

Effect of *Citrus maxima* on Hematological Parameters on 14th day in Normal and EAC Tumor Bearing Mice

SHIVANANDA A^{1*}, MURALIDHARA RAO D², AND JAYAVEERA KN³

¹Department of Zoology, SBGNSC, Chickballapur, Karnataka, India-562 101

²Department of Biotechnology, S.K.University, Anantapur, Andrapradesh, India

³Department of Chemistry, Jawaharlal Nehru Technological University Anantapur, Anantapuramu, Andrapradesh, India

ABSTRACT

The present study was aimed to investigate the hematological parameters by the activities of *Citrus maxima* in animal models. This investigation revealed the effect of CM-LF-ETH, CM-LF-ACET, CM-LF-WATE, CM-BRK-ETH, CM-BRK-ACET, CM-BRK- WATE, CM-FP-ETH, CM-FP-ACET and CM-FP- WATE 300 mg/kg b.w p.o., and CYP 25 mg/kg b.w i.p on hematological parameters against EAC induced animals estimated on 14th day of treatment. CM-LF-ETH, CM-BRK-ETH and CM-FP-ETH 300 mg/kg b.w p.o., showed better improvement in the hematological parameters than the rest of the doses among the compared groups. The total WBC count found increased in the EAC control group. All the test drugs when administered to the EAC bearing mice shoed the significant ($p < 0.001$) decrease in the WBC count(Figure 1.1) when compared with the EAC control group,. RBC count (Figure 1.2) and Hb content (Figure 1.3) in the EAC groups were significantly ($p < 0.001$) decreased as compared to the normal group. All the test drugs have showed the significant increase but CM-LF-ETH, CM-BRK-ETH and CM-FP-ETH 300 mg/kg b.w p.o., showed the better activity compared to rest of the drugs and doses.

Key words: Citrus maxima, hematological, tumor, mice

Introduction

Traditional medicine based on herbal remedies has always played a key role in the health systems of many countries. In India the native people are exploiting a variety of herbals for effective curing of various ailments. The plant parts used, preparation, and administration of drugs vary from one place to other. Ethno medicinal studies have offered immense scope and opportunities for the development of new drugs. Some modern drugs have been deducted from folklore and traditional medicines. The pharmaceutical industry continues to investigate and confirm the efficacy of many medicines used by traditional communities.

Cancer is a generic term for a group of over hundred diseases that can affect any part of the body. An important feature of cancer is the rapid creation of abnormal cells which grow beyond their usual boundaries and can invade adjoining parts of the body and spread to other organs, a process referred to as metastasis. Metastases are the major cause of death from cancer.

In the present study, with CM-LF-ETH, CM-BRK-ETH and CM-FP-ETH at the dose of 300 mg/kg b.w. p.o. has shown significant prolongation of lifespan, reduction in

tumor volume, improvement in the hematological parameters (W.B.C, R.B.C and Hemoglobin) when compared to the rest of groups.

The *Citrus maxima* tree, which is the most cold-intolerant citrus species, has a rounded crown and grows 5 to 15 m (15 to 50 ft) tall. The tree has large evergreen oblong to elliptic leaves, 10.5 to 20 cm (4 to 8 in) long, with winged petioles (leaf stems). The flowers and fruits are borne singly, in contrast to grape fruits (*C. X paradisi*), in which they grown in clusters of 2 to 20. The fruits, which vary from round to pear-shaped and ripen to yellow, orange, or red, are large--30 cm or more in diameter, and weighing up to 9 kg (20 lbs). The flesh of the fruit, which may be greenish yellow, yellow, pink, or red, is often juicy, and divided into 11 to 18 segments. The flavor is sweet to somewhat acidic.

The leaves, flowers, and rind are given for their sedative effect in cases of epilepsy, chorea and convulsive coughing. The hot leaf decoction is applied on swellings and ulcers. The fruit juice is taken as a febrifuge. The seeds are employed against coughs, dyspepsia and lumbago. The fruit include treatment of coughs, fevers, cardiotoxic, cancer and gastrointestinal disorders. The plant used as anti diarrhea.

*Address for correspondence:

Materials and Methods

Collection and authentication of plant material

The leaves, stem bark and fruit peels of *Citrus aurantium* Lin. (*Bitter orange*) were collected from the local gardens around Devanahalli, Bengaluru, Karnataka, India. Plant species were authenticated by Prof. B.V. Krishnappa, Government First Grade College, Chickballapur, Karnataka, India. And voucher specimens, SBGNSC/O14, 015 were deposited at the herbarium of Botany Department of SBGNS College, Chickballapur, Karnataka, India. The respective materials were washed thoroughly 2-3 times with running tap water. Then the materials were air and shadow dried and mechanically crushed into coarse powder of 40 μm size by using mixer grinder. Powders were stored separately in air tight bottles.

Preparation of extract

Extracts were prepared in order to study their antioxidant activity. Ethanolic, acetone and aqueous extracts of each of the leaves, stem bark and fruit peels of two different citrus plants were prepared by soaking the material in various solvents for 72h and after every 24h, the mixture was stirred with a sterile glass rod. After the completion of 72h, the extracts were filtered using a Buckner funnel and Whatman No. 1 filter paper and concentrated by vacuum drying.

Hematological Parameters

In order to detect the influence of three drugs on the hematological status of EAC bearing mice, comparison were made amongst all groups of mice on the 14th day after inoculation. The tumor bearing mice treated with *Citrus maxima* (p.o for first 9 days) and Cyclophosphamide (i.p for first 9 days).

Collection of Blood

Six Mice from each group were anaesthetized on 14th of the study. Blood samples were collected by sino-orbital puncture in sterilized, heparinized tubes. The heparinized blood was used for hematological evaluation. Following hematological parameters were estimated from blood were WBC, RBC and Hb.

White Blood Cell Count

Total leukocyte count in blood was carried by the method described by Joshi¹⁴. The blood was diluted 20 times with a suitable diluting fluid which destroys the red blood corpuscles and stains the nuclei of the white blood cells. The leucocytes are then counted in a hemocytometer and their number in undiluted blood was calculated.

Reagent: Turk's Fluid Composition: Glacial acetic acid 1.5 mL (for hemolysing RBCs), Gentian violet (1% solution in water) 1mL, Distilled water 100 mL.

Procedure

The blood was taken up to 0.5 mark in the WBC pipette and diluted with Turk's fluid up to 11 mark, mixed thoroughly. 1-2 drops were discarded and charged the diluted blood into counting chamber with cover slip. The cells were counted after 5 minute under low power objective in the compound microscope. Total WBC in undiluted blood was calculated and expressed as cell $\times 10^6$ /mL blood.

Red Blood Cell Count

Enumeration of Red blood corpuscles were carried by the method described by Joshi⁷⁴. The number of red cells in blood is very high. The blood was therefore, diluted 200 times, with an appropriate diluting fluid before the cells was counted in a hemocytometer. Their number in undiluted blood can then be calculated.

Reagent: Haeym's Fluid Composition: Sodium chloride 0.5 g (for isotonicity), Sodium Sulphate 2.5 g (as anticoagulant), Mercuric chloride 0.25 g (as preservative), and Distilled water 100 mL.

Procedure

The blood was taken up to 0.5 mark in the RBC pipette and diluted with Haeym's fluid up to 101 mark, mixed thoroughly. 1-2 drops were discarded and charged the diluted blood into counting chamber (hemocytometer) with cover slip. The cells were counted after 5 minute under high power objective in the compound microscope.

Total number of RBC in undiluted blood was calculated and expressed as cell $\times 10^9$ /mL blood.

Hemoglobin

Hemoglobin in blood was assayed by cyanmethemoglobin method using Beacon diagnostics kit, Navasari.

Principle

In alkaline medium Hemoglobin and its derivatives are oxidized in the presence of potassium ferric cyanide and get converted to methemoglobin which then reacts with potassium cyanide to form purple red colored cyanmethemoglobin complex. The intensity of the Complex which is measured at 546 nm or green filter.

Preparation of Working Reagent: Drabkin's Solution: Dilute 1 mL of the Reagent in 1 to 19 mL of deionised water.

Procedure: Rinse the Pipette with the Reaction mixture.

Table No : Hemoglobin Working Procedure

Pipette into tube marked	Blank	Test
Working Drabkin's Solution	5.0 mL	5.0 mL
Blood	20 μ L	20 μ L

Blood hemoglobin in gm %

$$\frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times 15.6 \text{ standard concentration as stamped on the vial} \times 0.251$$

The hemoglobin concentration in blood was expressed as g/ dl of blood.

Statistical Analysis

The results were expressed as mean \pm S.E.M (n=6). The statistical analysis involving 12 groups was performed by means analysis of variance (ANOVA) followed by Dunnett test. *p* value at < 0.05 was considered as statistically significant. Data were processed with graph pad prism version 5.00 soft ware.

RESULTS

Table 1: Effect of *Citrus maxima* on hematological parameters on 14th day in normal and EAC tumor bearing mice

Table-1 revealed the effect of CM-LF-ETH, CM-LF-ACET, CM-LF-WATE, CM-BRK-ETH, CM-BRK-ACET, CM-BRK- WATE, CM-FP-ETH, CM-FP-ACET and CM-FP-WATE 300 mg/kg b.w p.o., and CYP 25 mg/kg b.w i.p on hematological parameters against EAC induced animals estimated on 14th day of treatment. CM-LF-ETH, CM-BRK-ETH and CM-FP-ETH 300 mg/kg b.w p.o., showed better improvement in the hematological parameters than the rest of the doses among the compared groups. The total WBC count found increased in the EAC control group. All the test drugs when administered to the EAC bearing mice shoed the significant ($p < 0.001$) decrease in the WBC count(Figure 1.1) when compared with the EAC control group,. RBC count (Figure 1.2) and Hb content (Figure 1.3) in the EAC groups were significantly ($p < 0.001$) decreased as compared to the normal group. All the test drugs have showed the significant increase but CM-LF-ETH, CM-BRK-ETH and CM-FP-ETH 300 mg/kg b.w p.o., showed the better activity compared to rest of the drugs and doses.

Groups	Treatment	WBC (x10 ⁶ /mL)	RBC (x10 ⁹ /mL)	Hb (g%)
Group I	Normal Mice	7.24 \pm 0.23	5.67 \pm 0.24	12.9 \pm 0.44
Group II	EAC + solvent 20 mL/kg	21.78 \pm 1.31 ^c	3.55 \pm 0.04 ^c	8.1 \pm 0.28 ^c
Group III	EAC + CYP 25 mg/kg	10.71 \pm 0.27 ^{c,r}	5.26 \pm 0.09 ^r	12.08 \pm 0.40 ^r
Group IV	EAC + CM-LF-ETH (300 mg/kg)	14.76 \pm 0.16 ^{c,r,z}	4.79 \pm 0.11 ^{c,r,z}	9.25 \pm 0.11 ^{c,q,z}
Group V	EAC + CM-LF-ACET (300 mg/kg)	16.82 \pm 0.23 ^{c,r,y}	4.03 \pm 0.08 ^{c,p,z}	7.7 0 \pm 0.15 ^{c,y}
Group VI	EAC + CM-LF-WATE (300 mg/kg)	16.64 \pm 0.29 ^{c,r,y}	4.32 \pm 0.05 ^{c,r,z}	8.97 \pm 0.14 ^{c,p,z}
Group VII	EAC + CM-BRK-ETH (300 mg/kg)	12.28 \pm 0.28 ^{c,r,z}	4.91 \pm 0.02 ^{c,r,z}	11.29 \pm 0.09 ^{c,r,z}
Group VIII	EAC + CM-BRK-ACET (300 mg/kg)	15.59 \pm 0.24 ^{c,r,y}	4.13 \pm 0.14 ^{c,r,z}	8.99 \pm 0.13 ^{c,p,z}
Group IX	EAC + CM-BRK-WATE (300 mg/kg)	14.13 \pm 0.27 ^{c,r,z}	4.54 \pm 0.20 ^{c,r,z}	10.88 \pm 0.19 ^{c,r,z}
Group X	EAC + CM-FP-ETH (300 mg/kg)	11.93 \pm 0.08 ^{c,r,z}	5.07 \pm 0.09 ^{b,r,z}	11.01 \pm 0.22 ^{c,r,z}
Group XI	EAC + CM-FP-ACET (300 mg/kg)	14.90 \pm 0.20 ^{c,r,z}	4.23 \pm 0.05 ^{c,r,z}	9.34 \pm 0.13 ^{c,q,z}
Group XII	EAC + CM-FP-WATE (300 mg/kg)	13.69 \pm 0.31 ^{c,r,z}	4.50 \pm 0.07 ^{c,r,x}	10.19 \pm 0.29 ^{c,r,z}

Values are mean \pm S.E.M. (n=6).

p values: $p < 0.05$, $r < 0.001$ as compared with EAC control + solvent. $x < 0.05$, $y < 0.01$, $z < 0.001$, as compared to CYP (by one way ANOVA followed by Dunnett's multiple comparison test). CM-*Citrus maxima*, LF-leaf, BRK-bark, FP-fruit peel, ETH-ethanol, ACET-acetone, WATE-water.

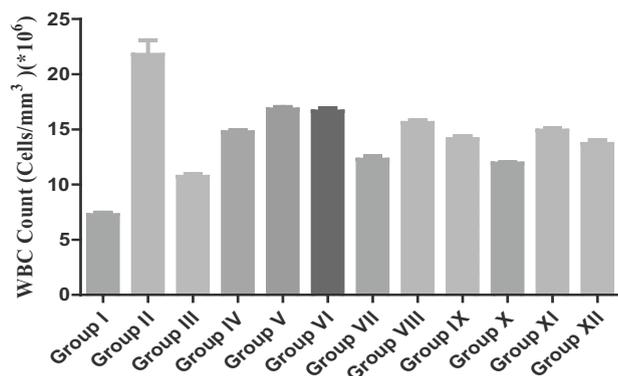


Fig. 1.1: Effect of Citrus maxima on WBC Count on 14th day in normal and EAC tumor bearing mice

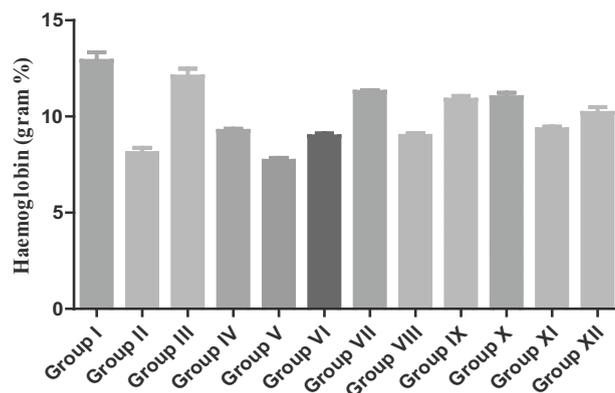


Fig. 1.2: Effect of Citrus maxima on RBC Count on 14th day in normal and EAC tumor bearing mice

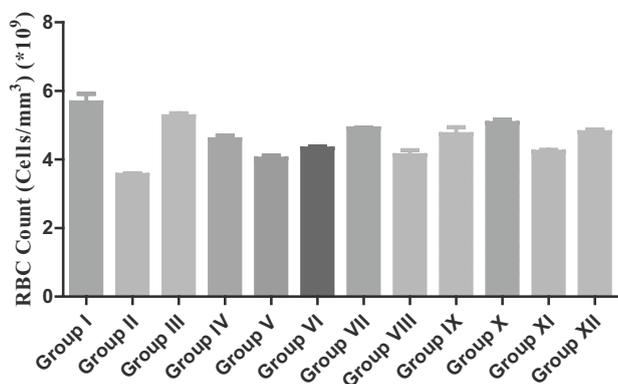


Fig. 1.3: Effect of Citrus maxima on Haemoglobin on 14th day in normal and EAC tumor bearing mice

DISCUSSION

Table No.5.1.2 revealed the effect CM-LF-ETH, CM-LF-ACET, CM-LF-WATE, CM-BRK-ETH, CM-BRK-ACET, CM-BRK-WATE, CM-FP-ETH, CM-FP-ACET, CM-FP-WATE, CA-LF-ETH, CA-LF-ACET, CA-LF-WATE, CA-BRK-ETH, CA-BRK-ACET, CA-BRK-WATE, CA-FP-ETH, CA-FP-ACET and CA-FP-WATE 300 mg/kg b.w. p.o dose of hematological parameters against EAC induced animals estimated on 14th day of treatment. CM-LF-ETH, CM-BRK-ETH and CM-FP-ETH, 300 mg/kg b.w. p.o., showed better improvement in the hematological parameters than the rest of the doses among the compared groups. The total WBC count found significantly ($p < 0.001$) increased in the EAC control group. All the test drugs when administered to the EAC bearing mice showed the significant ($p < 0.001$) decrease in the WBC count when compared with the EAC control group, with CM-LF-ETH, CM-BRK-ETH, CM-FP-ETH, 300 mg/kg b.w. p.o., showing the significant decrease in the WBC when compared to the normal ($p < 0.001$), cyclophosphamide ($p < 0.01$) and EAC control ($p < 0.001$) groups. RBC count and Hb content in the EAC groups were significantly ($p < 0.001$) decreased as compared to the normal group. Treatment with CM-LF-

ETH, CM-BRK-ETH, CM-FP-ETH, 300 mg/kg b.w. p.o., significantly ($p < 0.001$) increased the RBC and Hb content when compared with the EAC control. All the test drugs have showed the significant increase but CM-LF-ETH, CM-BRK-ETH, CM-FP-ETH, 300 mg/kg b.w. p.o. showed the better activity compared to rest of the drugs and doses

Conclusion

Furthermore, it may be that, the increase of lifespan of tumor-bearing mice by treatment is a positive result and supports the anti-cancer effect of *Citrus maxima* (Pomelo). The results of the present study are encouraging, as all the extracts have shown significant prolongation of lifespan, reduction in tumor volume, improvement in the hematological parameters of the hosts. The above parameters are responsible for the anti cancer activity of *Citrus maxima* (Pomelo).

Acknowledgement

The authors wish to thank management of SBGNSC, Chickballapur, Karnataka, India and The Director, Leads clinical research lab, Bengaluru, Karnataka, India for their technical support and providing laboratory facilities during the research work.

References

1. Brodys.; Human Pharmacology. New Delhi, Elsevier publications, **2005**, 374.
2. http://www.ninds.nih.gov/disorders/chronic_pain/detail_chronic_pain.htm#140543084. (Cited on 4th-Jan-2012).
3. <http://www.pain101.com/TreatmentMethods/HerbalRemedies.aspx>. (Cited on 18th-Nov-2012).
4. Vogel, H.G.; Drug discovery and evaluation. 2 ed. Germany, Spinger, **2002**.
5. Mohan, H.; Text book of pathology, 3 ed. New Delhi, **1998**.

6. Tripathi, K.D.; Essentials of medical pharmacology, 4 ed. New Delhi, jaypee publishers, **2002**.
7. Farsam, H.; Amanlou, M.; Dehpour, A. R.; Jahani, F.; J Ethnopharmacol, **71, 2000**, 443.
8. Morebisea, O.; Aweb, E.O.; Makinde, J.M.; and Olajided, O.A.; Fitoterapia, **72, 2001**, 1497.
9. Panthong, A.; Supraditaporn, W.; Kanjanapothi, D.; Taesotikul, T.; and Reutrakul V.; J Ethnopharmacol, **110, 2007**, 264.
10. "Moreira, S.A.; Piuvezam, M.R.; Arau, C.C.; and Thomas, G. ; J Ethnopharmacol, **67, 1999**, 171.
11. Adedapo, A. A.; Sofidiya, M.O.; Maphosa, V.; Moyo, B.; Masika, P.J.; and Afolaan, A.J.; Rec Nat Prod., **2(2), 2008**, 46.
12. Shinde, U.A.; Phadke, A.S.; Nair, A.M.; Mungantiwar, A. A.; Dikshit, V.J.; and Saraf, M.N.; J Ethnopharmacol, **65, 1999**, 21.
13. Trongsakul, S.; Panthong, A.; Kanjanapothi, D. ; and Taesotikul, T.; J Ethnopharmacol, **85, 2003**, 221.
14. Dongmo, A. B. ; Kamanyi, A. ; Anchang, M. S. ; Nkeh, B.C.A.; Njamen, D.; and Ngudefack, T.B.; J Ethnopharmacol, **77(2-3), 2001**, 137.
15. Ahamed, S.M.; Swamy, S.K.; Jayaveera, K.N.; Rao, V.; and Kumar, S.V.; Pharmacologyonline, **3, 2008**, 852.
16. Trovato, A.; Raneri, E.; Kouladis, M.; Tzakou, O.; Taviano, M.F.; and Galati, E.M.; IL Farmaco, **56, 2001**, 455.
17. Semnani, K.M.; Saedi, M.; Hamidian, M.; Vafamehr, H.; and Dehpour, A.R.; J Ethnopharmacol, **80, 2002**, 181.
18. Lu, T.S.; Ko, Y.Z.; Huang, H.W.; Hung, Y.H.; Lin, Y. C.; and Pen, W.H.; J Ethnopharmacol, **113, 2007**, 142.
19. Hajhashemia, V.; Sajjadi, S.E.; and Heshmatib, M.; J Ethnopharmacol, **124, 2009**, 475.
20. Sayyah, M.; Hadidi, N.; Kamalinejad, M.; J Ethnopharmacol, **92, 2004**, 325.
21. Hajhashemi, V.; Ghannadi, A.; and Sharif, B.; J Ethnopharmacol, **89, 2003**, 67.
22. Leea, K.H.; Choib, E.M.; J Ethnopharmacol, **120, 2008**, 103".
23. Intahphuak, S.; Panthong, A.; Kanjanapothi, D.; Taesotikul, T.; Krachangchaeng, C.; and Reutrakul, V.; J Ethnopharmacol, **90, 2004**, 69.
24. Owoyele, V.B.; Oloriegbe, Y.Y.; Balogun, E. A.; and Soladoye, A.O.; J Ethnopharmacol, **99, 2005**, 153.
25. Perazzo, F.F.; Souza, G.H.B.; Lopes, W.; Cardoso, L.G.V.; Carvalho, J.C.T.; and Nanayakkara, N.P.D.; J Ethnopharmacol, **99, 2005**, 215.
26. OAdeyemi, O.; Okpo, S.O.; and Ogunti, O.O.; Fitoterapia, **73, 2002**, 375.
27. Blank, M.D.F.; Dmitrieva, E.G.; Franzotti, E.M.; Antonioli, A.R.; Andrade, M.R.; and Marchioro, M.; J Ethnopharmacol, **91, 2004**, 215.
28. Zhang, G.Q.; Huang, X.D.; Wang, H.; Leung, A.K.; Chan, C.L.; and Fong, D.W.; J Ethnopharmacol, **118, 2008**, 290.
29. Franzotti, E.M.; Santos, C.V.F.; Rodrigues, H.M.S.L.; Moura, R.H.V.; Andrade, M.R.; and Antonioli, A.R.; J Ethnopharmacol., **72, 2000**, 273.
30. Owoyele, V.B.; Wuraola, C.O.; Soladoye, A.O.; and Olaleye, S. J Ethnopharmacol, **90, 2004**, 317.
31. Dharmasiri, M.G.; Jayakody, J.R.A.C.; Galhena, G.; Liyanage, S.S.P.; and Ratnasooriya, W.D.; J Ethnopharmacol, **87, 2003**, 199.
32. <http://www.flowersofindia.net/catalog/slides/Pomelo.html> . (Cited on 28th-Jan-2013).
33. Bailey, L.H.; Bailey, E.Z.; and Bailey, L.H.; Hortatorium. 1976. *Hortus Third: A concise dictionary of plants cultivated in the United States and Canada*. New York: Macmillan, **1976**, 276.
34. Morton, J.; Pummelo. *Citrus maxima* In: Fruits of warm climates. Available online from <http://www.hort.purdue.edu/newcrop/morton/pummelo.html>., **1987**, 147-151.
35. Van Wyk, B.E.; 2005. "*Citrus maxima*." Food Plants of the World: An Illustrated Guide. Portland, OR: Timber Press, **2005**, 141.
36. Manthey, A.; & Grohmann, K.; J. Agri. Food Chem., **44, 1996**, 811.
37. Manthey, A.; Grohmann, K.; J. Agri. Food Chem, **49, 2001**, 3268.
38. Nogata, Y.; Sakamoto, K.; Shiratsuchi, H.; Ishii, T.; Yano, M. ; and Ohta, H.; Biosci. Biotech. Biochem, **70, 2006**, 178.
39. Chung, S.K.; Kim, S.H.; Choi, Y.H.; Song, E.Y.; and Kim, S.H.; Food Industry and Nutrition, **5, 2000**, 42.
40. Xu, G.; Liu, D.; Chen, J.; Ye, X.; Ma, Y.; and Shi, J.; Food Chem., **106, 2008**, 545.

