were prepared seeded with the organisms in question. Later, the cups of 10 mm size were made by punching the agar plate aseptically, 0.1 ml (10^{-3}m) of the test compound in dimethylformamide was added using sterile pipettes. The plates were incubated at 37°C for 24 hr. The extent of zone of inhibition produced was measured. The plates with only solvent (DMF) were used as control. The bacteriological results are summarized in table 1.

The ligand PASA was moderately active and the complexes of Co(II) and Ni(II) were inactive against these micro-organisms. Hg(II) complex was highly active against *Sarcina lutea* and *E. coli* compared to other metal complexes. Th(IV) complex showed high activity against *E. coli* and less activity or same activity as that of ligand.

Table 1 *Bacteriological results of schiff bases and their metal complexes*

Ligand/ complex	Activity against *E. coli*	*S. lutea*	*B. sublitis*	*K. pneumoniae*
Ligand (PASA)	b	—	b	b
Co(PASA) Cl	—	—	—	—
Ni(PASA) Cl	—	—	—	—
Cu(PASA) Cl	b	—	b	—
Zn(PASA) Cl	a	a	b	—
Cd(PASA) Cl	a	a	—	—
Hg(PASA) Cl	c	c	b	b
Sn(PASA) Cl_4	a	b	b	b
Th(PASA) Cl_4	c	b	b	b
Ligand(PASP)	b	b	a	b
Co(PASP)Cl	b	c	c	a
Ni(PASP) Cl	—	—	—	—
Cu(PASP) Cl	b	c	b	b
Zn(PASP) Cl	b	a	—	a
Cd(PASP) Cl	b	a	a	—
Hg(PASP) Cl	c	c	b	b
Sn(PASP) Cl_4	c	b	a	b
Th(PASP) Cl_4	c	b	b	b
Ligand(PAST)	—	—	a	a
Co(PAST) Cl	b	—	b	—
Ni(PAST) Cl	—	—	—	—
Cu(PAST) Cl	b	c	b	b
Zn(PAST) Cl	a	—	a	—
Cd(PAST) Cl	a	—	a	—
Hg(PAST) Cl	b	c	c	b
Sn(PAST) Cl_4	a	b	a	—
Th(PAST) Cl_4	c	b	c	b

Inactive (< 13 mm); a. Less active (13-16 mm); b. Moderately active (17-20 mm); c. Highly active (21-27 mm).

Among the complexes of PASP, most of them are moderately or highly active against these micro-organisms. Co(II), Cu(II) and Hg(II) complexes are highly active against *Sarcina leutea* whereas they are less active against *K. pneumoniae*. Ni(II) complexes showed no activity against these bacteria. The ligand PASP shows moderate activity against *E. coli*, *S. lutea* and *K. pneumoniae*, less activity against *B. sublitis*. Th(IV) complexes showed moderate activity against *S. lutea*, *B. sublitis* and *K. pneumoniae* and high activity against *E. coli*. The ligand PAST and its Ni(II) complex showed no activity against these bacterial types. However, the ligand showed less activity against *B. subtilis* and *K. pneumoniae*. Cu(II), Hg(II) and Th(IV) complexes are moderately or highly active against these bacteria.

The azoschiff bases (ligands) with their metal complexes tested against four organisms showed different ranges of activity. They were found active against both gram-positive and gram-negative organisms and hence can be regarded as active antibacterial agents.

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ANTI-LIPID PEROXIDATIVE EFFICACY OF PLUMBAGIN AND MENADIONE: AN *IN VITRO* STUDY

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LIPID peroxidation is implicated in the damage to cellular constituents. The process of *in vivo* lipid

peroxidation resembles non-enzymic oxidation (auto-oxidation) of biological materials and is usually studied *in vitro* using tissue homogenate or subcellular fractions[1].

Plumbagin and menadione having anticancer effects[2,3] have been shown to be powerful antioxidants in microsomes[4,5]. Cytotoxic effects of menadione have been shown to be due to the intracellular generation of hydrogen peroxide[6] which has been shown to produce lipid peroxides in tissue homogenates[7]. The antioxidant nature of plumbagin and menadione was investigated against the lipid peroxidation induced by two oxidants—ascorbate and H_2O_2. DL-α-tocopherol and butylated hydroxy toluene (BHT) were used as positive controls.

Male wistar rats (100–120 g) were fasted overnight prior to use. The animals were killed and the liver and the heart removed immediately, washed and homogenized in potassium phosphate buffer (100 nM, pH 7.4). These homogenates were rapidly filtered through gauge and aliquots were immediately assessed for the malonaldehyde content (basal level). Aliquots were incubated at 37°C for 2 hr with or without oxidant stressers. Different concentrations of plumbagin and menadione were added to the tubes containing oxidant stressers. Malonaldehyde content of the homogenates was measured at the end of incubation by thiobarbituric acid reaction[8]. Protein was measured by the method of Lowry *et al*[9].

At 1 mM concentration both plumbagin and menadione prevented the oxidative stressers-induced lipid peroxidation in liver and heart homogenates. DL-α-tocopherol acetate and BHT also inhibited at the concentration used. But as can be seen from tables 1 and 2, plumbagin was slightly less active than menadione.

Menadione has been suggested to prevent NADPH and ascorbate-induced microsomal lipid peroxidation by forming hydroquinones which may trap the free radical species involved in catalysing the propagation of lipid peroxidation[10]. Plumbagin which has one more hydroxyl group than menadione may also follow the same mechanism.

A surprising observation was that plumbagin and menadione showed powerful anti-lipid peroxidative effect against H_2O_2 induced lipid peroxidation. The novel way by which plumbagin and menadione can kill the cells by intracellular H_2O_2 production while at the same time inhibit H_2O_2 mediated lipid peroxidation is not very clear. H_2O_2 mediated cell death has been reported to follow two mechanisms, one which was dependent on lipid peroxidation and

Table 1 *Effect of plumbagin and menadione on in vitro lipid peroxidation induced by ascorbate (100 mM) and H_2O_2 (8 mM) in rat liver homogenate. Values are expressed as mean ± S.D from 6 experiments in each group*

Additions	Ascorbate	H_2O_2
None	1324 ± 6.10^a	1025 ± 8^a
Plumbagin		
50 μM	1250 ± 11.5	1023 ± 12^a
100 μM	887 ± 12^a	998 ± 13^a
250 μM	425 ± 11.3^a	570 ± 12.1^a
500 μM	178 ± 7.8^a	278 ± 11.2^a
1 mM	152 ± 9.7^{NS}	192 ± 10^a
Menadione		
100 μM	980 ± 10.3^a	490 ± 11.4^a
500 μM	165 ± 11.1^a	248 ± 10.7^a
1 mM	142 ± 12^{NS}	148 ± 11.0^{NS}
DL-α-tocopherol acetate (0.01%)	147 ± 12^{NS}	152 ± 9.8^{NS}
Butylated hydroxy toluene (1 mM)	138 ± 13^{NS}	148 ± 8.9^{NS}

The values are expressed as nmol of malonaldehyde/100 mg protein; Basal malonaldehyde level was 139.2 ± 12.3; $^aP < 0.001$; NS = Non-significant.

Table 2 *Effect of plumbagin and menadione on in vitro lipid peroxidation induced by ascorbate (100 mM) and H_2O_2 (8 mM) in rat heart homogenate. Values are expressed as mean ± S.D from 6 experiments in each group*

Additions	Ascorbate	H_2O_2
None	1528 ± 13^a	1218 ± 13.2^a
Plumbagin		
50 μM	1328 ± 12.7^a	1017 ± 10.2^a
100 μM	725 ± 12.0^a	810 ± 12.8^a
250 μM	470 ± 12.4^a	510 ± 13.1^a
500 μM	195 ± 11.7^b	210 ± 12.1^a
1 mM	182 ± 10.4^a	168 ± 10.8^{NS}
Menadione		
100 μM	578 ± 10.5^a	452 ± 13.1^a
500 μM	192 ± 8.7^b	200 ± 12.4^a
1 mM	168 ± 11.1^{NS}	170 ± 12.1^{NS}
DL-α-tocopherol acetate (0.01%)	174 ± 10.5^{NS}	172 ± 11.4^{NS}
Butylated hydroxy toluene (1 mM)	172 ± 10.7^{NS}	174 ± 10.3^{NS}

The values are expressed as nmol of malonaldehyde/100 mg protein; Basal malonaldehyde level was 165 ± 12.4; $^aP < 0.001$; $^bP < 0.05$; NS = Non-significant.

which can be prevented by antioxidants and the second that was independent of lipid peroxidation[11]. Hence we tentatively suggest that plumbagin and menadione may act by preventing the first mechanism.

An important application of this may be that plumbagin and/or menadione in combination with adriamycin while increasing the tumour cell death, may prevent at the same time the lipid peroxidation associated cardiac toxicity of adriamycin. Further investigations are in progress to check this.

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IN VITRO REGENERATION IN *SOLANUM NIGRUM*

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When plants are regenerated from cultured tissues or protoplasts, they are not always genetically identical to the material from which the cultures were initiated. This phenomenon of genetic variability, termed as somaclonal variation[1] is characteristic of *in vitro* growth involving a callus phase, and it has been reported in several species[2-6]. The origins of genetic variability during culture are not yet understood[7]. Changes in both chromosome number[8,9] and structure have been recorded in regenerated plants and these may be responsible for some of the observed phenotypic differences. Plant cell and tissue cultures due to the presence of this genetic variability, therefore, represent an extraordinary reservoir of genetic information to be exploited for genetic and breeding research[10]. The present report deals with the occurrence of polyploidy in a species of medicinal value *Solanum nigrum* regenerated *in vitro*.

Different explants (leaf) of *S. nigrum* were collected from 4-month-old plants growing in the campus of this University. Meristematic portions of the leaf base were cut into rectangular explants measuring (10 × 15 mm). They were washed with running tap water for about 30 min, rinsed with distilled sterile water several times and finally surface-sterilized with 0.1% (W/V) mercuric chloride for 5 min followed by thorough washing with distilled sterile water. Explants were inoculated in culture tube (18 × 150 mm) containing solidified Murashige and Skoog's (MS) medium[11], supplemented with various combinations of coconut milk (1%, 5% and 10%) and IAA (0.1, 1 and 10 μM). Cultures were maintained at 25 ± 2° C in the dark. For each treatment 12 replicates were kept and all experiments were repeated thrice. The freshly initiated callus tissues were periodically transferred to basal medium. For cytological investigation *in vitro* regenerated roots of 5-week-old plantlets obtained on MS medium were pretreated with *p*-dichlorobenzene for 4 hr followed by overnight fixation in acetic:ethanol (1:3). For softening the tissue was hydrolysed for 12–15 min in IN HCl at 50–60°C and for staining the usual 2% aceto orcein: NHCl (9:1) method was used. A minimum of 1000 cells were screened for cytological analyses.

The leaf discs cultured on MS medium with all combination of coconut milk and IAA produced rooting. IAA at low concentration (0.1 μM) failed to induce callusing in the explants with either 1%, 5% or 10% coconut milk. However, IAA at 1 μM concentration iduced callusing with 5% and 10% coconut milk within 8–20 days of incubation indicating the requirement of a higher concentration of IAA and coconut milk for callusing. IAA at the highest concentration (10 μM) induced the best