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Anti-Acute Myelogenous Leukaemia Effect of the Ethanolic Extract of the Indian Rose

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ABSTRACT

Leukaemia has emerged as one of the most lethal of the cancers in the modern era. Out of all Leukaemia, Acute Myelogenous Leukaemia (AML) is the most notorious. The treatment regime being expensive & toxic has led to a world-wide hunt for alternate sources of anti-AML agents. In the various traditional systems of medicines, the Indian rose (Rosa indica) has been recorded as having various health benefits. In this paper, the Ethanolic extract of Rose was investigated for its anti-AML activity. The investigators treated both the AML cell line, HL-60 & the Normal Human Embryonic Kidney cell lines (NHEK) with varying doses of the Rosa indica's Ethanolic extract for 24 h. Based on the current observations, the Rosa indica's Ethanolic extract induced growth inhibition having IC50 44.44 µg/ml. Moreover, the ethanolic extract showed negligible activity against normal cell lines. Based on these findings, it can be safely assumed that the ethanolic fraction of the Indian Rose can be utilized as an anti-AML agent.

Keywords: Apoptosis, Acute Myelogenous Leukaemia (AML), Cells, Ethanolic fraction, IC50 value, HL-60, Reactive Oxygen Species (ROS), Rosa indica

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INTRODUCTION

Among adults, Acute Myelogenous Leukaemia (AML) is a common form of leukaemia afflicting them. Though advances in modern medicine has led to an improvement in the life span of the younger population, yet people who are above the age of 65 bear the brunt of this disease. Though, AML can occur in patients with a pre-existing haematological disorder, or due to chemotherapy (involving radiation, alkylating agents, topoisomerases II etc.) though it appears many a times as de novo malignancy in erstwhile healthy people in many cases. It must be added that, the occurrence of AML entails the anomalous differentiation & proliferation of a doppelgänger populace of transformed stem cells of myeloid origin which occurs due to a cascade of various signalling molecules [1–5]. WHO has classified AML according to some parameters which have been disclosed in Table 1 while Table 2 discloses the molecular & cytogenic profiling of risk groups?

Various sub-types of AML (AML) have been tabulated in this table based upon their chromosomal aberrations. As on 2016, the WHO classification of AML was revised to include particulars of genetics including clinical manifestations & immune phenotype with morphology to underscore major six subtypes of AML, which are as follows: AML having recurrent genetic flaws, AML like myelodysplasia, AML that is therapy related, unspecified AML, Myeloid sarcoma & AML related to Down syndrome. (The abbreviations used in this table are as follows: AML is acute myeloid leukaemia, ML is myeloid leukaemia, APL is acute pro-myelocytic leukaemia, & WHO is world health organization) [Table 1].

Table 1. Classification of AML as per the WHO.

Genetic anomalies	Types
AML having t(8:21)(q22;q22); RUNX1-RUNXITI	AML having periodic heritable abnormalities
AML having inv(16)(p13.1q22) or t(16;16)(p 13.1;q 22); CBFB-MYH11	
APL having PML-RARA	
AML having t(9;11)(p21.3;q23.3);BMLLT3-KMT2A	
ML having t(6,9)(p23;q34.1); DEK-NUP 214	
AML having inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2); GATA2, MECOM	
AML having minimal differentiation	AML having myelodysplasia-centered alterations
AML in absence of maturation	Thyroid centered myeloid neoplasms
AML in presence of maturation	
AML-M4	
AML-M5	
Category M6 leukaemia	
AML-M7	
Type M0 leukaemia	
Transient abnormal myelopoiesis	Myeloid sarcoma
ML in combination of trisomy 21	Combination of Myeloid proliferation and trisomy 21

In AML, the major factor for the phenomenon of overall survival (OS) & complete remission (CR) is cytogenetic changes. Based on the profile of the cytogenetics, incidents of AML could be grouped into: adverse, median or favourable prognostic groups. The rearrangements of chromosomes t (8;21), t (15;17) or inv (16) involves a prospects which is favourable whereas chromosomal changes like t (6;9), inv (3) or 11q & monosomy 5 or 7 changes other than t(9;11) are categorized as high risk groups. This table tabulates the various jeopardy sets categorized over their chromosomal outline [Table 2].

Table 2: Molecular & Cytogenic profiling of risk groups.

Molecular anomalies & cytogenetic profile	Cytogenetic profile	Prognostic-risk factor
T(8:21)(q22;q22) lacking c-KIT alteration Inv(16)(p13;1q22) t(15;17)(q22;q12)	T(8:21)(q22;q22) Inv(16)(p13;1q22) t(15;17)(q22;q12)	Favourable
Altered NPM1 in absence of FLT3-ITD(CN-AML) Altered biallelic CEPBA (CN-AML) T(8:21)(q22;q22) having altered c-KIT CN-AML except those involved within beneficial o0r meagre predictive assembly t(9;11)(p22;q23)	CN-AML t(9,11)(p22;q23) Cytogenetic aberrations excluded within complimentary or poor predective jeopardy aspects	Median
Cytogenetic anomalies excluded within beneficial or poor predictive menace sets TP53 alteration notwithstanding with cytogenetic outline CN having FLT3-ITD CN having DNMT3A CN having KMT2A-PTD Inv(3)(q21q26.2) t(6;9)(p23;q34) 11q anomalies apart from t(9;11) -5 or del (5q) -7 Intricate karyotype	Inv(3)(q21 q26.2) t(6;9)(p23;q34) 12q anomalies excluding t(9;11) -5 or del (5q) -7 Intricate karyotype	Adverse

Common kinase signalling alterations

The most important mutations in the kinase signalling pathway, involves the genes encoding the trans-membrane receptors of tyrosine kinase such as FLT3 & KIT, or the genes encoding the guanosine triphosphatases which is a member of the RAS family, (Figure 1). Upon DNA sequencing of clinical samples, such mutations have been observed in 30% to 75% of patients [2].

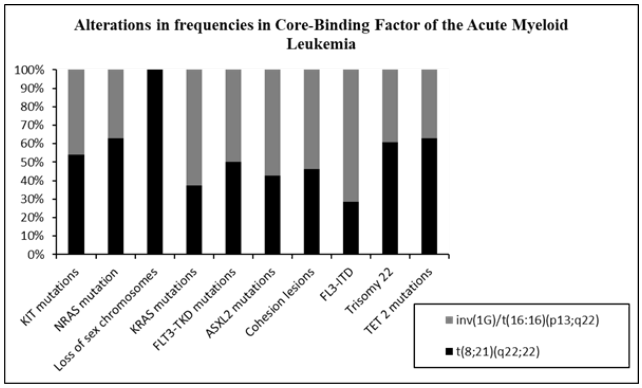


Figure 1. Mutation landscape analysis of clinical samples (A) Genetic landscape & (B). Alterations in genetic mutation frequencies in AML [2].

Secondary AML

AML occurring due to a previous myeloid malignancy or the one that occurs after previous radiation therapy or chemotherapy for a separate malignancy are known as secondary or 2° AML. Secondary AML befalls upon senior citizens & studies based upon population have suggested that such AML comprises of nearly 25% AML cases, in which 17% to 25% evolves via preceding myeloid ailment & 6% to 10% because of remedy induced (Figure 2) [2].

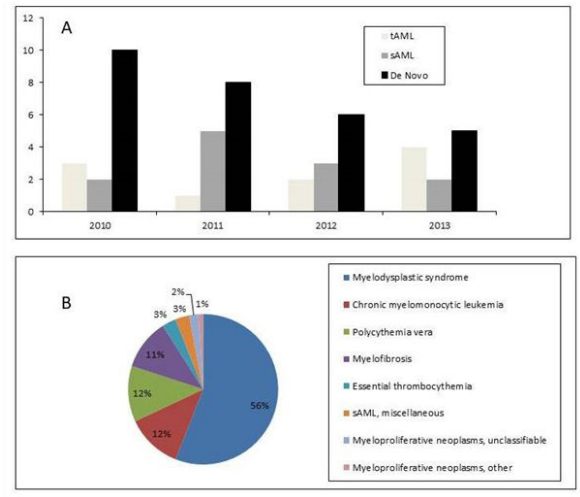


Figure 2. (A) Incidence of AML occurrence based upon their origin: Secondary, De Novo & cancer therapy induced (AML) in a Population of Denmark on- 3,000 non-selected Patients having AML, (B) Spread of previous blood borne Disease in 600 Patients possessing 2° AML [2].

TP53 mutated AML

In AML, mutations in TP53 gene are mainly found in the DNA-binding domain. Interestingly, among AML patients, TP53 gene mutations arise within about 11% to 18% of the AML instances. Among the genes that define AML, reduced frequencies of co-occurring mutations were reported in FLT3 & NPM1. Similar reduction in frequencies was reported in genes namely; WT1, DNMT3A, IDH1, RUNX1 & IDH2 along with a spurt in the frequency of JAK2 deviants. There is also a report about the TP53 mutation which reacted to decitabine, & a DNMT3A mutation was noted in a different clone which inflated during remission & was then removed at relapse (Figure 3A). In an earlier report, about 65 obtainable cases with simultaneous mutations in DNMT3A, N/KRAS FLT3 or TET2 (which are one of the most co-mutated common genes) wherein, TP53 deviant allele prevalance & simultaneous alteration were publically obtainable were determined (Figure 3B).

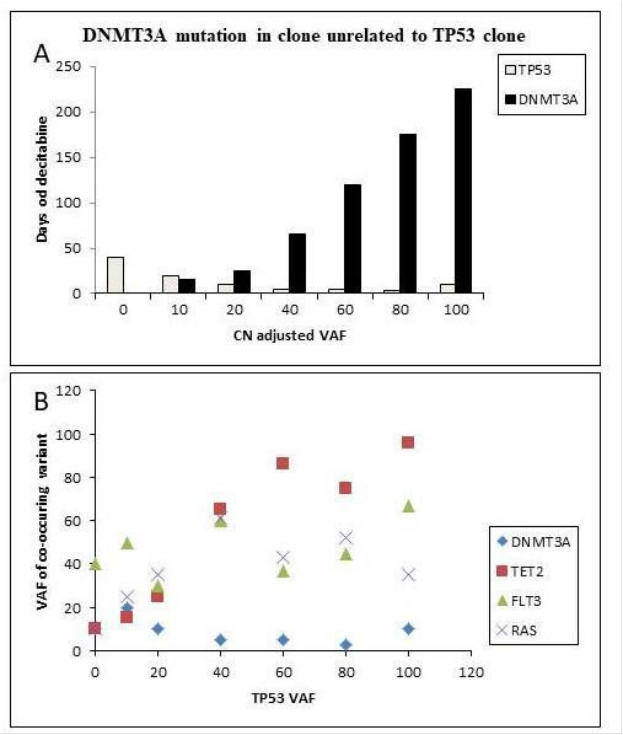


Figure 3. Meta-Analysis of previously reported AML cases having mutation in TP53. (A) Identification of genes involved in AML progression. Columns represents valetudinarian. Rows denotes genes, while coloured boxes represent mutations. (B) Detection of mutation in TP53. (C) Observation of deviant allele frequencies in various reported cases having mutations in DNMT3A, TP53, TET2, KRAS, FLT3 or NRAS [2].

LITERATURE REVIEW

In the traditional Indian system of medicine various preparation of the Indian Rose (Rosa indica) has been described for its usage as an astringent, mild laxative, treatment of sore throat etc. [6] Extracts of Rosa canina has also been reported for its anti-cancer activity

towards Human Colorectal cancer cells lines; CaCo-2 [7]. There are now many reports regarding the composition & activity of the constituents found in rose hips [8]. In this paper, the anti- AML has been described.

Among the parts of rose that are considered to be rich in phyto-chemicals are Rose hips which are the accessory fruits rose hips have been lately been associated with phyto-chemicals such as phenolic acids, anthocyanins, vitamins. Owing to these phyto-chemicals, rose hips have been utilized for many in-vitro & in-vivo studies (Table 3, 4 & 5) [8]. It must be added that many of the phyto-chemicals found in rose hips are also found in rest of the aerial parts of the Indian rose as well [6].

Rose hips are rich in phyto-chemicals. There are many traditional medicinal usages of rose hips which have prompted an analysis of its phytochemical content, some of which has been tabulated in this table. However, it can be assumed that the same chemical constituents can be found in all the Ariel parts of the Indian Rose [Table 3].

Table 3. Phyto-chemical composition of Rose hips.

Components	Phytochemical class
Cynidine-3-glucoside, hydroxycinnamic acid & derivatives (3-p-coumaroylquinic acid, 4-p- coumaroylquinic acid 1-2-5-p- coumaroylquinic acid, Taxifolin pentoside	Anthocyanin
(+) Catechin, catechin hexoside, PA-dimer-gly 1-4, (-)-Epicatechin	Flavanols
Quercetin-3-galactoside, quercetin-3-glucoside, quercetin pentoside, quercetin rhamnoside, quercetin hexoside	Flavonols
Quercetin, quercetrin	Flavonols-glycoside
Phloridzin	Dihydrochalcone-glycoside
Eriodytol hexoside 1,2, naringenin hexoside 1-5, hesperidin	Flavanones
Apigenin derivative 1,2	Flavone
Lycopene, Leutin, ß carotene, epimers of neochrome, zeaxanthin	Carotenes
Linoleic acid, Palmitic acid, Linolenic acid,	Fatty Oil
Rutin, Isoquercetin, kempferol, Quercetin,	Flavonoids
Glucose, fructose, sucrose, citric acids, malic acid	Sugars & Organic acids
Elagitanins	Tanins
Iron, Nitrogen, Magnesium, Potassium, Calcium,	Inorganic compounds
Folates, K, abscorbic acid, Vitamin E	Vitamins

The rose hips are reported to be affluented in phytochemicals myriads which plays an important role in various traditional medicinal preparations of rose hips. Elucidation of their chemical structures would help in designing of effective lead agents against various diseases [Table 4].

Table 4. Representation of phyto-chemicals structure found in Rose hips.

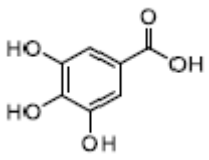
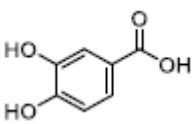
Number	Compounds denomination	Layouts	Race
	Phenocarboxylic acids		
1	Gallic acid		*Rosa canina & Rosa sempervirens*
2	Protocatechuic acid		*Rosa canina & Rosa sempervirens*

Table 5. Hydroxybenzoic acids.

Number	Name of compounds	Species
1	Ellagic acid pentoside 1-3	*Rosa rugosa & Rosa canina*
2	Ellagic acid hexoside 1-3	*Rosa rugosa & Rosa canina*
3	Methyl ellagic acid pentoside	*Rosa rugosa & Rosa canina*
4	Methyl gallate acetyl d hexoside	*Rosa rugosa & Rosa canina*
5	Methyl gallate hexoside	*Rosa rugosa & Rosa canina*
6	Methyl gallate rutinoside	*Rosa rugosa & Rosa canina*
7	Methyl gallate pentoside	*Rosa rugosa & Rosa canina*

Table 6. Biological activity documented for Rose hips (in-vitro & in-vivo).

Number	Activity/Disease	Rose species	Active constituents/ preparations	Study design	Results
1	Ethanol actuated peptic ulcer	*Rosa canina*	Aqueous extract of fresh fruits	Rat model	Gastro-protective effect
2	Anti-Helicobacter pyroli function	*Rosa canina*	Carotenoids	In-vitro study	Comparable to metronidazole
3	Ethanol actuated gastric damage	*Rosa canina*	Hydroalcoholic pure fruit extract	Rat model with aqueous solution of extract	Protection of gastric mucosa
4	Chronic CCl4 induced liver damage	*Rosa canina*	Aqueous-ethanolic fruits extract	Rat model	Liver protection
5	Anti-diabetic	*Rosa canina*	Aqueous extracts	In-vitro ß cell lines	Significant ß cell proliferation
6	Calcium Oxalate (CaOx) kidney stone avertion	*Rosa canina*	Hydro-methanolic extracts	Rat model in combination with Hypodicarbonous acid	Improved kidney & Liver function test. Decrease in the size & no. of CaOx stones
7	Streptozotocin induced diabetes	*Rosa canina*	Ethanolic extract	Oral rat model	Decrease in the level of fasting blood sugar

Among the various traditional medicinal uses & the various biological activities as reported by the researchers, none of them are regarding AML.

Thereby this paper has attempted to report the anti-AML activity of rose hips from Rosa indica.

RESEARCH QUESTIONS

The ethanolic extract of Rosa indica would be evaluated against AML cell lines.

MATERIALS & METHODS

Experimental design

The experiment design is inspired from earlier reported publications [9]. Firstly, ethanolic extracts of rose stems & leaves would be prepared & the AML cell line HL-60 & the normal human embryonic kidney 293 (nHEK-293) cell lines would be treated with various doses of ethanolic extracts for 24 h to determine its IC50 value. The results would be analysed for statistical significance.

Chemicals and reagents

Foetal bovine serum, Cell culture media, MTT, Antibiotics, DMSO etc. that were used for this study were as described earlier [9].

Preparation of ethanolic extracts of Rosa indica

Firstly, the rose leaves & stems (aerial parts) were isolated from Rosa indica, & then dried under the sun to remove moisture. The dried rose hips would be pulverized under mortar & pestle to powder form. Ethanolic extracts would be prepared by mixing 30 g of the pulverized powder into 200 ml of ethanol & Soxhlet extraction would be performed to obtain the ethanolic extract. The ethanolic extract so obtained would be lyophilized & the residues so obtained were kept at 4°C in a method as described earlier [6, 7].

Cell culture

Human AML cell line, HL-60 & normal human embryonic cell lines (nHEK) bought from "National Facility of Animal Tissue & Cell Culture" ("NCCS"), Pune, India. All cells were further subjected to RPMI (Roswell Park Memorial Institute) media to obtain cultures as per manufacturer's instructions [9, 10].

Cell viability assay

Ethanolic extract of rose was firstly dissolved in DMSO & the solution was then made up with RPMI following which both the HL-60 & nHEK-293 cells (1×104) were administered with varying concentrations of Rose's ethanol extract (0, 10, 20, 30, 40 & 50 µg/ml) for 1 day. Viable cells were confirmed by MTT assay reported earlier.

Statistical study

Experiments were performed at least thrice & statistically significance was obtained at $p < 0.05$.

RESULTS & DISCUSSION

Apoptotic effects of Spergulin A on HL-60 cells

Growth inhibitory activity of the Ethanolic extract of rose towards AML was observed using the MTT assay. After 24 h treatment, it was found that Ethanolic extract of Rose showed a concentration dependent growth inhibition in HL-60 cell lines, with an IC50 value of 44.44 µg/ml (Figure 4) without any growth inhibitory activity on the nHEK-293 cell lines (data not shown). This implies that ethanolic extract of rose unlike on AML cells has no growth inhibitory activity on normal cells.

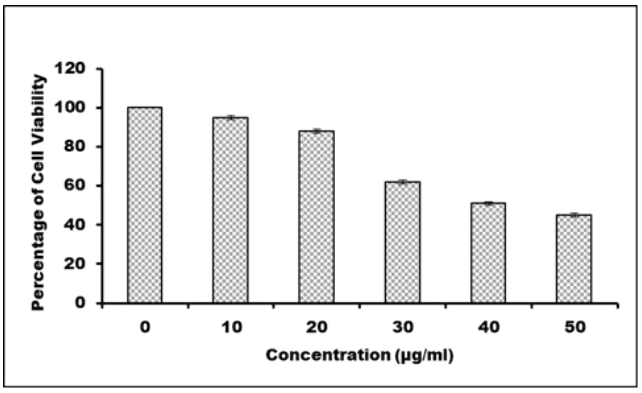


Figure 4: Graph showing growth inhibitory efficacy of the Ethanolic extract of Rose. The AML cells; HL-60 showed amount reliant decrease within percentage of viable cells after 24 h. Values were statistically significant with respect to the control or un-treated cells with P<0.05.

CONCLUSION

In this paper, the anti-AML activity of an ethanolic extract of Indian Rose has been described. Firstly, Ethanolic extracts of the leaves & stems of the Indian rose (Rosa indica) were prepared. The Ethanolic extract was then first dissolved in DMSO & then was reconstituted in RPMI media. The growth inhibitory activity of the Ethanolic extract of Rose on an AML cell line; HL-60 & on a normal human embryonic kidney cell line; nHEK-293 cells was noted in amount reliant way for 24 h.

Ethanolic extract induced growth inhibition on HL-60 cell line in a concentration dependent manner after 24 h, with the IC50 value at 44.44 µg/ml whereas no growth inhibition was observed in nHEK cells. These results thereby hint at a possibility of the Ethanolic extract being used as a potential lead agent against AML. As the Indian Rose is commonly available in India, thereby this plant has the potential to produce anti-AML agent at a commercial scale.

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