka S, Khan MT and Evans FJ (2002). Effect of methanol extract from flower petals of *Tagetes patula* L. on acute and chronic inflammation model. *Phytother. Res*., **16**: 217-222.

Lee TT, Huang CC, Shieh XH, Chen CL, Chen LJ and Yu B (2010). Flavonoid, phenol and polysaccharide contents of *Echinacea purpurea* L. and its immunostimulant capacity *in vitro*. *IJESD,* **1**(1): 5-9.

Modarai M, Silva E, Suter A, Heinrich M and Kortenkamp A (2011). Safety of Herbal Medicinal Products: Echinacea and Selected Alkylamides do not Induce CYP3A4 mRNA Expression, *Evid-based. Compl. Alt.*, pp.1-7.

Miller SC (2005). *Echinacea*: A miracle herb against aging and cancer evidence *in vivo* in mice. *eCAM,* **2**(3): 309-314.

Oliveira FA, Vieira-Júnior GM, Chaves MH, Almeida FRC, Florˆencio MG, Lima RCP, Silva RM, Santos FA and Rao VSN (2004). Gastroprotective and anti-inflammatory effects of resin from *Protium heptaphyllum* in mice and rats. *Pharmacol. Res.*, **49**: 105-111.

Otuki MF, Vieira-Lima F, Malheiros A, Yunes RA and Calixto JB (2005). Topical anti-inflammatory effects of the ether extract from Protium kleinii and a-amyrin pentacyclic triterpene. *Eur. J. Pharmacol.*, **507**: 253-259.

Raduner S, Majewska A, Chen JZ, Xie XQ, Hamon J, Faller B, Altmann KH and Gertsch J (2006). Alkylamides from Echinacea are a new class of cannabinomimetics. *J. Biol. Chem.*, **281**(20): 14192-14206.

Xiao XQ, Wang S, Luo C and Liu XQ (2008). Experimental study on anti-inflammation and decreasing pain effects of extracts of *Periplaneta americana. MAS.,* **2**(4): 12-16.

Yuan G, Wahlqvist ML, He G, Yang M, Li D. (2006). Natural products and anti-inflammatory activity. *Asia Pac. J. Clin. Nutr.,* **15**(2): 143-152.