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Original Research Article

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Determination of cellular protection and leukemic cell inhibition by herbal extracts in cow urine.

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Abstract

Cow urine is used as a therapeutic agent and a most valuable secretion of animal origin and herbs have been used as a antioxidant and chemotherapeutic agents,therefore we evaluated the cell protective and cell inhibitory activity of different herbs/spices which was prepared in cow urine(in order to enhance the activity) by MTT assay, and performed the above assay in blood cancer cells viz.ALL, AML, CML type of cancers.The results suggest that revealed that the drugs tested Ajwain, garlic, Dhania, Satavar, and Guduchi prepared in cow urine showed significant antioxidant activity or cell proliferative activity whereas, the extracts such as Dalchini, evaporated cow urine showed an excellent cell inhibition activity in all the three types of cancers viz. ALL, AML and CML. Kalmegha is showing cell inhibitory property for ALL cells but not for AML and CML cells, similarly Garlic showed cell inhibition property for AML but not for ALL and CML cells.

Keywords: AML-Acute myeloid leukemiaALL-Acute lymphoblastic leukemia CML-chronic myelogenous leukemia

Introduction

Cytotoxicity is the quality of being toxic to cells. Cells exposed to a cytotoxic compound can respond in a number of ways. The cells may undergo necrosis, in which they lose membrane integrity and die rapidly as a result of cell lysis; they can stop growing and dividing or they can activate a genetic program of controlled cell death, termed apoptosis. [1]Cytoprotection is a process by which chemical compounds provide protection to cells against harmful agents. [2] Antioxidants are compounds that are naturally produced by the body for protection against harmful molecules we are exposed to every day. They stabilize free radicals by donating an electron to the free radical before it can damage other cell components, and at the same time, do not become free radicals themselves.[3] Leukemia is a type of cancer that results in the body making too many abnormal white blood cells. This uncontrolled production results in an excessive amount of white blood cells that may be immature (acute leukemia) or mature (chronic leukemia). The leukemic cells may not function well to fight infection and may interfere with the production of red [8]

Cell viability is a determination of living or dead cells, based on the total cell sample. Cell viability measurement may be used to evaluate the death or life of cancerous cell and the rejection of implanted organs.[13] The MTT assay and the MTS assay are colorimetric assays for measuring the activity of enzymes that reduce MTT or close dyes(XTT,MTS,WSTs) to formazon dyes, giving a purple colour. A main application allows assessing the viability (cell counting) and the proliferation of cells (cell culture assays).[4] Cow urine helps the lymphocytes to survive and to repair the damaged DNA and thus it is effective for Cancer Therapy. [5] It is reported to prevent the pathogenic effects of free radicals through cow urine therapy[6]. Cow Urine is used as a therapeutic agent. A study found that cow urine distillate i.e. Go-mutra ark has anti-oxidant potential and this might be because of volatile fatty acids present in it, which act as anti-oxidants.[7] Herbal medicine is still in great interest because the plant extract may contain compounds of novel therapeutic efficacy and they may also appear to be more natural and thus more acceptable to patients than man-made drugs. [10]Therefore, as a part of broader investigation it was thought worthwhile to study the cell protective activity and cell inhibition activity of 7different herbal plants in combination with Cow urine so as to prove this drug as a activity enhancer. PLANT DESCRIPTION-[15]

1) *Tinospora cardifolia* (Guduchi),Family-Menispermacea, a glabrous climbing shrub,leaves are heart shaped. Alkaloids, terpenoids, lignans and steroids are present in it. It is one of the best bitter tonic useful in fevers.

2)Andrographis paniculata (Kalmegha),Family-Acanthaceae, commonly known as “Kings of bitter”.The medicinal value of this plant is due to presence of active ingredients viz. andrographolide and neoandrographolide which are derivative of diterpenoids. Diterpenoids and Flavanoids are the main chemical constituents.

3)Asparagus racemosus, (Satavar),Family-Asparageacea , mainly known for its Phytoestrogenic properties. It is considered as a Female Reproductive Tonic. Active constituents are steroidal saponins present in roots. Shatavarin IV has immunomodulatory effects.
4)Trachyspermum ammi(Ajwain), Family-Apiaceae, roots are diuretic in nature and seed possess execellent aphrodisiac and contain 2-4%oil, main component of this oil is thymol.
5)Allium sativum (Garlic), Family-Liliaceae, produces hermaphrodite flowers. Allicin is the main biological component. Allixin and organo-selenium are the other compounds of garlic have anti-oxidant effects.
6)Cinnamon cassia (Dalchini),Family-Lauraceae, Bark is used as spice and leaves have free radical scavenging activity. It contain 1-2% oil, primary component of it is 65-80% cinnamonaldehyde and less amount of eugenol, mucilage, starch and tannin
7)Corriander sativum (Dhania),Family-Apiaceae, is considered both an herb and a spice since both its leaves and seeds are used as a seasoning condiment.Seeds contain 0.5-1% essential oil and rich in phytonutrients Oil found in leaves have antimicrobial properties. It have flavanoids and phenolic acid compounds.
These plants were very well established immunomodulatory agent and proven antioxidant herb. That's why we are intended to evaluate its cytotoxic effects on blood cancer cells by MTT Assay.

Plant Collection

Satavar and Kalmegha collected from Indranikunj nursery,Bhopal.
Garlic,Ajwain,Dhania and Dalchini were collected from local market,Bhopal.
Guduchi collected from botanical garden of JNCH&RC,Bhopal.

Extract Preparation

The dust particles were removed and crushed in mechanical grinder then the extract were soaked in cow urine and kept in incubator for 4-5 days.After this filteration was done with the help of guage bed and funnel and kept in refrigerator.

Cell Viability and Cell Toxicity Assay

Leukocyte rich plasma is collected from whole blood and over layed with 1ml of Hisep TM LSM solution and then centrifuged .Lymphocyte formed a gray coloured fluffy layer at interface of blood plasma and separation medium. The layer of lymphocyte was aspirated with a clean micropipette/spinal needle. The cells were counted and their viability was determined by Trypan Blue Exclusion Staining. Cells were then cultured in flask for 24hrs at 37˚c in CO_2 incubator for proper proliferation of lymphocyte.

MTT/Cytoprotective Assay-

30µl Cells in 150 µl RPMI 1640 media was plated per well in a 96 well plate.media alone was taken as blank whereas cell with media as control and media alone were taken as control.50µl of different drugs extract were plated. Cells were also incubated with standard as Cyclophosphamide (50µg) and antioxidant forte B in a few no. of wells.96-well plate were incubated for 48hrs in CO2 incubator at 37˚c. MTT is prepared in PBS. plated in each well and incubated at for 4hrs.After incubation media is discarded and the formazon produced is re-suspended in 5%DMSO.The plate was removed and absorbance was measured at 492nm and 640nm in ELISA Reader.The % proliferation and % inhibition were calculated by following formula.

$$\% \text{ Proliferation} = \frac{\text{Absorbance (test) x100.}}{\text{Absorbance (control)}}$$

$$\% \text{ Cell inhibition} = 100 - \frac{(At-Ab)}{(Ac-Ab)} \times 100$$

Observation and Results

In-vitro Cell proliferative and cytotoxic assay

The PI for control group was considered as 1; whereas, the standard control i.e Cyclophosphamide showed no proliferation and found to exhibit significant inhibitory activity on lymphocyte proliferation .In CML and AML, the p value was found to be extremely significant with $p<0.001$. Whereas, in ALL it is only significant with Guduchi ($p<0.05$) extract while all the other drugs were found to be not significant. ($p>0.05$).
In CML type of blood cancer, the p value was found to be significant for Cow urine, Ajwain, Kalmegha ,Satavar, Dhania, Guduchi, and garlic with $p<0.001$ and not significant for evaporated cow urine, when compared to standard Cyclophosphamide.
In AML type of blood cancer, the p value were found to be extremely significant for Cow urine, evaporated cow urine, Ajwain, Kalmegha, Satavar, Dhania and Guduchi ($p<0.001$)and not significant for Dalchini and garlic when compared to standard cyclophosphamide.
In CML, the p value of antioxidant was found to be extremely significant ($p<0.001$) with Ajwain and Dalchini and moderately significant with evaporated cow urine , Satavar and Dhania and is not significant with cow urine , Kalmegha, garlic and Guduchi.
In AML, the p value was found to be extremely significant with evaporated cow urine, Ajwain, Dalchini and garlic ($p<0.001$), and moderately significant with Dhania and Satavar and not significant to cow urine, Guduchi, and Kalmegha.
In ALL, the p value was found to be extremely significant with Kalmegha only, and moderately significant with Ajwain, Dalchini, cow urine and Satavar whereas not significant for Guduchi, garlic and Dhania.

Table.1. .MTT: Analysis of leukemic cell inhibition[CML] by different extract

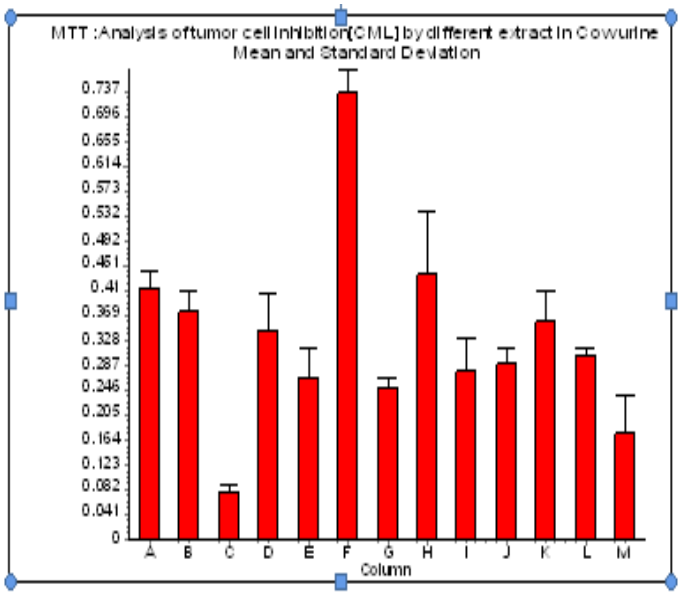
S. N	GROUPS	% CELL SURVI	% CELL INHIBI
1.	CELLS ONLY (A)	99.99	.1
2.	CELLS + ANTIOXIDANT (B)	90.9	9.1
3.	CYCLOPHOSPHAMIDE(C)	18.5	81.5
	CELLS +COW URINE (D)	83.7	16.3
5.	CELLS +EVAPORATED CU	64.4	35.6
6.	CELLS+AJWAIN (F)	More than 100	-77.7
7.	CELLS + DALCHINI (G)	60.5	39.5
8.	CELLS + KALMEGHA (H)	More than 100	-5.5
9.	CELLS +SATAVAR (I)	66.8	33.2
10.	CELLS + DHANIA (J)	69.7	30.3
11.	CELLS +GARLIC (K)	86.4	13.6
12.	CELLS + GUDUCHI (L)	72.8	27.2
13.	BLANK (M)	42.1	57.9

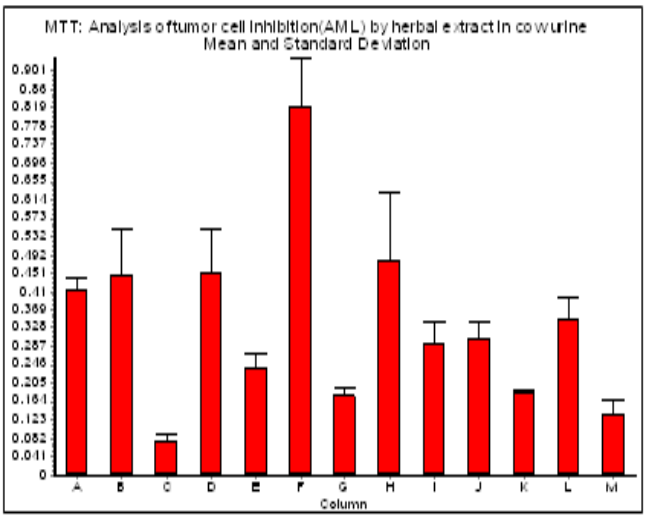
Table 2 mtt: analysis of leukemic cell inhibition [aml] by different Extract in cow urine (% cell inhibition

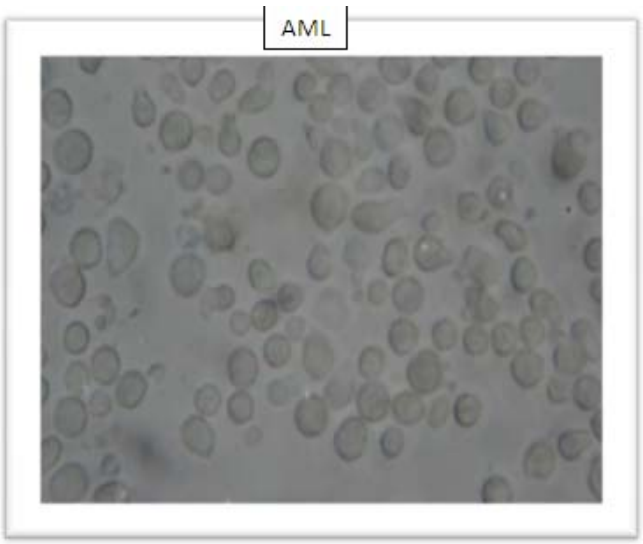
S.NO.	GROUPS	% CELL SUR	% CELL INHIBI
1.	CELLS ONLY (A)	100	
2.	CELLS+ ANTIOXIDAI	108%	-9%
3.	CYCLOPHOSPHAMID	18.5%	81.5%
4.	CELLS+COW URINE	109.4%	-9.4%
5.	CELLS +EVAPORATED	57.38%	42.62%
6.	CELLS+AJWAIN (F)	199.5%	-99.5%
7.	CELLS + DALCHINI	42.6%	57.4%
8.	CELLS + KALMEGHA	116.2%	-16.2%
9.	CELLS +SATAVAR (I	70.4%	29.6%
10.	CELLS + DHANIA (J	73.12	26.88%
11.	CELLS +GARLIC (K)	44.3%	55.7%
12.	CELLS + GUDUCHI	83.5%	16.5%
13.	BLANK (M)		

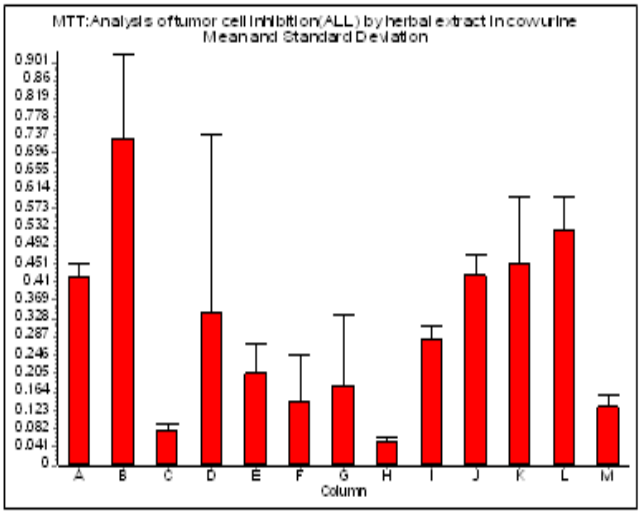
Table 3, MTT : Analysis of leukemic cell inhibition[ALL] by different extract in Cow urine.

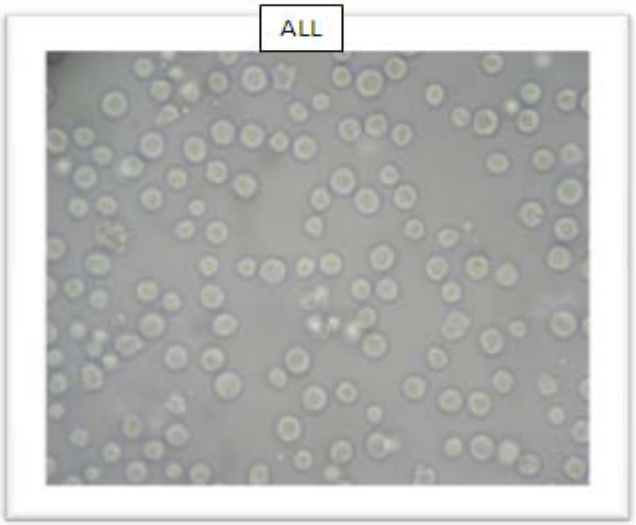
S.NO.	GROUPS	% CELL SURVIV	% CELL INHIBIT
1.	CELLS ONLY (A)	100	
2.	CELLS + ANTIOXIDAI	More than 100	-74.4
3.	CYCLOPHOSPHAMI	18.42	81.58
4.	CELLS +COW URIN	81.3	18.7
5.	CELLS +EVAPORATE (E)	49.28	50.72
6.	CELLS+AJWAIN (F)	34.2	65.8
7.	CELLS + DALCHINI	42.3	57.7
8.	CELLS + KALMEGH	12.4	87.6
9.	CELLS +SATAVAR	67.7	32.3
10.	CELLS + DHANIA (	More than 100	-1.4
11.	CELLS +GARLIC (K	More than 100	-7.4
12.	CELLS + GUDUCHI	More than 100	-25.1
13.	BLANK (M)		



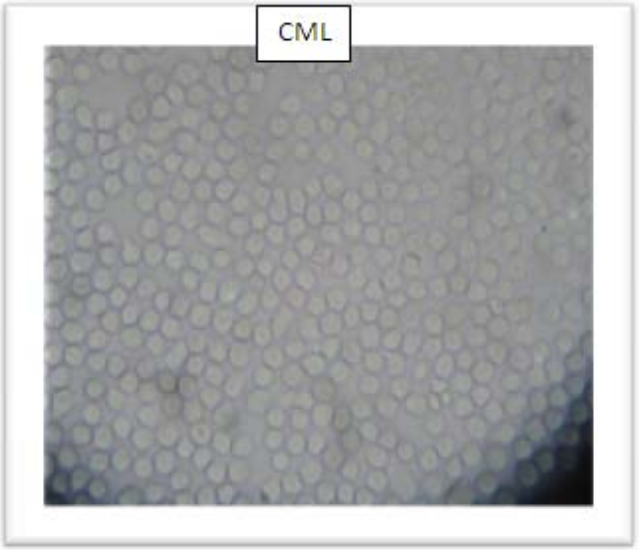


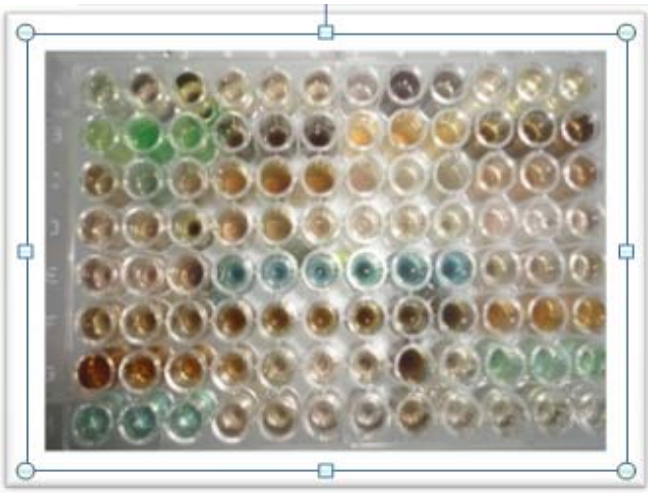






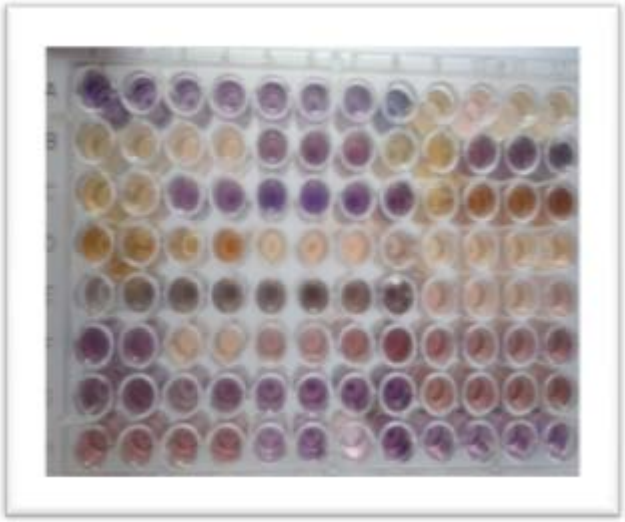
Trypan Blue Cell Viability Assay





Cml plate after adding mtt

All plate after adding mtt



aml plate after adding mtt

Phytochemicxalscreening

TEST	SATAVAR	KALMEGHA	AJWAIN	GUDUCHI	DHANIA	DALCHINI	GARLIC
Alkaloids							
1.Dragendorf	-	-	-	-	-	-	-
2.Mayer's test	-	-	-	-	-	-	-
3.Wagner test	-	-	-	-	-	-	-
AminoAcids							
1.Millons test	+	+	+	+	+	+	+
2.Ninhydrine test	-	-	-	-	+	-	-
Carbohydrates							
1.Molisch test	-	-	-	-	-	-	-
2.Barfoed test	-	-	-	-	-	-	-
Fats	-	-	-	-	-	-	+
Flavanoids							
1.Alkaline reagent test	+	-	-	-	+	+	+
Glycosides							
1.Fehling test	-	-	-	-	-	-	-
2.Brontager test	-	-	-	-	-	-	-
Proteins							
1.Biuret test	-	-	-	-	-	-	+
2.Heat test	-	-	-	-	-	-	+
Steroids and triterpenc							
1.Salkowskitest	+(triterpenoids)	-	+(steroids)	+(triterpenoids)	+(triterpenoids)	-	+(steroids)

Discussion and Conclusion

The present study revealed that the drugs tested Ajwain, garlic, Dhania, Satavar, and Guduchi prepared in cow urine showed significant antioxidant activity or cell proliferative activity whereas, the extracts such as Dalchini, evaporated cow urine showed an excellent cell inhibition activity in all the three types of cancers viz. ALL, AML and CML. Kalmegha is showing cell inhibitory property for ALL cells but not for AML and CML cells, similarly Garlic showed cell inhibition property for AML but not for ALL and CML cells. The present study observed that the drugs enhanced the antioxidant status of lymphocytes they would be expected to show increased resistance to oxidative damage when challenged *in vitro* with an oxidant.Therefore, the present study conclude that to make more validate and viable the primary therapeutic drugs with regard to cancer treatment the Asian indigenous herbs/spices

have its own role to act as an adjuvant to potentiate the properties. This work will not only lead a pathfinder for unanswered queries in the field of pharmacological research. Such indigenous herbs/spices will be poor men friendly and in near future molecular mechanism will definitely enlighten to help to cure the patient/ cells under oxidative stress.

References

[1]. Tulassay Z. Herszenyi L.,"Gastric mucosal defense and cytoprotection."Best pract Res Clin Gastroenterol. 2010;24 (2):99-108.

[2]. Wallace J L.,"Prostaglandins,NSAIDs and cytoprotection." Gastroenterol. Clin North America,1992; 21 (3):631-41.

[3]. Seirifried H E and Anderson D E.,Areview of the interaction among dietry antioxidants and reactive oxygen species.,Journal of Nutritional Biochemistry,2007.

[4]. Bernas T, and Dobrucki J.,"Mitochondrial and non-mitochondrial reduction of MTT:Interaction of MTT with TMRE,JC-1 and NAO mitochondrial fluorescent probes,2010;47 (4):236-242.

[5]. Kumar A, Kumar P, Singh L K and Agrawal DK. Pathogenic effects of free radicals and their prevention through cowpathy. The Indian Cow,2004;6:27-34.

[6]. Prabhar K,Structural dynamics of apoptosis in avian lymphocytes.M.V.Sc.thesis submitted to college of veternery sciences.GB plant University of agriculture and technology ,Panta nagar,India.

[7]. Krishnamurthy K, Dutta D and Chakrabarti T. Protective effects of distillate and redistillate of cow's urine in human polymorphonuclear leukocytes challenged with established genotoxic chemicals.,Biomed Environ Sci,2004;3 (6):123-126.

[8]. Vijayalakshmi R and Sarayna V T K.,"Effects of Go-Mutra on plant growth and its Antifungal and Antimicrobial activity, International Journal of Pharmaceutical Sciences and research, 2010;6:1-11.

[9]. Kong Y C and Chen D S,Elucidation of Islamic drugs in hui hui yao fang:a linguistic and pharmaceutical approach, journal of ethnopharmacology,1996;54:85-102.

[10]. Krishnamurthy K, Dutta D and Chakrabarti T. Protective effects of distillate and redistillate of cow's urine in human polymorphonuclear leukocytes challenged with established genotoxic chemicals.,Biomed Environ Sci,2004;3 (6):123-126.

[11]. Pathak Ak, Agrawal A, Jain DC, Howarth OW :NMR studies of 20 hydroxyedysome,a steroid isolated from T.cardifolia:Indian journal of chemistry.,1995;34:674-678.