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Anti-Hairy Cell Leukaemia Effect of Aqueous Extract of *Momordica dioica*

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ABSTRACT

The unique feature of Hairy Cell Leukaemia (HCL) is that it's prone to relapse once treatment stops. As it's a rare form of leukaemia, most of its mechanism and the mode of treatment are still shrouded in mystery. Nowadays, a lot of focus has come on the role of common vegetables as a panacea for various diseases including cancer. Momordica dioica or Spiny gourd is common Indian vegetable which is rich in phytochemicals and some medicinal uses have attributed to it as well. In this paper, the anti-HCL property of Momordica dioica was investigated. Firstly, the aqueous extract of Momordica dioica was prepared and its anti-HCL activity was observed on a HCL cell line; Mo-B on a concentration or dose dependent manner. An IC50 value of 38.46 µg/ml was obtained after 24 hours on Mo-B cells but no effect was seen on the normal Human Embryonic Kidney cells (nHEK). It implies that the aqueous extract of Momordica dioica can be used as an anti-HCL agent. As Momordica dioica is a common vegetable found in India, thereby the anti-cancer components of the plant can be easily isolated and subsequently produced at an industrial level, making the anti-cancer medicines affordable to the common person.

Keywords: Apoptosis, Cells, Hairy cell leukaemia, IC50value, *Momordica dioica*, Mo-B cell lines, MTT assay, normal Human Embryonic Kidney cell (nHEK)

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INTRODUCTION

As per the WHO classification, Hairy Cell Leukaemia (HCL) has been classified as a chronic lymphoid peripheral B-cell neoplasm. One of the major issues with HCL is that though it is prevalent in low number and patients respond positively to chemotherapy with analogues of purine for example, pentostatin and cladribine, yet they have suffer several relapses. Patients suffering from HCL tend to suffer from infiltration of mature B cells into their Liver, Spleen and Bone marrow. In HCL, mature B cells have hair like cytoplasmic corona like projections with the co-expression of CD11c1, CD25 and CD103 as surface markers [1].

In the year 2011, a point mutation in a kinase-encoding proto-oncogene BRAF involves replacement of a Thymine (T) with an Adenine (A) in the exon 15 of the BRAF at the 1799th position located in chromosome number 7q34. This mutation then produces change in the amino acid from Valine (V) to Glutamate (E) at position number 600 (V600E) of the protein sequence (Figure 1), thereby leading to an aberrant activation of the oncogenic kinase called BRAF which in turn activate the downstream ERK-MEK signalling pathway (Figure 1). In HCL patients, the mutation in V600E-BRAF is heterozygous [2,3].

A major component of the ERK-MEK-RAS–RAF signalling route is the proto-oncogene called BRAF (Figure 1) that finally phosphorylates ERKs which helps in the spread and growth of neoplastic cells. The level of phosphorylated ERK and MEK suffers a sharp decrease in their expression following treatment of HCL cells with dabrafenib and/or vemurafenib which down-regulates HCL particular expression identification (Figure 1). The HCL cells upon treatment with the inhibitors of BRAF down-regulated their number of cytoplasmic corona or hair-like projections (Figure 1) and later on undergoes cell death or apoptosis (Figure 1) [4,5].

The clinical recognition of HCL involves cytoplasmic features as shown in Figure 1. Various HCL like cancers which have found a place under the WHO system of classifications have been tabulated in Table 1.

Nowadays, vegetables have gained importance among cancer researchers owing to their richness in phyto-molecules have been proven to have medicinal roles. As mentioned above, the frequent relapses of HCL tend to increase the costs of treatment greatly. In order to find a new source of anti-HCL agents, this paper attempts to evaluate the anti-HCL property of the aqueous extract of *Momordica dioica*.

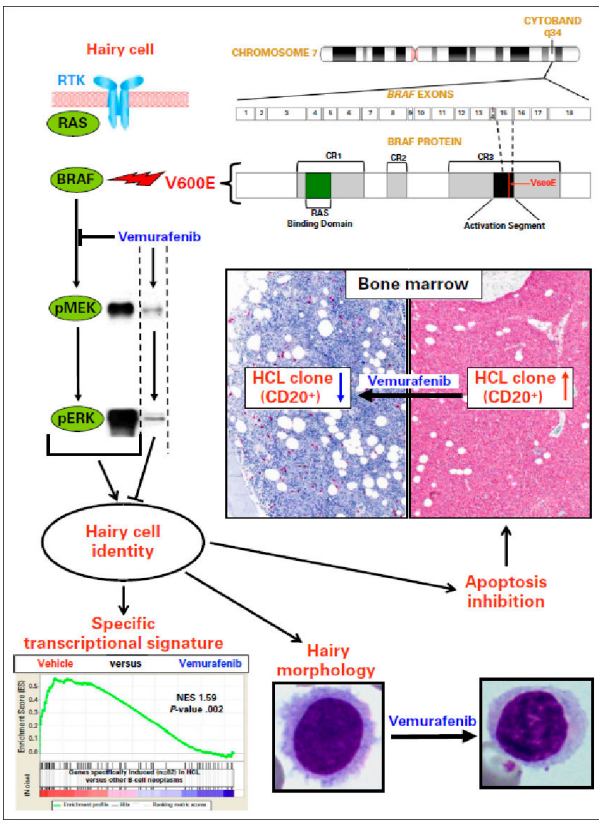

Figure 1. The BRAF-V600E mutation and Hairy Cell Leukaemia (HCL) pathogenesis. The Figure discusses about the signalling pathways activated in Hairy cell leukaemia, the presence of mutated genes on Chromosome 7, specific transcriptional signature upon treatment with Vemurafenib, specific bone marrow histology and cellular morphology upon treatment with Vemurafenib (Figure courtesy).

Table 1. Genetic wounds in HCL and HCL-Like Neoplasms with are occurring frequently. Following are the mutations which most likely drive up HCL and its variants.

Genetic Lesion	SDRPSBCL	HCL	SMZL	HCL-v
BRAF-V600E aberration or mutation	Absent	Present (>97%)	Absent	Absent
CDKN1B aberration or mutations	NA	Present (>16%)	NA	NA
KLF2 aberration or mutations	NA	Present (>16%)	Present (20%-40%)	Absent
KLF2 deletions	NA	NA	Present (11%)	NA
MAP2K1 aberration or mutations	Rare	Absent	Rare	Present (48%)
NOTCH2 aberrations or mutations	Absent	Absent	Present (10%-25 %)	NA
7 q deletions	Present (18%)	Present (<10%)	Present (~30%)	Present (15%)
TP53 deletions &/ aberration or mutations	Rare	Rare	Present (15%-20%)	Present (33%)
NF-κB genetic aberrations	NA	NA	Present (35%)	NA

Abbreviations are as follows: HCL as hairy cell leukemia; HCL-v as Hairy cell leukemia variant; NA as being not assessed by targeted analyses regarding the concerned gene(s); NF-kB as Nuclear Factor-kB; SDRPSBCL as Splenic Diffuse Red Pulp Small B-cell Lymphoma; SMZL as Splenic Marginal Zone Lymphoma. *MAP2K1 as mutations that has been observed in BRAF wild-type cases displaying a flow-cytometry immune phenotype compatible with HCL but are almost always carrying an unmutated or lowly mutated IGHV4-34 rearrangement which seems to define a separate genetic group of IGHV4-34+ HCL-like neoplasms that are characterized by a poorer response to purine analogs and by a flow-cytometry immune phenotype which can be either that of HCL-variant or HCL. †Including TNFAIP3, IKBKB, MAP3K14, TRAF3, BIRC3 and TRAF2.

LITERATURE REVIEW

Medicines of herbal or botanical origin have been known for their various medicinally useful activities including anti-cancer activities since ancient times. Even the World Health Organization has acknowledged that 80% of all the medicines consumed by the rural folks are of herbal or botanical origin [6].

Even many chemical agents from medicinal plants get their place in the manufacture of modern medicines. Table 2 tabulates about the various medicinal plants and their important parts which are used for anti-cancer studies.

Table 2. Tabulation of medicinal plants and their parts having role as a source for anti-cancer agents. Most of the anti-cancer drugs in the market as of today have molecules of herbal origin.

S.No.	Clan or Family	Medicinal Plant	Useful portions
1	Araceae	*Acoruscalamus*	Rhizome
2	Rutaceae	*Aegelemarmelos*	Whole plant
3	Agapanthaceae	*Agapanthus africanus*	
4	Agavaceae	*Agave americana*	Leaves
5	Asteraceae	*Ageratum conizoides*	Roots
6	Meliaceae	*Aglailaflaveolata*	Leaves, stem and bark
7	Meliaceae	*Aglailaslyvestrae*	Fruits
8	Rosaceae	*Agrimoniapilosa*	Herb
9	Poaceae	*Agropyronrepens*	Rhizome
10	Simaraubaceae	*Ailanthus altisima*	Bark
11	Lardizabalancae	*Akebiaquinata*	Fruit
12	Liliaceae	*Aliumcepa*	Bulb
13	Liliaceae	*Aliumsativum*	Bulb
14	Liliaceae	*Aloe barbedensis*	Leaf juice
15	Liliaceae	*Aloe vera*	Leaves
16	Zingiberaceae	*Alpinia galangal*	Rhizome
17	Meliaceae	*Amoorarohituka*	Bark
18	Acanthaceae	*Andragraphispaniculata*	Dried leaves
19	Anemarrhaenaceae	*Anemarrbenaasphodeloides*	Root
20	Annonaceae	*Annonasquamosa*	Seeds

RESEARCH QUESTIONS

Hairy Cell Leukaemia (HCL) is peculiar form of leukaemia that is marred by multiple relapses which makes its continuous treatment a costly affair. This paper has investigated whether an aqueous extract of the fruit of *Momordica dioica* could be an effective anti-HCL agent.

MATERIALS AND METHODS

Experimental design

The experiment design regarding utilizing *Momordica dioica* was inspired from earlier reported methods [8]. Firstly, the aqueous extract of the fruit of *Momordica dioica* was prepared.

The Hairy Cell Leukaemia (HCL) cell line Mo-B and the normal Human Embryonic Kidney 293 (HEK-293) cell lines were treated with various doses of the aqueous extract of the fruit of *Momordica dioica* for 24 hours to determine its IC50 value. The results were analysed for statistical significance.

Chemicals and reagents

MTT [3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide], cell culture media, Foetal Bovine Serum (FBS), antibiotics and all other chemicals and reagents that were used in this study were of the highest grade commercially available.

Preparation of aqueous extract of the fruit of Momordica dioica

Firstly, the fruits (3 kg) of *Momordica dioica* were deseeded and then dried in an oven or in the sun for 48 hrs. The left over dried fruit was then shredded and soaked in water for 24 hours.

The following water extract was filtered and the filtrate was lyophilized and the lyophilized powder was kept in an airtight container at 4°C for future use. The aqueous extract of the fruit of *Momordica dioica* was then reconstituted in phosphate buffer saline prior to its use on the cell lines.

Cell culture

From the National Facility of Animal Tissue and Cell Culture, Pune, India, human hairy cell leukaemia cell line Mo-B and the normal human embryonic cell line (NHEK) were purchased. Cells were cultured in RPMI 1640 medium with 10% heat inactivated FBS, under 5% CO2 conditions with antibodies at 37°C in a method reported by Mallick et al. and Chatterjee et al. [9,10].

Cell viability assay

Both the Mo-B and NHEK-293 cells (1×104) were treated with different concentrations of the aqueous extract of the fruit of Momordica dioica (0, 10, 20, 30, 40 and 50 µg/ml) for 24 hours following which, cell viability was determined by MTT assay as reported earlier.

Statistical analysis

All the experiments were performed three times and a value of $p<0.05$ was regarded as statistically significant with respect to the control cells.

RESULTS AND DISCUSSION

Growth inhibitory effect of aqueous extract of the fruit of Momordica dioica on Mo-B cells

To evaluate the efficacy of the aqueous extract of the fruit of Momordica dioica against HCL, the compounds were tested for their growth inhibitory effect on Mo-B cell lines using the MTT assay. After 24 hours treatment, it was found that the aqueous extract of the fruit of *Momordica dioica* showed a concentration dependent growth inhibition in Mo-B cell lines, with an IC50 values of 38.46 µg/ml, respectively (Figure 2) without any growth inhibitory activity on the Normal human embryonic kidney 293 (HEK-293) cell lines (data not shown). This implies that the aqueous extract of the fruit of Momordica dioica unlike that on HCL cells has no growth inhibitory activity on normal cells.

Momordica dioica being a commonly used vegetable has been used in Indian cooking for many years. In this paper, the aqueous extract of this vegetable has been tested for its growth inhibitory effect on a panel of HCL cells; Mo-B. The aqueous extract showed a concentration dependent decrease in the viability of Mo-B cells after 24 hours indicating that aqueous extract must have constituents to prevent cancer growth. Moreover, the aqueous extract being soluble in Phosphate Buffer Saline (PBS) and its negligible activity against the normal human cells, means it can be developed as an oral medication.

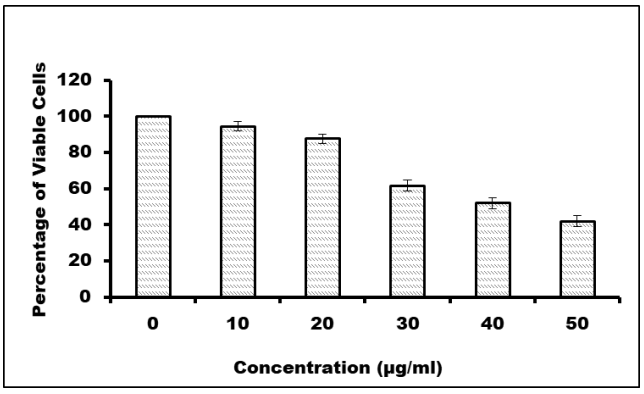

Figure 2. Graph showing growth inhibitory efficacy of aqueous extract of the fruit of Momordica dioica. The HCL cells; Mo-B showed a dose dependent decrease in the percentage of viable cells after 24 hours with an IC50 values of 38.46 µg/ml. The values were statistically significant with respect to the control or un-treated cells with P<0.05.

CONCLUSION

Hairy cell leukaemia is responsive to chemotherapy but is marred by frequent relapses which in turn tend to drive up the cost of treatment substantially. One of the major

solutions would be find anti-cancer agents from commonly available sources. Thereby, the aim of every cancer biologist is to acquire anti-cancer agents from everyday vegetables as vegetables have been found to be rich in phyto-molecules having medicinal roles. In this regard there have been some research in the medicinal value of Kantola or Kakrol (*Momordica dioica*) which formed the basis of this investigation that whether Kantola or Kakrol can be used as an anti-Hairy cell leukaemia agent or not.

In order to evaluate the growth inhibitory role of *Momordica dioica*, the fruit of the Kantola or Kakrol was deseeded and then dried to remove the moisture content. Then the dried out fruit was extracted in water for 24 hours. The extract was then filtered and lyophilized and the resultant powder was stored at 4°C in an airtight container. The resultant powder was reconstituted in Phosphate Buffer Saline (PBS) for treating the cultured Hairy cell leukaemia cells, Mo-B cells for 24 hours in a concentration dependent manner. Moreover, the growth inhibitory potential of the aqueous extract was also evaluated on the normal Human Embryonic Kidney cells (nHEK) in a concentration dependent manner for 24 hours.

Upon treatment with the aqueous extract the Mo-B cells but not nHEK cells showed a decrease in cell viability upon increase in the concentration of the aqueous extract of the fruit of Momordica dioica after 24 hours. This indicates the aqueous extract has constituents which have anti-HCL activity, being PBS soluble and having negligible activity towards nHEK cell lines means that oral therapy from Momordica dioica can be developed. Moreover, in future, the near universal availability of the Kantola or Kakrol can be used as a source of anti-hairy cell leukaemia agent(s) on a commercial scale.

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