REVIEW ARTICLE

Accepted 12 December 2001
Revised 21 November 2001
Received 11 May 2001
Indian J. Pharm. Sci., 2002, 64(3): 183-190

Inducible Nitric Oxide Synthase (iNOS) Inhibitors from Plants

NEERJA PANT AND D. C. JAIN*
*Medicinal Plants Chemistry Division, Central Institute of Medicinal and Aromatic Plants (CIMAP), P. O. CIMAP, Lucknow-226 015.

Nitric Oxide (NO), a gas at temperatures below -152° has long since been known as a toxic smog pollutant. Present reports furnish the fact that nitric oxide is synthesized in many cells endogenously. While the endogenous production of NO may significantly benefit an organism, its excessive production has been implicated in the pathogenesis of many diseases involving the cardiovascular, immune and nervous system. Inhibition of NO formation may have therapeutic benefit in patients with septic shock or inflammatory diseases. Phytochemicals isolated from various medicinally important plants provide valuable agents that suppress the expression of inducible nitric oxide synthase (iNOS) enzyme which are hence useful for the prevention of various diseases. Systematic work performed during the last decade has been reviewed and tabulated in this article.

Nitric oxide (NO) was honored as the molecule of the year in 1992[1]. Nitric oxide which was earlier considered as an environmental pollutant and a highly toxic gaseous free radical, is now known to play a crucial bioregulatory role as an intercellular messenger in a number of physiological processes such as vasodilatation, neurotransmission, platelet aggregation, in the cytostatic and cytotoxic action of macrophage and neutrophils[2,3]. An important chemical property of NO is that it rapidly and spontaneously reacts with a superoxide anion (O_2^-) to form a peroxynitrite anion ($ONOO^-$) and its conjugate acid, peroxynitrous acid (ONOOH) which is more toxic to biological system than O_2^- or NO alone[4].

Biosynthesis of NO:

Nitric oxide (NO) is biosynthesized in living organisms by the oxidation of L-arginine to NO and citrulline via an intermediate N^{ω}-hydroxy-L-arginine (NHA). The entire process is catalyzed by a remarkable family of enzymes, the nitric oxide synthases (NOS). The reaction is overall a five-electron oxidation of L-arginine using nicotinamide adenine dinucleotide phosphate (NADPH) as the source of electrons. The NO thus generated is rapidly converted to stable products of nitrogen oxide, nitrite and nitrate[5].

*For correspondence:
E-mail: cimap@satyam.net.in

Assay of nitric oxide:

Nitric oxide analysis in activated macrophages is done with the help of Griess reaction. NO released from the cells is detected and quantified photometrically as its stable product nitrite by a simple colorimetric reaction. (Griess reaction)[6]. In this reaction the cellular production of NO is determined by measuring its stable product nitrite in cell culture supernatants. Griess reagent [sulfanilamide/N-(1-naphthyl)-ethylenediamine dihydrochloride] added to various cell culture supernatants converts nitrite into a purple azo dye which can be quantified photometrically and thus used as a parameter for the NO synthesis of cultured cells[7].

Nitric oxide synthases (NOS):

NOS is a heme-containing enzyme with a sequence similar to cytochrome P-450 reductase[8]. Several isoforms of NOS are now known to exist which are either constitutive NOS (cNOS) or inducible (iNOS). The cNOS include the endothelial (eNOS) or neuronal (nNOS) isoforms that are calcium-dependant and release small and regulated amount of NO required for various physiological functions. On the

other hand, the iNOS is Ca^{++} independent and produces large amounts of NO continuously for longer periods of time and is responsible for the cytotoxicity of NO[9].

Inducible Nitric oxide synthase (iNOS):

Inducible nitric oxide synthase (iNOS) is an inflammation-induced enzyme that catalyzes the production of nitric oxide (NO), a molecule that may lead to carcinogenesis. Macrophages and some other cells have a transcriptionally inducible form of NOS (iNOS) that remains undetectable until these cells are activated. Interferon-γ and bacterial lipopolysaccharides (LPS) are the most potent activators of the iNOS gene in murine macrophages[10]. Tumor necrosis factor -α (TNFα), originally discovered by its anti-tumor activity, is one of the most pleotropic cytokines acting as a host defense factor in immunologic and inflammatory responses[11]. However, high production of NO by iNOS may induce host cell death and inflammation.

Inhibitors of iNOS:

Dirsch *et al.* stated that in the field of inflammation research the inducible nitric oxide synthase (iNOS) has become an important pharmacological target, since over-production of nitric oxide (NO) after induction of this enzyme seems to be associated with numerous pathological conditions[7]. Over expression of iNOS by various stimuli, resulting in over-production of NO, contributes to the pathogenesis of septic shock and some proinflammatory effects including vasodilatation, edema, cytotoxicity and autoimmune diseases[12]. Therefore, it is valuable to develop inhibitors of iNOS for potential therapeutic use. Thus, agents that suppress the expression of iNOS mRNA or enzyme protein will be useful for the prevention of various diseases. These agents have therapeutic potential in treating the hyperfunctioning of the NO pathway. Such agents should inhibit iNOS selectively without inhibiting the constitutive NO release[13].

Among the most widely used drugs in antiinflammatory therapies, synthetic glucocorticoid, dexamethasone is highly effective in controlling inflammation and this may be in part, due to its ability to inhibit iNOS expression. Glucocorticoid inhibition of NO production was described in cytokine- stimulated mesangial cells[14,15]. Di Rosa *et al.* first demonstrated that dexamethasone and hydrocortisone also inhibit the production of NO in the lipopolysaccharides and IFN-γ stimulated macrophage cell line J 774[16]. Walker *et al.* studied the mechanisms by which these synthetic glucocorticoids suppress IFN-γ stimulated iNOS expression in RAW –264.7 cells[17]. Several analogues of L-arginine are now known to act as synthetic iNOS inhibitors[3,9,18,19].

One potential source for novel iNOS inhibitors is the diverse area of natural products. Compounds isolated from plants that are iNOS inhibitors showed inhibition of nitric oxide (NO) synthesis in a dose-dependent manner in murine macrophage-like RAW 264.7 cells stimulated with interferon-γ plus lipopolysaccharides. Murine macrophage-like cell line, RAW 264.7, is a suitable cell model to perform *in vitro* studies of the iNOS system. Since the systematic work on the isolation of compounds inhibiting the excess NO production has been reported during the last few years and there is no such compilation of NOS inhibitory compounds. Hence, we have reported the iNOS inhibitory compounds isolated from different plant parts of various families described in Table 1 and their structures, in fig. 1. This review summarizes the work reported till 2001.

NATURAL INHIBITORS

Alkaloids:

Two quinazoline alkaloids dehydroevodiamine (1) and evodiamine (2) isolated from Evodia rutaecarpa inhibited NO production in IFN-γ/LPS-stimulated RAW macrophages in concentration-dependent manner. Compound 1 inhibits NO production in almost equipotent manner, whether added before IFN-γ or before or after LPS application, indicating that the compound suppresses the activity of iNOS at multiple levels where as compound 2 affects only the IFN-γ related actions. These compounds account for the antiinflammatory property of this plant[20].

Coumarins:

Till now eight coumarins have been tested for NO inhibitory activity. Of these, three coumarins 5-[(6',7'-dihydroxy-3',7'-dimethyl-2-octenyl)oxy]psoralen (**4**), 5-geranyloxypsolaren (**5**) and oxypeucedanin (**9**) isolated from *Citrus hystrix* act as inhibitors of LPS, IFN-γ induced NO generation in RAW 264.7 cells. Compound 5 was found to be highly active (IC_{50} = 14 μM) where as other coumarins (**4**,**9**) bearing isoprenyl (IP) or geranyl (GR) chains with hydroxyl groups were drastically less active (IC_{50} values as 130 μM and 310 μM, respectively). The isolated compounds showed no detectable cytotoxicity at every concentration tested. The structural difference among these coumarins were found only in the side chains. Thus, these compounds can further be used in chemo-preventive activity in rodents and humans[22]. 5-Geranyloxypsolaren (**5**), 8-geranyloxypsolaren (**6**) and 5-geranyloxy-7-methoxycoumarin (**7**) isolated from the 80 % methanol-wa-

TABLE 1: LIST OF INDUCIBLE NITRIC OXIDE SYNTHASE (i NOS) INHIBITORY COMPOUNDS.

Name of compounds	Plant name	Family	Plant part	Ref
ALKALOIDS				
1 Dehydroevodiamine (**1**)	*Evodia rutaecarpa*	Rutaceae	Fruit	20
2 Evodiamine (**2**)	*Evodia rutaecarpa*	Rutaceae	Fruit	20
COUMARINS				
1 Deltoin (**3**)	*Saposhnikovia divaricata*	Umbelliferae	Dried roots rhizomes	21
2 5-[(6',7'-Dihydroxy-3',7'-dimethyl-2-octenyl) oxy]psoralen (**4**)	*Citrus hystrix*	Rutaceae	Fresh fruit	22
3 5-Geranyloxypsolaren (**5**)	*Citrus limon*	Rutaceae	Peel	4
	Citrus hystrix	Rutaceae	Fresh fruit	22
4 8-Geranyloxypsolaren (**6**)	*Citrus limon*	Rutaceae	Peel	4
5 5-Geranyloxy-7-methoxycoumarin (**7**)	*Citrus limon*	Rutaceae	Peel	4
6 Imperatorin (**8**)	*Saposhnikovia divaricata*	Umbelliferae	Dried roots rhizomes	21
7 Oxypeucedanin (**9**)	*Citrus hystrix*	Rutaceae	Fresh fruit	22
8 Scopoletin (**10**)	*Fraxinus*	Oleaceae	Bark	23
	rhynchophylla	Asteraceae	Aerial	13
	Artemisia feddei		parts	24
9 Cnidicin (**11**)	*Angelica koreana*	Umbelliferae	Roots	24
DIARYLHEPTANOIDS				
1 (5S)-1,7-Bis-(3,4-dihydroxyphenyl)-heptane-5-hydroxy-3-one (Hirsutanonol) (**12**)	*Alnus hirsuta*	Betulaceae	Leaves	2
2 (5S)-1,7-Bis-(3,4-dihydroxyphenyl)-heptane-3-one-5-O-b-D-xylopyranoside (oregonin) (**13**)	*Alnus hirsute*	Betulaceae	Leaves	2
DITERPENES				
1 Andrographolide (**14**)	*Andrographis paniculata*	Acanthaceae	Leaves	25
2 Ent-6a,8a,18-trihydroxy-13(16),14-Labdadiene (Andalusol) (**15**)	*Sideritis foetens*	Lamiaceae	Aerial parts	26
3 Kamebanin (**16**)	*Isodon japonicus*	Labiatae	Whole plant	27
4 Kamebacetal A (**17**)	*Isodon japonicus*	Labiatae	Whole plant	27
5 Kamebakaurin (**18**)	*Isodon japonicus*	Labiatae	Whole plant	27

6	Excisanin A (**19**)	*Isodon japonicus*	Labiatae	Whole plant	27
FLAVONOIDS					
1	5,4'-Dihydroxy-6,7,8,3',5'-pentamethoxyflavone (**20**)	*Cleome droserifolia*	Capparidaceae	Aerial parts	28
2	5,4'-Dihydroxy-6,7,8,3'-tetramethoxyflavone (**21**)	*Cleome droserifolia*	Capparidaceae	Aerial parts	28
POLYACETYLENES					
1	Falcarindiol (**22**)	*Saposhnikovia divaricata*	Umbelliferae	Dried roots rhizomes	29
2	Falcarinone (**23**)	*Saposhnikovia divaricata*	Umbelliferae	Dried roots rhizomes	29
3	Panaxydol (**24**)	*Saposhnikovia divaricata*	Umbelliferae	Dried roots rhizomes	29
4	Panaxynol (**25**)	*Saposhnikovia divaricata*	Umbelliferae	Dried roots rhizomes	29
5	Panaxytriol (**26**)	*Saposhnikovia divaricata*	Umbelliferae	Dried roots rhizomes	29
SESQUITERPENES					
1	Dehydrocostuslactone (**27**)	*Saussurea lappa*	Compositae	Roots	30
2	Yomogin (**28**)	*Artemisia princeps*	Asteraceae	Whole herb	31
3	Costunolide (**29**)	*Magnolia grandiflora*	Magnoliaceae	Dried leaves	32
4	Parthenolide (**30**)	*Magnolia grandiflora*	Magnoliaceae	Dried leaves	32
		Tanacetum parthenium	-	-	33
TANNINS					
1	Epi- gallocatechin-3-gallate (EGCG) (**31**)	*Green tea*	-	-	34
MICELLANEOUS					
1	Curcumin (**32**)	*Curcuma zanthorrhiza*	Zingiberaceae	Roots	7,35, 36
2	Epi-rhododendrin (**33**)	*Acer nikoense*	Aceraceae	Stem Leaves	37
3	Ferulaldehyde (**34**)	*Fraxinus rhynchophylla*	Oleaceae	Bark	23
4	Honokiol (**35**)	*Magnolia obovata*	Magnoliaceae	Stem bark	38
5	Magnolol (**36**)	*Magnolia obovata*	Magnoliaceae	Stem bark	38
6	Rhododendrol (**37**)	*Acer nikoense*	Aceraceae	Stem Leaves	37
AQUEOUS EXTRACT					
1	Aqueous extract	*Tinospora tuberculata*	Menispermaceae	Stem	39

*Compound structure number is given in parenthesis.

ter portion of the lemon peel extract exhibited inhibitory activity toward tumor promoter 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced Epstein-Barr virus (EBV) activation at a concentration of 10 µM in Raji cells. Compound **6** and **7** inhibit the LPS/IFN-γ triggered iNOS expression pathway or iNOS enzyme activity. In all, compound *6* was indicated to have higher inhibitory activity towards EBV activation, O_2^- and NO generation than compounds **5** and compound **7**[4]. Furanocoumarins, imperatorin (**8**) and deltoin (**3**) had been suggested to be the major components of *S. divaricata* to inhibit NO production in RAW 264.7 cells. The IC_{50} values of compounds **8** and **3** (64 µM and 35 µM, respectively) are much higher than for the polyacetylene, falcarindiol (**17**) and panaxynol (**20**)[21]. Scopoletin (**10**) a coumarin, isolated from

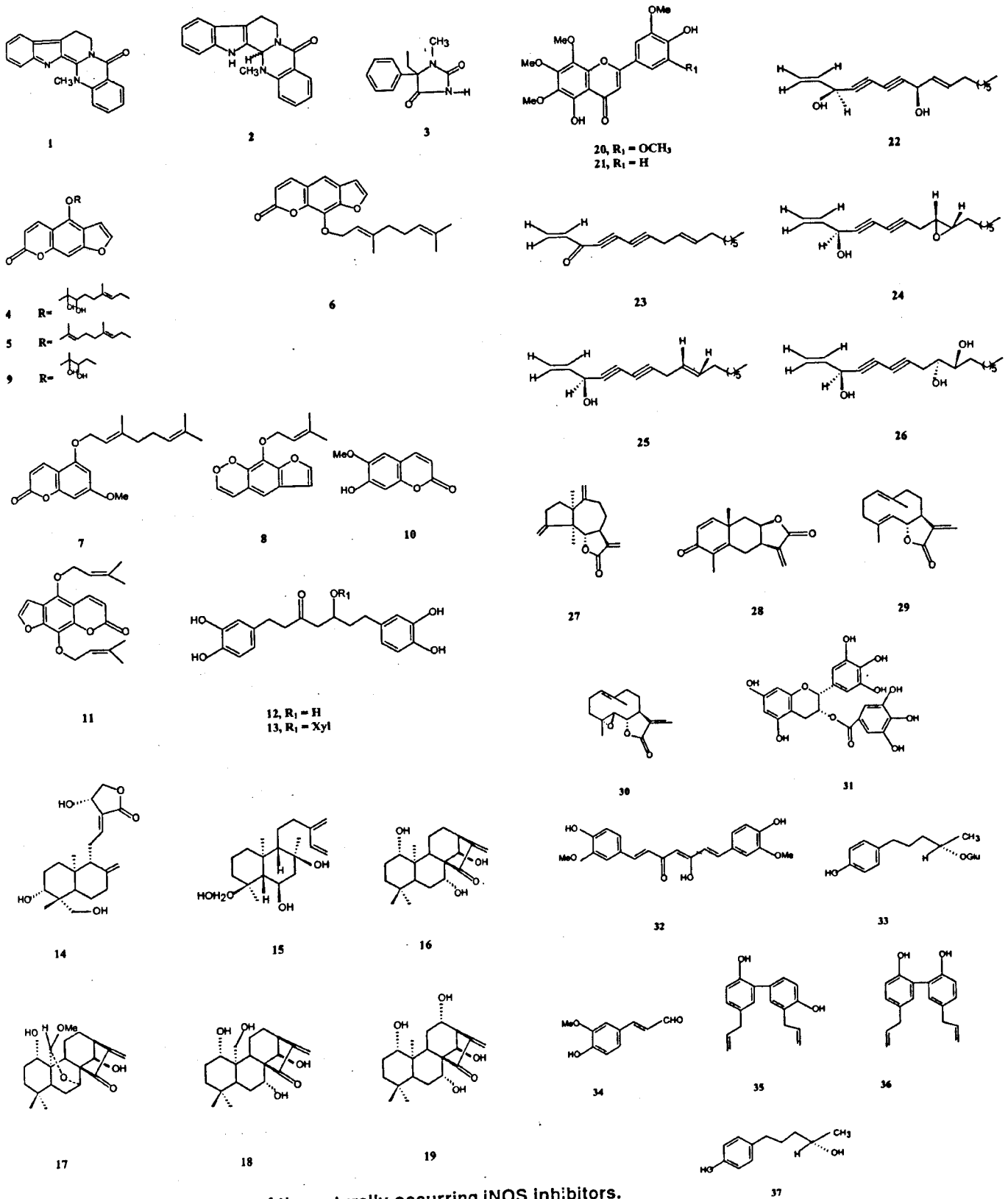

Fig. 1: Chemical structures of the naturally occurring iNOS inhibitors.

A. feddei inhibits the production of NO-induced by IFN-γ plus LPS in RAW 264.7 macrophages. The mechanism for the inhibition of NO production was due to suppression of the expression of iNOS mRNA as well as enzyme protein[13]. Scopoletin has also been isolated from the dried bark of *Fraxinus rhynchophylla.* The compound inhibited NO production by IFN-γ plus LPS-stimulated RAW 264.7 cells with IC_{50} value of 52 μM. Compound did not show cytotoxicity within concentration of 1-50 μg/ml.[23] Cnidicin (**11**), a coumarin isolated from the root extract of *Angelica koreana* inhibited the expression of nitric oxide synthases in RAW 264.7 cells with IC_{50} of 7.5μM[24].

Diarylheptanoids:

Two diarylheptanoids, hirsutanonol (**12**) and oregonin (**13**) isolated from fresh leaves of *Alnus hirsuta*, an indigenous species growing in Korea, were found to be potent iNOS inhibitors. They showed inhibition of NO production in interferon-γ (IFN-γ) and LPS-activated RAW 264.7 cells in a dose-dependent manner. Their IC_{50} values were 3.8 and 14.3 μM, respectively. This activity was due to the suppression of expression of iNOS mRNA[2].

Diterpenes:

Andrographis paniculata utilized in traditional system of medicine is used for the treatment of bacterial infection and inflammatory diseases (e.g. Rheumatoid arthritis). Andrographolide, (**14**) a bicyclic diterpenoid lactone, isolated from this plant display NO synthesis inhibitory effect. The inhibitory effect was due to the inhibition of iNOS protein induction and this inhibition of iNOS synthesis may contribute to the beneficial haemodynamic effects of andrographolide in endotoxic shock[24]. Andalusol (**15**) was isolated from the acetone extract of *Sideritis foetens* (aerial parts). Compound **15** did not cause direct inhibition of iNOS activity, but rather affected the expression of the enzyme during the initial 0-6 h after LPS stimulation. This shows the anti-inflammatory property of this compound[25]. Four known kaurane diterpenes (**16-19**) were isolated by activity-guided fractionation from the plant *Isodon japonicus* with IC_{50} values of 0.02 (0.06), 0.21 (0.58), 0.05 (0.15) and 0.12 mg/ml (0.35 mM), respectively[27].

Flavonoids:

5,4'-Dihydroxy-6,7,8,3',5'-pentamethoxyflavone (**20**) and 5,4'-dihydroxy-6,7,8,3'-tetramethoxyflavone (**21**) isolated from methanol extract of *Cleome droserifolia* suppressed the NO production in dose-dependent manner. IC_{50} values for the suppression of NO production by **20** and **21** were 50.5 and 85.5 mM, respectively. The activity of compound **21** was weaker than that of compound 20. The compounds might be immunosuppressive constituent of *C. droserifolia* and have a potential to be antiinflammatory and immunomodulatory agents[26].

Polyacetylenes:

Five polyacetylenes, falcarindiol (**22**), falcarinone (**23**), panaxydol (**24**), panaxynol (**25**) and panaxytriol (**26**) isolated from the ethylacetate extract of roots of *S. divaricata* inhibited nitrite production by iNOS. The IC_{50} value of falcarindiol, falcarinone, panaxydol, panaxynol and panaxytriol were 1.98, > 20, 6.58, 2.23 and 9.58 mM, respectively. Among these, falcarinone showed a marginal effect on NO production suggesting that the hydroxy group at C-3 plays a critical role in their inhibitory effect[27].

Sesquiterpenes:

Ethyl acetate soluble fraction of *Saussurea lappa* yielded compound dehydrocostuslactone (**27**), a sesquiterpene lactone that reduced the level of NO production in LPS-activated macrophages cell culture systems by inhibiting iNOS expression. The IC_{50} value was 3.0 μM. This compound may have potential in the treatment of endotoxemia[28]. Yomogin (**28**), a eudesmane sesquiterpene isolated from dichloromethane soluble fraction of *Artemisia princeps* was tested for NO production in LPS-activated murine macrophages. It exhibited potent inhibition on NO production with IC_{50} value calculated as 3±0.14 μM. This result suggested an anti-inflammatory activity of compound **28**[29]. Sesquiterpene lactones, costunolide (**29**) and parthenolide (**30**) were isolated from *Magnolia grandiflora.* Comparable activities for both the compounds in inhibition of NO production was with an IC_{50} value of 0.43 μM for compound **29** and 0.56 μM for the compound **30.**

Tannins:

A catechin, which is a polyphenolic phytochemical, epigallocatechin-3-gallate (EGCG) (**31**) isolated from green tea is the most potent in terms of antioxidative capacity and has been ascribed to have the predominant role in cancer chemoprevention. It has been found that it also inhibits the NO production and iNOS gene expression[30].

Miscellaneous:

Curcumin (**32**), a dietary polyphenolic, isolated from *Curcuma zanthorrhiza* inhibits the NO production in a concentration-dependent manner with an IC_{50} of 6 μM. Curcumin decreases the activity and protein levels of iNOS by reduc-

ing the expression of iNOS mRNA. Exact mechanism for inhibition of iNOS induction by curcumin is not known[7,31,32]. Rhododendrol (**37**) and its glucoside, epi-rhododendrin (**33**) isolated as active principle from ethylacetate soluble and *n*-butanol soluble fractions of *Acer nikoense* also suppressed the NO production. Compound **37** significantly reduced the maximal level of NO release in the LPS-stimulated macrophage when given p.o. at a dose of 50 mg/kg/day and did not show any cytotoxic effect toward the macrophage, where as its glucoside (**33**) suppressed the NO production by 35 % when p.o. administered at a dose of 30 mg/kg/day and the activity was weaker than that of compound **37**. Thus, the compounds **33** and **37** have a potential to be anti-inflammatory drugs[33]. Ferulaldehyde (**34**) was isolated from dried bark of *Fraxinus rhynchophylla*, which was screened for NO inhibitory activity. The compound inhibited NO production by interferon-γ (IFN-γ) plus LPS-stimulated RAW 264.7 cells with IC_{50} of 90 μM. The compound did not show cytotoxicity in the concentration range of 1-50 μg/ml. The inhibition of NO production of **34** was due to suppression of the expression of iNOS protein[23]. Two inhibitors of NO were isolated from the methanol extract of stem bark of *Magnolia obovata*. Their structures were elucidated as honokiol (**35**) and magnolol (**36**) with IC_{50} value of 16.8 and 6.4 μM, respectively. Both reduced the inducible level of iNOS and TNF-α in the LPS-activated macrophage cell culture system. Thus, these compounds may be used in treatment of endotoxemia and inflammation accompanied by the overproduction of NO and TNF-α[34]. The aqueous extract of *Tinospora tuberculata* exerts inhibitory effects on enhanced NO formation in both cell and cell-free systems. Thus, the compounds present in this extract may be responsible for antiinflammatory and antiinfective activity of this plant[35].

CONCLUSIONS

Till now thirty compounds of different classes have been isolated from various plants of different families for NOS inhibitory activity. They all inhibited either the expression of iNOS enzyme or inhibition of NO production by IFN-γ plus LPS –stimulated RAW 264.7 cells. Among these the coumarins are the major group of compounds isolated as NOS inhibitors. Till now eight coumarins have been isolated from five different plants. The coumarins that bear a geranyloxy group appear to be potent NOS inhibitors. These naturally occurring NOS inhibitors may be used as antiinflammatory drugs or in the treatment of endotoxemia. Therefore, it would be worth while to isolate naturally occurring compounds, which may act as potent and selective inhibitors of iNOS for potential therapeutic use.

ACKNOWLEDGEMENTS

Authors thank the Director, CIMAP for constant encouragement and for the award of Senior Research Fellowship to Ms. Neerja Pant.

REFERENCES

1. Kulkarni, S.K. and Gupta, M., **Indian Drugs**, 2000, 37, 65.
2. Lee, M.W., Kim, N.Y., Park, M.S., Ahn, K.H., Toh, S.H., Hahn, D.R., Kim, Y.C., and Chung, H.T., **Planta Medica**, 2000, 66, 551.
3. Moncada, S., Palmer, R.M.J. and Higgs, E.A., **Pharmacol. Rev.**, 1991, 43, 109.
4. Miyake, Y., Murakami, A., Sugiyama, Y., Isobe, M., Koshimizu, K. and Ohigashi, H., **J. Agric. Food Chem.**, 1999, 47, 3151.
5. Adams, D.R., Brochwicz-Lewinski, M. and Butler, A.R., In; Herz, W., Falk, H., Kirby, G.W., Moore, R.E. and Tamm. C.H., Eds., Progress in the chemistry of natural Products, Vol. 76, Springer Wein, New York, 1999, 1.
6. Green, L.C., Wagner, D.A., Glogowski,J., Skipper, P.L., Wishnok, J.S. and Tannenbaum, S.R., **Anal. Biochem.**, 1982, 126, 131.
7. Dirsch, V.M., Stuppner, H. and Vollmar, A.M., **Planta Medica**, 1998, 64, 423.
8. Marletta, M.A., **Cell**, 1994, 78, 927.
9. Moncada, S., Higgs, A. and Furchgott, R., **Pharmacol. Rev.**, 1997, 49, 137.
10. Kamijo, R., Harada, H., Matsuyama, T., Bosland, M., Gerecitano, J., Shapiro, D., Le, J., Koh, S.I., Kimura, T., Green, S.J., Mak, T.W., Taniguchi, T. and Vilcek, J., **Science**, 1994, 263, 1612.
11. Habtemariam, S., **Planta Medica**, 2000, 66, 303.
12. Mineo, M., Nakamura, N., Kitajima, K., Ueda, M., Tsutsumishita, Y., Futaki, S. and Takaishi, Y., **Biochem. Biophys. Res. Commun.**,1997, 239, 367.
13. Kang, T.H., Pae, H.O., Jeong, S.J., Yoo, J.C., Choi, B.M., Jun, C.D., Chung, H.T., Miyamoto, T., Higuchi, R. and Kim, Y.C., **Planta Medica**, 1999, 65, 400.
14. Pfeilschifter, J. and Schwarzenbach, H., **FEBS Lett.**, 1990, 273, 185.
15. Pfeilschifter, J., **Eur. J. Pharmacol.**, 1991, 195, 179.
16. Di Rosa, M., Radomski, M., Carnucci, R. and Moncada, S., **Biochem. Biophys. Res. Commun.**,1990, 172, 1246.
17. Walker, G., Pfeilschifter, J. and Kunz, D., **J. Biol. Chem.**, 1996, 271, 16679.
18. Marletta, M.A., **J. Med. Chem.**, 1994, 37, 1899.
19. Moncada, S. and Higgs, E.A., **FASEB J.** , 1995, 9, 1319.
20. Chiou, W.F., Sung, Y.J., Liao, J.F., Shum, A.Y.C. and Chen, C.F., **J. Nat. Prod.**, 1997, 60, 708.
21. Wang, C.C., Chen, L.G. and Yang, L.L., **Cancer Lett.**, 1999, 145, 151.
22. Murakami, A., Gao, G., Kim, Oe K., Omura, M., Yano, M., Ito, C., Furukawa, H., Jiwajinda, S., Koshimizu, K. and Ohigashi, H., **J. Agric. Food Chem.**, 1999, 47, 333.
23. Kim, N.Y., Pae, H.O., Ko, Y.S., Yoo, J.C., Choi, B.M., Jun, C.D., Chung, H.T., Inagaki, M., Higuchi, R. and Kim, Y.C., **Planta Medica**, 1999, 65, 656.

24. Ryu, S.Y., Kou, N.Y., Choi, H.S., Ryu, H., Kim, T.S. and Kim, K.M., **Planta Medica**, 2001, 67, 172.
25. Chiou, W.F., Lin, J.J. and Chen, C.F., **Brit. J. Pharmacol.**, 1998, 125, 327.
26. Heras, R.D.L., Navarro, A., Diaz-Guerra, M.J., Bermejo, P., Castrillo, A., Bosca, L. and Villar, A., **Brit. J. Pharmacol.**, 1999, 128, 605.
27. Hwang, B.Y., Lee, J-H., Koo, T.H., Kim, H.S., Hong, Y.S., Ro, J.S., Lee, K.S. and Lee, J.J., **Planta Medica**, 2001, 67, 406.
28. Fushiya, S., Kishi, Y., Hattori, K., Batkhuu, J., Takano, F., Singab, A.N.B. and Okuyama, T., **Planta Medica**, 1999, 65, 404.
29. Wang, C.N., Shiao, Y.J., Kuo, Y.H., Chen, C.C. and Lin, Y.L., **Planta Medica**, 2000, 66, 644.
30. Lee, H.J., Kim, N.Y., Jang, M.K., Son, H.J., Kim, K.M., Sohn, D.H., Lee, S.H. and Ryu, J.H., **Planta Med.**, 1999, 65, 104.
31. Ryu S.Y., Oak M.H. and Kim K.M., **Planta Medica**, 2000, 66, 171.
32. Koo, T.H., Lee, J-H., Park, Y.J., Hong, Y-S., Kim, H.S., Kim, K-W. and Lee, J.J., **Planta Medica**, 2001, 67, 103.
33. Wong, H.R. and Menendez, I.Y., **Biochem. Biophys. Res. Commun.**, 1999, 262, 375.
34. Chan, M.M.Y., Fong, D., Ho, C.T. and Huang, H.I., **Biochem. Pharmacol.**, 1997, 54, 1281.
35. Chan, M.M.Y., Huang, H.I., Fenton, M.R. and Fong, D., **Biochem. Pharmacol.**, 1998, 55, 1955.
36. Brouet, I. and Ohshima, H., **Biochem. Biophys. Res. Commun.**, 1995, 206, 533.
37. Fushiya, S., Kabe, Y., Ikegaya, Y. and Takano, F., **Planta Medica**, 1998, 64, 598.
38. Son, H.J., Lee, H.J., Yun-Choi, H.S. and Ryu J.H., **Planta Medica**, 2000, 66, 469.
39. Yokozawa, T., Wang, T.S., Chen, C.P. and Hattori, M., **Phytother. Res.**, 2000, 14, 51.