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COMPARATIVE STUDY OF AMELIORATIVE EFFECT OF ETHANOLIC AND AQUEOUS EXTRACT OF CALLUS OF *OROXYLUM INDICUM* [L.] VENT. ON TOBACCO EXTRACT INDUCED DAMAGE IN HUMAN LYMPHOCYTES

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ABSTRACT

Oroxylum indicum was analysed for its ameliorative effects as callus is known as a reservoir of metabolites of that plant from which it is developed.This study was conducted to study the ameliorative effect of ethanolic and aqueous extract of callus of *Oroxylum indicum* [L.] Vent. on tobacco extract induced cell damage in human peripheral blood lymphocytes (PBL). The cytotoxicity of tobacco extract on human PBL was tested at two hours exposure then aqueous (crude and 50%) and 70% ethanol (crude and 50%) extracts of callus were used to assess their ameliorative effects by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay.Aqueous and 70% ethanol extract of all types of callus were analysed for fresh weight, dry weight, presence of TPC (total phenol content), TFC (total flavonoid content) and antioxidant activity by 2, 2-diphenyl- 2- picrylhydrazyl (DPPH) method. Both extracts have been chemically analysed by GC-MS to observe the occurrence and area % of different compounds present. Isolated human lymphocyte cells were treated with TE for two hrs followed by cell viability test by MTT assay in an ELISA microplate reader and absorbance was read at 600 nm. After treatment with various callus extracts for 24 Hours (hrs.), the viability of lymphocytes was analysed. 't test' was used for statistical analysis. It was observed that cell viability was significantly enhanced when TE injured cells were treated with 70% ethanol (crude and 50%) extract as compared to aqueous extract. However crude *i.e.,* 70% ethanol extract showed better results. GC-MS chromatogram showed presence of flavonoid baicalein in 70% ethanol extract only.Survival rate of damaged lymphocytes might have been enhanced after callus extract treatment due to the presence of flavonoid baicalein in 70% ethanol extract.

KEY WORDS: **Tobacco extract, human peripheral blood lymphocytes, callus extract, *Oroxylum indicum*, ameliorative effect**

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INTRODUCTION

Oroxylum indicum (L.) Vent. is a medium sized vulnerable tree which has long been used for the preparation of ayurvedic medicines like Dashmoolarisht, Chyavanprash and Panchmulayaadiquath. Every part of this tree contains metabolites that possess medicinal value[1]. A number of secondary metabolites including flavonoids, glycosides, alkaloids, tannins, terpenoids etc. have been reported from various parts of the plant. Roots and root bark are reported to contain chrysin (5, 7–dihydroxyflavone), baicalein (5,6,7– trihydroxyflavone) and oroxylin–A (5,7–dihydroxy–6–methoxy flavone)[2].Various studies have proved anticancer potential of *Oroxylum indicum* using various models. *In vitro* plant cell cultures offer the possibility of obtaining desirable medicinal compounds as well as ensuring sustainable conservation and rational utilization of biodiversity. It is considered as an alternative for producing valuable phytochemicals [3-4]. Hence this study was conducted to observe ameliorative effects of metabolites present in callus of *Oroxylum indicum.*It is well known that tobacco contains a variety of toxic substances such as nicotine, nitrosamines, polycyclic aromatic hydrocarbons, formaldehyde, hydrogen etc. The carcinogenetic potential of tobacco is well known.[5] Hence, in the present study, tobacco extract was used to induce toxicity on human peripheral blood lymphocytes and ameliorative effect of ethanolic and aqueous extract of callus of *Oroxylum indicum* [L.] Vent. was studied.

MATERIALS AND METHODS

The research was performed *in vitro* on human peripheral blood lymphocytes.

Extraction of secondary metabolites from callus of Oroxylum indicum

Secondary metabolites were extracted from 2 month old callus initiated and developed on Murashige and Skoog media supplemented with BAP ($1mgL^{-1}$) and $AgNO_3$ ($2mgL^{-1}$) with various concentrations of sucrose (2, 3, 4, 5, 6, 7%) by cold method. The dried callus was ground into fine powder form. The powder (5gm) was dissolved in 100 ml of 70 % ethanol and water in separate 250 ml conical flasks. Both suspensions were incubated in an orbital shaker for 24 hours on 110 rpm. After incubation period, both extracts were filtered and used as 70 % ethanol and aqueous extracts.

Determination of Total Phenolic Content (TPC) in callus extract of Oroxylum indicum

The total phenolic content was estimated by the Folin-Ciocalteu colorimetric method [6]. Callus extract (0.5 ml) was mixed with equal amount of Folin-Ciocalteu reagent and deionized water (7.5 ml). The mixture was kept at room temperature for five minutes (min, and then 5 ml of 7% sodium carbonate was added and incubated for 90 min at room temperature. After incubation, the absorbance against the reagent blank was determined at 760 nm. The total phenolic content of the callus extract was expressed as gallic acid equivalent (mg/g dry weight of callus). The sample was analyzed in triplicate.

Determination of Total Flavonoid Content (TFC) in callus extract of Oroxylum indicum

The TFC was measured by spectrophotometeric method [7]. Callus extract (0.5 ml) was diluted with 2 mL water in a 10 ml volumetric flask. Initially, 5% $NaNO_2$ solution (0.15 ml) was added to the volumetric flask. After five mins, 10% $AlCl_3$ (0.15 ml) was added and at sixth min 1.0 M NaOH (1 ml) was added and mixed well. Absorbance of the reaction mixture was read at 510 nm. TFC was determined as quercetin equivalent (mg/g of dry weight of callus). Three readings were taken and the results averaged.

Determination of 2, 2-diphenyl- 2- picrylhydrazyl (DPPH) radical scavenging activity

The DPPH radical scavenging activity was performed according to the assay system of Mensor *et al* (2001) [8]. DPPH methanol solution (2.5 ml of 0.1 mM) was added to 0.5 ml solution of the each callus extract and allowed to react at room temperature for 30 min. The absorbance (Abs) of the resulting mixture was measured at 518 nm and converted to percentage antioxidant activity (AA%), using the formula:

$$AA\% = [(Abs\ control - Abs\ sample)/Abs\ control \times 100]$$

Preparation of tobacco extract used for toxicity testing

Tobacco extract was prepared in a weight/volume of 1:10 from pulverized tobacco[9]. The extract was prepared by defatting the material with diethyl ether (boiling point 34°C). A 1:5 weight/volume extract was prepared by stirring the defatted material in phosphate buffered saline (PBS) for 72 h at 4°C. The extract was centrifuged and the supernatant was dialyzed for 48 h against PBS and after that for 24 h against distilled water. Subsequently, the supernatant was filtered under sterile conditions. The filtered extract was stored at −20°C and designated as 100% aqueous extract of tobacco. The 100% aqueous TE was appropriately diluted with PBS under sterile conditions to give 6, 9, 12.5% solutions of TE.

Isolation of lymphocytes from whole blood

Blood (3 ml) from healthy volunteer donors (with their written pre informed consent) of 27- 44 yrs age groups was collected in sterile heparinized vials. This was diluted with an equal volume of PBS (1×). Three milliliter of HiSep™ Lymphocyte Separation Medium (LSM) 1077 (Hi Media) was transferred aseptically into a centrifuge tube. This was then carefully overlaid with 6 ml of diluted blood. It was centrifuged at 400 × g at room temperature (RT) for 20 min. Erythrocytes were sedimented and the lymphocytes formed a layer above the Hi Sep layer. Most of the supernatant was aspirated out and then the lymphocyte layer along with half of the Hi Sep layer was carefully aspirated into a separate centrifuge tube. It was then washed twice with isotonic PBS. The cells

were counted in a heamocytometer. 0.5 ml of the suspension was appropriately diluted in TC 199 medium (Hi Media) supplemented with fetal bovine serum to give a final concentration of 5 × 10^5 cells/ml.

MTT assay

MTT assay was done according to protocol specified by Khanna *et al* (2014) [10]. Aliquots (180 µl) of the prepared lymphocyte suspension (5 × 10^5 cells/ml) were seeded into a 96-well polystyrene tissue culture plate in ten replicates. One row containing only medium and cells served as a control. Aliquots of 40 µl of the TE (6, 12.5, 25, and 50% solutions) were added to the wells. Each concentration was tested in ten replicates. The plate were incubated for 2 hrs. at 37°C. After 2 hrs the damage to the cells injured by TE were allowed to be reversed, by keeping them with 40 µl of various callus extracts separately for overnight. Cells incubated in culture medium alone served as control for cell viability which was taken as 100%. After overnight incubation, 20 µl aliquots of MTT solution (5 mg/ml in PBS) were added to each well and reincubated for 2 h at 37°C. Dimethyl sulfoxide (DMSO) (100 µl) was added to each well to dissolve the formazan crystals followed by incubation at 37°C for 30 min. The culture plates were then placed in an ELISA microplate reader and absorbance was read at 600 nm. The amount of color produced was directly proportional to the number of viable cells. OD of various concentrations of TE and of various fractions were noted and the final OD was calculated after making the due adjustment for these factors. Cell viability rate was calculated as the % of MTT absorption as follows:

% survival = (Mean experimental absorbance/Mean control absorbance) × 100.

GC-MS Analysis

Gas chromatography mass spectrometry (GC-MS) analysis was carried out for aqueous and 70% ethanol extracts . The analysis were performed at Advanced Instrumentation Research Facility (AIRF), JNU, New Delhi. GC-MS analysis of the crude was performed on a Shimadzu GC MS-QP-2010 plus system using Rx-5 SilMS column (30 m × 0.25 mm id × 0.25 film thickness) was used for the analysis. The operating conditions of the column were as follows: oven temperature program from 80°C– 250°C at 5°C/ min with holding time of 4 min and from 250°C– 310°C at 20°C/ min with holding time of 5 min, and the final temperature was kept for 24 min. The injector temperature was maintained at 270°C, pressure 81.7 kPa, total flow 16.3 ml/ min, column flow 1.21 ml/ min, linear velocity 40.5 cm/ sec, purge flow 3.0 ml/ min, split ratio 10.0, ion source temperature 230°C, scan mass range *m*/*z* 40–600, and interface line temperature 280°C. The identification of compounds was performed by comparing the mass spectra with data from NIST (National Institute of Standards and Technology, US), WILEY, Pesticide Library 3rd edition, Drug Library, GC/MS Metabolite Mass Spectral Database and FFNSC (Flavour and Fragrance Natural and Synthetic Compounds) libraries.

RESULTS AND DISCUSSIONS

Total phenolic (TPC) and flavonoid (TFC) content of Oroxylum indicum callus

Fresh weight of the callus increased with the increase in sucrose concentrations from 2 to 7%. Fast increase in dry weight up to 4% sucrose concentration was observed, followed by gradual decrease on higher concentrations. The TPC and TFC activities of 70% ethanol extract of callus (developed on various concentrations of sucrose), were analysed. Extract from callus developed on 4% sucrose yielded maximum 835 mg GAE (gallic acid equivalent)/g of phenolic content and 87.37 mg QE (quercetin equivalent) /g of flavonoid content. Maximum total flavonoid content 92.45 mg QE (quercetin equivalent) /g and antioxidant activity were delivered by extract of callus on 3% sucrose. Total phenolic (TPC), flavonoid (TFC) content and antioxidant activity in different extracts of *Oroxylum indicum* callus can be observed from Table-1.

Table 1
Total phenolic (TPC), flavonoid (TFC) content and antioxidant activity in different extracts of Oroxylum indicum callus.

	70% Ethanol extract of callus developed on various concentrations of Sucrose (%)	FW (mg)	DW (mg)	TPC (mg GAE/g)	TFC (mg QE/g)	Antioxidant activity (%) *
Callus extract	2	2150±0.77	462±0.54	311±1.70	42.63±0.34	39.51±0.44
	3	2810±0.43	834±0.67	750±0.39	92.45±0.44	79.79±1.12
	4	2910±0.23	1416±0.23	835±0.22	87.37±1.65	76.51±0.96
	5	5310±0.09	862±1.12	500±0.06	85.25±0.84	53.77±0.78
	6	5740±1.33	882±0.95	371±0.78	50.41±1.33	47.88±0.45
	7	5080±0.34	851±0.57	215±1.27	31.24±0.69	32.14±0.78
	Aqueous extract of callus developed on 3%concentration of Sucrose	-	-	410±0.96	65.04±0.34	74.79±0.92

*as compared to ascorbic acid standard

Table 2

Ameliorative effect of aqueous and 70% ethanol callus extracts (overnight exposure) of O. indicum on tobacco extract (TE) (2hrs) induced damage on lymphocytes

No.	TE Conc. (%)	Sample	Viability (%)
1	Cell + media	Control	100±0.00
2	6 (%)	Cell+media+TE	68.60±1.80
		Cell+media+TE+aq extract	81.19±1.15
		Cell+media+TE+aq extract (50%)	72.79±1.90
		Cell+media+TE+70% ethanol	88.19±1.07
		Cell+media+TE+70% ethanol (50%)	82.48±1.72
3	9 (%)	Cell+media+TE	59.19±1.24
		Cell+media+TE+aq extract	82.45±1.27
		Cell+media+TE+aq extract (50%)	72.13±2.38
		Cell+media+TE+70% ethanol	87.4±1.87
		Cell+media+TE+70% ethanol (50%)	71.36±1.05
4	12.5 (%)	Cell+media+TE	48.11±2.08
		Cell+media+TE+aq	67.05±1.92*
		Cell+media+TE+ aq extract (50%)	53.96±2.09*
		Cell+media+TE+70% ethanol	74.29±1.98*
		Cell+media+TE+70% ethanol (50%)	50.67±2.02*

Results are expressed as mean±S.D t' test significant at p<0.01 * at p<.05

Table 3

Compounds isolated in GC-MS analysis in 70% ethanol Extract of callus of Oroxylum indicum.

Peak Report TIC

Peak#	R.Time	Area	Area%	Name
1	6.878	697819	1.92	5-ISOPROPYL-2-METHYLBICYCLO[3.1.0]HEX-2-ENE
2	6.998	132785	0.37	2(3H)-FURANONE, DIHYDRO-
3	7.366	610378	1.68	2-Cyclopenten-1-one, 2-hydroxy-
4	8.892	155480	0.43	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one
5	9.188	7876745	21.69	Phenol
6	12.073	91122	0.25	PYRAZINE, TETRAMETHYL-
7	14.087	3456902	9.52	2,3-DIHYDRO-3,5-DIHYDROXY-6-METHYL-4H-PYRA
8	15.547	97959	0.27	BENZENE, 1-METHOXY-4-(2-PROPENYL)-
9	17.122	332092	0.91	5-OXOPROLINE
10	21.481	641375	1.77	1'-HYDROXY-4,3'-DIMETHYL-BICYCLOHEXYL-3,3'-D
11	22.330	168202	0.46	Butyl(dimethyl)silyloxycyclohexane
12	24.163	215283	0.59	Phenol, 3,5-bis(1,1-dimethylethyl)-
13	24.487	811592	2.23	2-D,2-PENTADECYL-1,3-DIOXEPANE
14	25.625	341218	0.94	Silane, [(1,1-dimethyl-2-propenyl)oxy]dimethyl-
15	26.676	71069	0.20	Asarone
16	27.299	756132	2.08	Ethyl .alpha.-d-glucopyranoside
17	27.559	471353	1.30	1,2,3-PROPANETRICARBOXYLIC ACID, 2-HYDROXY-
18	29.851	1668884	4.60	Tetradecanoic acid
19	33.920	2425876	6.68	HEXADECANOIC ACID
20	36.728	316922	0.87	Phytol
21	37.160	195713	0.54	9,12-Octadecadienoic acid, methyl ester
22	37.241	224671	0.62	cis-Vaccenic acid
23	37.655	335586	0.92	Octadecanoic acid
24	41.620	187106	0.52	HEXANEDIOIC ACID, BIS(2-ETHYLHEXYL) ESTER
25	44.091	10553847	29.06	1,2-BENZENEDICARBOXYLIC ACID
26	45.472	1643024	4.52	Flavone, 5,7-dihydroxy-8-methoxy/Vogonin/Wogonin
27	46.835	1840182	5.07	9-OCTADECENAMIDE
		36319317	100.00	

Table 4
Compounds isolated in GC-MS analysis in aqueous extract of callus of Oroxylum indicum.

Peak Report TIC

Peak#	R.Time	Area	Area%	Name
1	7.087	93578	0.63	2(3H)-FURANONE, DIHYDRO-
2	7.454	275921	1.87	2-Cyclopenten-1-one, 2-hydroxy-
3	8.943	88183	0.60	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one
4	9.810	142807	0.97	But-3-enyl (E)-2-methylbut-2-enoate
5	12.206	188009	1.27	2-PYRIMIDINONE, 1,2,5,6-TETRAHYDRO-1-METHYL-
6	12.691	409605	2.78	4-METHYLOXAZOLE
7	14.094	2549008	17.28	2,3-DIHYDRO-3,5-DIHYDROXY-6-METHYL-4H-PYRA
8	15.280	51542	0.35	CYCLOPENTANECARBOXYLIC ACID
9	19.784	315338	2.14	cis-3-Nonen-1-ol, trifluoroacetate
10	20.792	230954	1.57	5-OXO-PYRROLIDINE-2-CARBOXYLIC ACID METHY
11	21.473	447819	3.04	1'-Hydroxy-4,3'-dimethyl-bicyclohexyl-3,3'-dien-2-one
12	22.426	1621445	10.99	1,2-ETHANEDIOL, 1-PHENYL-
13	24.159	1238665	8.40	NONANOIC ACID
14	24.468	1038077	7.04	2-D,2-PENTADECYL-1,3-DIOXEPANE
15	25.615	178192	1.21	SILANE, (CYCLOHEXYLOXY)TRIMETHYL-
16	26.067	204123	1.38	Trimethylsilyoxybenzene, 3-(2-hydroxyethyl)-
17	26.483	91164	0.62	S-TRIAZOLO[3,2-A]PYRIMIDINE, 5-METHYL-
18	27.126	1703302	11.55	.beta.-D-Glucopyranoside, methyl
19	29.828	383271	2.60	Tetradecanoic acid
20	33.908	197668	1.34	HEXADECANOIC ACID
21	34.303	1678624	11.38	3,7,11,14,18-Pentaoxa-2,19-disilaeicosane, 2,2,19,19-tetram
22	44.081	1060563	7.19	1,2-BENZENEDICARBOXYLIC ACID
23	46.842	564137	3.82	9-OCTADECENAMIDE
		14751995	100.00	

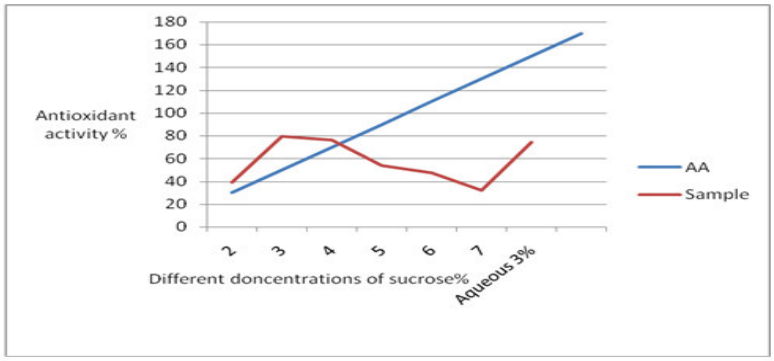

Figure 1
Antioxidant activity of 70%ethanolic extract of callus developed on different concentrations (from2-7%) of sucrose & in aqueous extract of callus on 3% sucrose in comparison with standard ascorbic acid (AA)

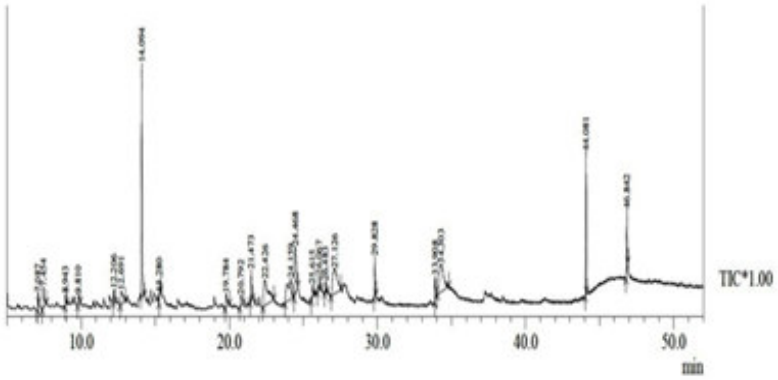

Figure 2
GC-MS chromatogram of 70% ethanol extract of Oroxylum indicum root

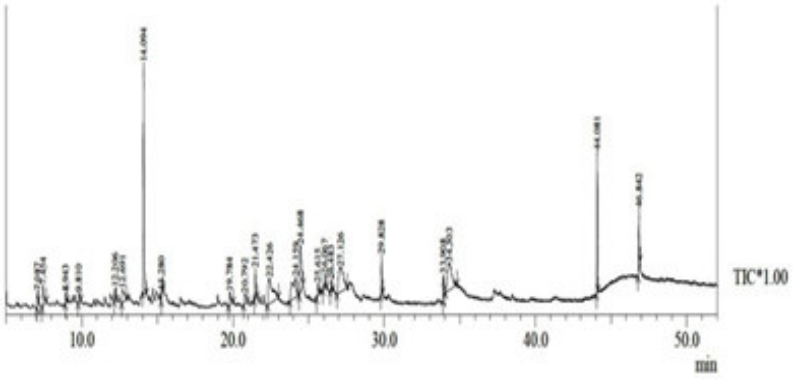

Figure 3
GC-MS chromatogram of aqueous extract of Oroxylum indicum root

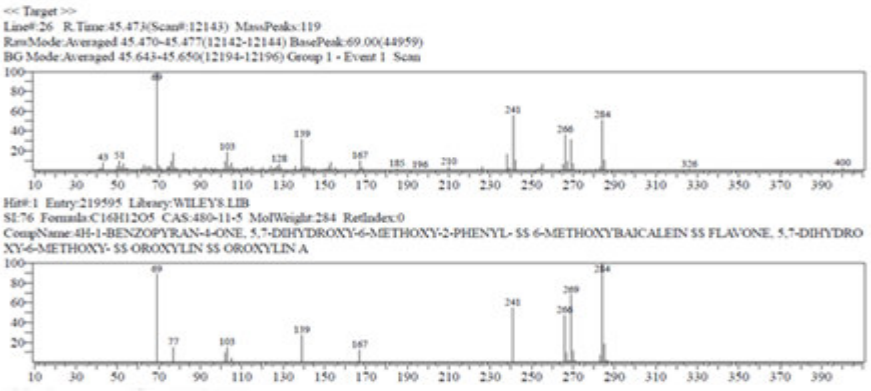

Figure 4
Base peak showing presence of baicalein compound in 70% ethanol extract of Oroxylum indicum root

DppH assay

The radical scavenging activity of the extract was observed from the decrease in absorbance of the DPPH at 518 nm. This is manifested in the rapid discoloration of the purple DPPH to light yellow, suggesting that the radical scavenging activity of different extracts of callus of *O. indicum* was due to its proton donating ability. The DPPH antioxidant activity of the extract was estimated from the absorbance of the free radicals. Different concentrations (10, 30, 50, 70, 90, 110, 130, 150, 170, 190 µg/ ml) of ascorbic acid were used as standard (Fig. 1). It was observed that antioxidant values of the extract are generally higher with no significant difference from that of standard ascorbic acid. This suggests the involvement of phenolic compounds in *O. indicum* callus extract with the reduction of DPPH. Maximum scavenging activity value (79%) was recorded in 70% ethanol and aqueous extracts of callus developed on 3% of sucrose, followed by 76 % antioxidant activity in callus developed on 4% sucrose. Lower and higher concentrations of sucrose do not favor the antioxidant activity retention in callus (Table 1).

MTT assay

This experiment investigated the cytotoxic activity of the tobacco extract. Effect of various concentrations of

tobacco extract on mean % viability of lymphocytes was observed. The shape of the curve shows significant inhibition of cell proliferation in the normal human lymphocytes treated with different concentrations (6%, 9% and 12.5%) of tobacco extract after 2 hrs treatment. It is clear from Fig.1 that if the viability of the controls (lymphocytes without treatment with TE) was taken to be 100%, the viability dropped as the TE concentration increased. At 6% the mean viability was 68.6%, it decreased gradually as the TE concentrations increased. At 12.5% TE it reached a minimum viability of 48.11%. To overcome the toxic effect of different concentrations of TE after 2 hrs., lymphocyte cells were treated with two extracts aqueous (crude and 50%) and 70% ethanol (crude and 50%) of 2 month old callus (developed on MS medium containing BAP ($1mgL^{-1}$) and $AgNO_3$ ($2mgL^{-1}$) of *O. indicum* separately for 24 hrs. Cell viability of lymphocytes after treatment for 24 hours with four extracts showed that there was increase in cell viability. Crude 70% ethanol extract was capable to enhance cell viability significantly on all concentrations of TE (Table 2). The curative capacity of 70% extract was proved to be better than that of aqueous extract. In both cases crude extracts gave more cell viability enhancement than 50% extracts (Table 2).

GC-MS analysis

The compounds obtained from 70% ethanol and aqueous extract of callus of *O. indicum* were shown in Table 3 and 4. Total 27 compounds were isolated from 70% ethanol extract (Fig. 2, Table 2) and 23 compounds from aqueous extract of callus (Fig. 3, Table 3). Presence of flavonoid baicalein (6-ethoxybaicalein flavone; $C_{16}H_{12}O_5$; mol wt:284) was observed in GC-MS chromatograms of 70% ethanol extract on Rt 45.74 (Fig.4). Callus, an unorganized mass of cells, is a reservoir of many compounds, some of them new and not present in the mother plant. Since many of these products (secondary metabolites) are obtained by direct extraction from plants grown in natural habitat, several factors can alter their yield. The first part of present work is designed to see the effect of different concentrations of sucrose on the development and accumulation of total flavonoid and total phenolic content in callus by measuring the parameters like fresh weight (FW), dry weight (DW), total phenolic (TPC) and total flavonoid content (TFC). Sucrose as a carbon source supports growth of plant cells in culture[11]. A sucrose concentration of 1-5% is generally used for *in vitro* tissue culture, since it is also synthesized naturally by the tissue [12]. In *Oroxylum indicum* callus, slight enhancement (4%) in sucrose concentration elevated fresh weight (FW), dry weight (DW), total phenolic (TPC) and total flavonoid content (TFC). But increasing concentration of sucrose more than 4% does not favor enhancement in DW, TPC and TFC. Occurrence of maximum fresh weight in callus developed on 6% sucrose is an indication that higher sucrose concentration enhances the fresh weight but not dry weight. Total phenolic content is greater in all extracts of callus developed on various concentrations (2, 3, 4, 5, 6, 7%) of sucrose than total flavonoid content. Increasing sucrose concentration in the growth media resulted in increased phenolics in willow [13]. It was noted that elevated phenolic levels were associated with increased sucrose in oregano[14]. The photosynthetic activity of the plantlets of rose [15], carnation[16] and strawberry[17] grown *in vitro* depends on the amount of carbon in the medium. It was reported that the highest net photosynthetic rate was obtained when rose plantlets were grown in media containing less than 3% sucrose[15]. It was reported that willow plantlets cultured in vitro with 8% sucrose died within a few days without any growth[13]. At 6% sucrose, the plantlets grew well, but at 1.5% or less sucrose, they were slow-growing and root development was poor[18]. It was reported that sugar decreased the inhibitory effect of malto-dextrin and consequently increased blackening in apple[19].Antioxidant activity was analysed by DPPH stable free radical method which is an easy, rapid and sensitive way to survey the antioxidant activity of a specific compound or plant extracts[20]. Antioxidant and hepatoprotective activity of leaf of *O. indicum* in several *in vitro* models have also been observed[21]. It was found due to the presence of polar phenolic compound flavonoid, tannin in leaf of this plant. In the present work 70% ethanolic extract of callus is capable to show not only antioxidant activity but also to enhance cell viability in the cells damaged after tobacco extract treatment[10].Preventive and curative effects of various plant extracts on embryonic 293 human cells intoxicated by the herbicide was observed and it was seen that defined plant extracts can protect human cells against combined xenobiotic effects[22]. It was observed that plant extracts can prevent intracellular effects caused by environmental pollutants in two human cell lines. Ameliorative effects of ethanolic Neem extract on dietary aflatoxin induced hematological damage have also been reported[21].Presence of flavonoid baicalein is confirmed by GC-MS analysis in 70% ethanolic extract of callus. The work shows that the ameliorative effect of callus extract on TE damaged lymphocytes may be due to activity of flavonoids. Flavonoid baicalein is well known natural antioxidant[24-25] inhibiting the replication of human viruses[26-27] and suppressing proliferation of human cancer cells[28-29].

CONCLUSION

Ethanol extract contain an important flavonoid baicalein. To obtain the metabolites for pharmaceutical purposes the medicinal plant should not be destroyed. The callus extracts was found to have ameliorative effect on TE induced damage in lymphocytes.

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CONFLICT OF INTEREST

Conflict of interest declared none.

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