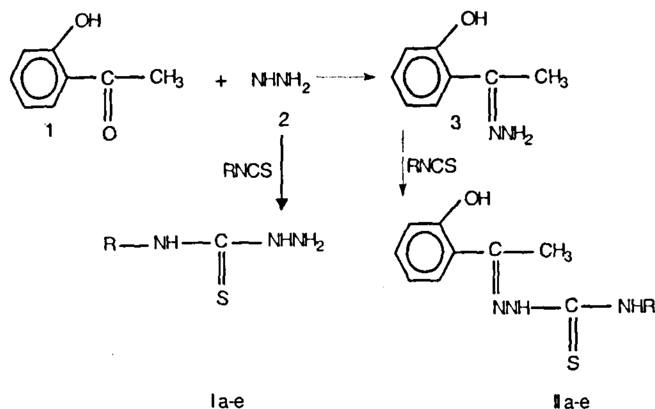

R= p-BrC_6H_5, C_6H_{11}, C_4H_9, C_2H_5, CH_3

Scheme 1: Synthesis of thiosemicarbazides and thiosemicarbazones.

coli. The results show that in series Ia-e the compound having methyl substitution (Ie) was most active compound. The replacement of methyl group with *p*-Br.Ph., butyl, ethyl and cyclohexyl groups decreases the activity while in series IIa-e the compound having *p*-Br.Ph. substituent (IIa) was most active compound and replacement of this group with cyclohexyl, butyl, ethyl and methyl groups decreases the activity.

REFERENCES

1. Sundane, A.R., Ranganath, S.H., Prayagraj, G., Rudresh, K. and Satyanarayna, N.D., **Orient. J. Chem.** 1998, 14, 251.
2. Hassan, S. and Honda, Y., **Bull. Pharm. Sci. Assint. Univ.**, 1999, 22, 97.
3. Pandeya, S.N., Sriram, D., Nath, G. and De clerq, E., **Eur. J. Pharm. Sci.**, 1999, 9, 25.
4. Salman, A., Ates, O., Cesur, N. and Otuk, G., **Archder pharmazie**, 1991, 324, 55.
5. Omer, A.M.M.E., Salma, H.M. and Eshba, N.H., **Farmaco. Ed. Sci.**, 1985, 40, 49.
6. Ananthanarayan, R. and Panikar, J.C.K., In; Textbook of Microbiology, 5th Edn., Orient Longman, Chennai, 1999, 578.

Antiinflammatory Effect of *Ocimum sanctum* Linn. and its Cultures

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Callus cultures from stem of *Ocimum sanctum* were induced on slightly modified Murashige and Skoog's medium and supplemented with 2,4-dichlorophenoxyacetic acid (2ppm) and kinetin (1ppm). Different extracts of stem, leaf and stem calli of *O. sanctum* were tested for antiinflammatory activity using carrageenan-induced rat paw oedema model in comparison with the standard indomethacin. The ethanol extract of callus tissue exhibited maximum significant antiinflammatory activity out of all extracts studied followed by ethanol extracts of leaves of *O. sanctum*.

Ocimum sanctum Linn. (Labiatae, *Tulsi*) is a widely grown plant. In herbal medicine it has been included as a general health promotor[1] and most of its activities that include antistress[2], adaptogenic[3], anticancer[4], antiinflammatory[5-6], antihyperlipidemic[7], antihypercholesteremic[8], hepatoprotective[9], radioprotective[10] and antimicrobial[11] have been investigated scientifically. No investigations to determine the antiinflammatory activity of the tissue culture samples of *O. sanctum* or the stem extracts of this plant have been carried out even though antiinflammatory effect has been reported with the leaf[12] and seeds[13] of *O. sanctum*. Hence in the present study, an attempt has been made to determine the antiinflammatory effect of cultured tissues and stems of *O. sanctum*.

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O. sanctum herb was collected from cultivated plants

grown in the medicinal plants garden of the University Institute of Pharmaceutical Sciences, Panjab University, Chandigarh. The stem portion was washed with running tap water, scrubbed clean with dilute detergent (1-2% Cleansol solution) for 2-4 min., washed again with tap water and finally with distilled water. It was sterilized with 0.1% w/v mercuric chloride for 3-5 min and washed with sterile distilled water (3-4 times).

Surface sterilized stem explants (10-15 mm) were inoculated under sterilized conditions on agar-solidified Murashige and Skoog's medium[14] with some modifications (MS) (ferric citrate was used in place of ferrous sulphate and manganese sulphate monohydrate in place of manganese tetrahydrate. Edamine was not used.). The medium was supplemented with 2% sucrose and growth regulators: 2,4-dichlorophenoxyacetic acid (2,4-D) (2 ppm) in combination with 6-furfuryl aminopurine (kinetin, Kn, 1 ppm). The pH of the medium was adjusted to 5.7-5.8. The cultures were maintained at a temperature of 25 ± 2^{0} for 12 h a day using white fluorescent tubes (0.6-m long, 20 watt each).

Stem callus (SC) tissue (6-month-old) dried in oven at 40^{0} for about 24 h was reduced to moderately coarse powder (8.57 g) and was extracted by refluxing with chloroform and ethanol (95% v/v) for 4 and 5 h, respectively. The dried marc was shaken with warm distilled water for 20h and filtered. Stem (St) and leaf (Lf) portions of parent plant (4-mo old) dried separately in shade were reduced to moderately coarse powder (#10) and 500 g each was soxhlet extracted for about 22 h with petroleum ether (60-80°), chloroform and ethanol (95% v/v). The dried marc was left in contact with distilled water for 48 h and filtered.

After removal of solvents from various extracts *in vacuo*, the percentage of various extracts obtained was: petroleum ether extracts (St-5.88, Lf-4.29), chloroform extracts (SC-4.41, St-1.84, Lf-6.52), ethanol extracts (SC-21.49, St-1.84, Lf-6.52) and water extracts (SC-15.75, St-2.51, Lf-6.85). Phytochemical screening[15-18] gave positive tests for saponins, sterols, triterpenoids, carbohydrates, tannins and proteins in SC, St and Lf while flavonoids were detected in St and Lf only.

Male Wistar rats weighing 150-250 g procured from the central Animal House of Panjab University, Chandigarh were used. The animals received a standard pelleted diet (M/S Hindustan Lever Foods, Calcutta, India) and water *ad libitum*, and were maintained under standard environmental conditions (25±5°, 12 h of light/dark cycle). The experimental protocols were approved by the Institutional Animal Ethics Committee.

Chloroform, ethanol and water extracts of callus tissue; petroleum ether, chloroform, ethanol and water extracts of stem; and petroleum ether, chloroform, ethanol and water extracts of leaves were tested. The antiinflammatory activity of these extracts (100, 200, 400 and 800 mg/kg, orally) was tested and compared with that of 30 mg/kg oral indomethacin (Jagsonpal Limited, Faridabad) using the carrageenan-induced rat paw oedema model.

Results were expressed as mean±S.E.M. and all the extracts were compared with indomethacin (standard) and control separately using one way analysis of variance (ANOVA) followed by Dunnett's test. $P<0.05$ was considered statistically significant.

Callus cultures were successfully induced on MS+2,4-D (2 ppm) + Kn (1 ppm). The colour of the callus developed was in patches of white, whitish-green and light green. It consisted of both nodular and non-nodular type of texture, which were soft and slightly hard, respectively.

Antiinflammatory activity studies indicated that chloroform extracts of callus tissue, stem and leaves of parent plant inhibited oedema at a dose of 800 mg/kg at 1, 2, 3, 4 and 5 h interval. Moreover, significant inhibition observed at the dose of 800 mg/kg was comparable to indomethacin as well as extracts of stem and leaf of parent plant (fig. 1). A dose of 400 mg/kg of chloroform extracts of callus tissue, stem and leaf also showed significant activity at 3, 4 and 5 h interval. Significant inhibition of oedema was also observed with leaf extract at a dose of 200 mg/kg at 3, 4 and 5 h time points after administration.

Antiinflammatory effect of ethanol extract of stem callus was comparable to that of leaf extract at all doses as significant oedema suppressant activity was observed in both cases during the entire 5-h study. While in case of stem extract, only the 400 and 800 mg/kg doses showed antiinflammatory effect equivalent to that of leaf extract. However, ethanol extract of stem was found to possess significant activity at doses of 100 and 200 mg/kg only at 4 and 5 h interval. The point of significance is that ethanol extract of callus tissue and leaf were found to be more effective than indomethacin at a dose of 800 mg/kg (fig. 2) While at this dose stem extract showed activity comparable to indomethacin.

Water extracts of callus and leaf were found to pos-

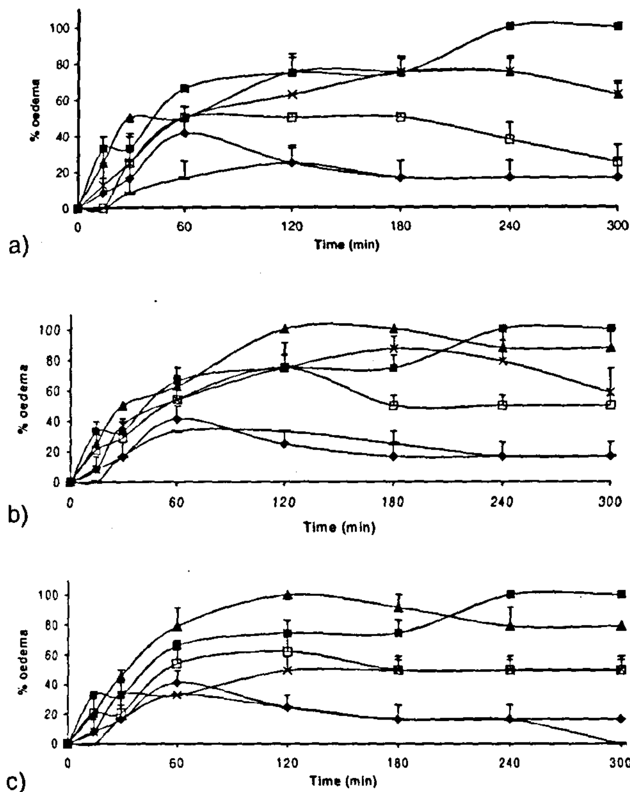

Fig. 1: Antiinflammatory activity of chloroform extracts of *O. sanctum*.

Antiinflammatory activity of chloroform extracts of (a) stem callus (b) stem and (c) leaves *O. sanctum* at doses of (-▲-) 100, (-x-) 200, (-□-) 400 and (-■-) 800 mg/kg in comparison with (-□-) control and (-◆-) the standard indomethacin. Data is expressed as Mean±S.E.M.; *P<0.05 versus control (ctrl); **P<0.05 versus standard (indo: indomethacin). Vertical lines show mean±S.E.M. (n=5-7).

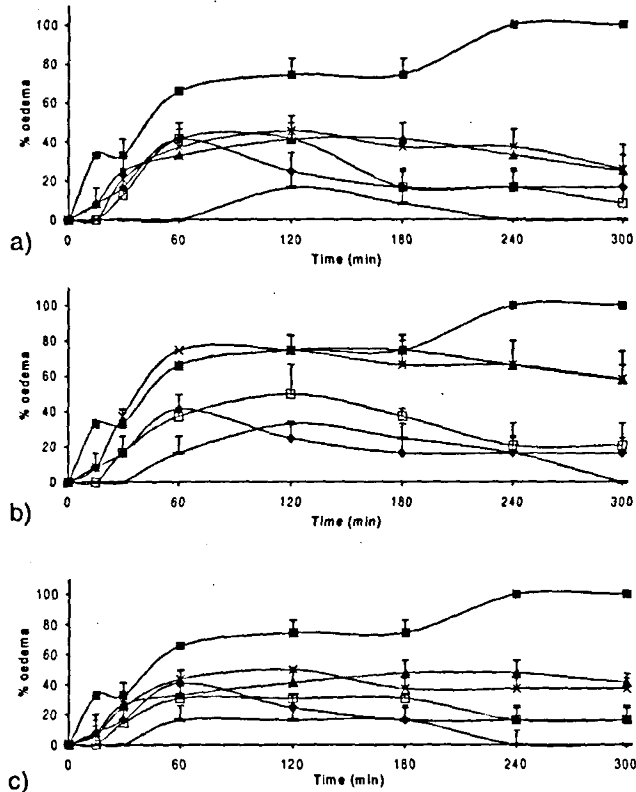

Fig. 2: Antiinflammatory activity of ethanol extracts of *O. sanctum*.

Antiinflammatory activity of ethanol extracts of (a) stem callus (b) stem and (c) leaves *O. sanctum* at doses of (-▲-) 100, (-x-) 200, (-□-) 400 and (-□-) 800 mg/kg in comparison with (-■-) control and (-◆-) the standard indomethacin. Data is expressed as Mean±S.E.M.; *P<0.05 versus control (ctrl); **P<0.05 versus standard (indo: indomethacin). Vertical lines show mean±S.E.M. (n=5-7).

sess significant activity at doses of 400 and 800 mg/kg. However, water extract of stem didn't show antiinflammatory activity at any dose. Petroleum ether extracts of stem and leaf showed significant oedema suppressant activity at a dose of 800 mg/kg during the entire 5 h study. Leaf extract at a dose of 800 mg/kg also showed comparable activity to indomethacin at 4 and 5 h interval. Doses 200 mg/kg and 400 mg/kg of leaf and stem extracts showed significant oedema suppressant activity at 4 and 5 h after the carrageenan injection.

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REFERENCES

1. Rai, M.K., **Fitoterapia**, 1993, 65, 485.
2. Vaidya, A.B., **Indian J. Pharmcol.**, 1997, 29, S-340.
3. Sembulingam, K., Sembulingam, P.A. and Namasivayam, A., **Indian J. Physiol. Pharmacol.**, 1999, 43, 137.
4. Aruna, K. and Sivaramakrishnan., V.M., **Food Chem. Toxicol.**, 1992, 30, 953.
5. Chattopadhyay, R.R., Sarkar, S.K., Ganguly, S. and Basu, T.K., **Fitoterapia**, 1994, 65,146.

6. Singh, S., **Indian J. Expt. Biol.**, 1996, 54, 19.
7. Ravi, V. and Mani U.V., **J. Nutr. Environ. Med.**, 1997, 7, 113.
8. Sarkar, A., Lavania, S.C., Pandey, D.N. and Pant, M.C., **Indian J. Physiol. Pharmacol.**, 1994, 38, 311.
9. De, S., Ravishankar, B. and Bhassar G.C., **Indian Drugs**, 1993, 30, 355.
10. Uma Devi, P., Bishot, K.S. and Vinitha M., **Brit. J. Radiol.**, 1998, 71, 782.
11. Rajindhran, J., and Arun, M.M., **Geobios**, 1998, 25, 280.
12. Godhwani, S., Godhwani, J.L. and Vyas, D.S., **J. Ethnopharmacol.**, 1987, 21, 153.
13. Singh, S., Majumdar, D.K. and Rehan, H.M.S., **J. Ethnopharmacol.**, 1996, 54, 19.
14. Murashige, T. and Skoog, F., **Physiol. Plantarum**, 1962, 15, 473.
15. Evans, W.C., Eds., In; Trease and Evans' Pharmacognosy, 14th Edn., Gopsons Papers Limited, Noida, 1996, 340.
16. Farnsworth, R.N., **J. Pharm. Sci.**, 1996, 55, 225.
17. Evans, W.C., Eds., In; Trease and Evans' Pharmacognosy, 14th Edn., Gopsons Papers Limited, Noida, 1996, 191.
18. Evans, W.C., Eds., In; Trease and Evans' Pharmacognosy, 14th Edn., Gopsons Papers Limited, Noida, 1996, 218.

Synthesis and Multiple Biological Activities of Azomethines and 4-Thiazolidinones

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4-(2-Methoxy-5-methylphenyl)-1-(substituted benzal) thiosemicarbazides IIa-m and 2-(arylidene-hydrazono)-3-(2-methoxy-5-methylphenyl)-4-thiazolidinones IIIa-m have been prepared by the condensation of 2-methoxy-5-methylphenyl thiosemicarbazide I with different aromatic carboxaldehydes, which on cyclocondensation with chloroacetic acid in presence of sodium acetate in glacial acetic acid yielded 4-thiazolidinones IIIa-m. The characterisation of the compounds have been done on the basis of elemental analyses, IR, IH NMR and mass spectral study. All the compounds have been evaluated for their *in vitro* growth inhibiting activity against several microbes and most of the compounds have also been screened for antitubercular activity against *Mycobacterium tuberculosis* $H_{37}Rv$. Some selected compounds have been evaluated for their *in vitro* anticancer screen aimed at identifying agents having cell type specificity using batteries of cell lines derived from human solid tumor at National Cancer Institute, U.S.A.

Considerable interest has been shown in the field of thiazolidinone chemistry due to their wide range of therapeutic activities such as anticancer[1], antimicrobia[2], herbicida[3] and antithyroid[4]. Thiosemicarbazide derivatives have become attractive target in organic synthesis because of their reactivity and biological significance such as anticancer[5], antitumor[6], antimicrobia[7], antiviral[8] and antiiflammatory[9]. Continuous increase in bacterial resistance to existing drugs has been resulted due to wide spread use of antibiotics leading to research on new substances possessing antimicrobial activity.

Awareness of wide spread tuberculosis epidemic and the emergence of MDRTB have stressed the urgent need for new, effective antitubercular drugs. Because of spread of HIV infection, the menace of TB has reached alarming properties world wide[10]. To achieve this goal and on the basis of above considerations, some new 4-thiazolidinone derivatives have been investigated.

The key intermediate 2-methoxy-5-methylphenyl thiosemicarbazide I, ammonia and carbon disulphide with

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