



Research Article

Volume 5 Issue 1 - January 2018
DOI: 10.19080/JCMAH.2018.05.555655

J Complement Med Alt Healthcare

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A Novel Biologically Active Mixture (Bam) with Anti-Tumoral and Anti-Atherosclerotic Effects: Report of *In Vitro* Efficacy

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Submission: November 30, 2017; Published: January 25, 2018

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Abstract

Purpose: The role of food nutrients in the protection and slowing of chronic disease states like cancer and atherosclerosis is not clearly defined; proper nutrition is believed to play a larger role in the protection of atherosclerosis than that of cancer. We aimed to test the effect of a Biologically Active Mixture (BAM) that includes specifically selected active nutrients on the process of atherosclerosis and on cancer cells.

Material and Methods: After a comprehensive literature search, 8 super food items were selected. We utilized MTT tests *in vitro* to determine the effect of the combination of these super food items, BAM, on atherosclerosis and cancer cells. The amount of each super food item in BAM was calculated on the basis of data from literature if direct human data existed, and by extrapolation from animal studies at the absence of human data. BAM contained 2 types of fruits, 2 types of spices, 2 kinds of grains and 2 types of plant tea. To test the *in vitro* cytotoxic effects of BAM, smooth muscle cell culture isolated from rat arteries, HEK293 kidney cells, and A549 lung cancer cell lines were used.

Results: BAM exerted direct cytotoxicity in all 3 cell lines. The per cent cell inhibition rates by BAM were as follows: 65% in rat artery smooth muscle cell culture, 74% in HEK293 kidney cell line, and 71% in A549 lung cancer cell line.

Conclusion: This preliminary data suggests BAM has anti- atherosclerotic and anti- cancer effects. Thus, BAM may have potential complementary preventive and therapeutic effects for a number of chronic disease states. Given the huge burden of chronic disease states on health outcomes, further clinical studies are needed to comment further on the therapeutic and preventive effects of BAM, as well as the mechanism of these effects.

Keywords: Cancer; Atherosclerosis; Cytotoxicity; Super food

Introduction

It is well known that chronic inflammation is an age related process ("inflamm-aging") and linked with metabolic, cardiovascular, neurological diseases as well as cancer, osteoporosis and frailty [1-8]. In addition, diet is also known to be associated with inflammation where a diet high in refined starches, trans-fats and low in omega-3 fatty acids, fibers and whole grains is known to promote inflammation [9]. Likewise, participants of Nurses' Health Study who consumed a more 'westernized' diet had higher CRP and IL-6 levels [10]. Interestingly, diet is also a source of some active compounds, like Apigenin, a flavonoid, which suppresses inflammation, retards cell proliferation, and may be preventive against chronic inflammatory states including cancer [11]. In parallelism, there are some natural "super food" items containing active ingredients that have been shown to inhibit both cancerogenesis and atherogenesis in laboratory

and animal models with or without data from clinical studies. Inflammation and immunity are multifaceted phenomena and inflammation, especially in its acute form, also serves important functions to restore health [12]. Thus, in order to develop a natural form of potential anti-inflammatory mixture derived from natural nutrients, with a desirable inhibitory activity against cancer cells and atherosclerosis, we aimed to test *in vitro* the effect of a Biologically Active Mixture (BAM).

Materials and Methods

Selection of super food items and construction of BAM

We searched the literature for food items with reported results showing efficacy both in cancer and atherosclerosis models, and also possessing anti-inflammatory compounds, with data derived from either cell culture experiments and/or animal

models. Literature search yielded 8 super food items, which were then used to construct BAM. The 8 super food components of BAM contained 2 types of fruits, 2 types of spices, 2 kinds of grains and 2 types of plant tea. In order to decide on the amount of each of the 8 components in the final BAM preparation, we used direct data from literature, if data from human studies existed. On the other hand, at the absence of human data, where only animal studies existed, the amount of each component in the BAM preparation was calculated by extrapolation from weight ratio of the daily consumed dose of the component over average total weight of the animal in that experiment, assuming an average human weight of 70kg.

Preparation of BAM

Components of BAM were used either in the form of dry powder, dehydrated fruit, freshly squeezed fruit juice or plant tea. See Table 1 for the description and amount of components used in BAM.

Table 1: Components in the daily of BAM. (250 cc mixture/day).

Component	Type	Form
1	Spice	Dry Powder
2	Fruit	Dried fruit
3	Fruit	Fruit juice
4	Plant tea	Tea
5	Spice	Dry Powder
6	Plant tea	Tea
7	Grain	Dry Powder
8	Grain	Dry Powder

During the preparation phase, firstly component 2 was minced in a dedicated machine, and then mixed with components

7 and 8. Components 4 and 6 are first brewed, and then waited until their heat drops down to room temperature and after this stage they are added to other components. The resultant mixture is then homogenized with the help of a standard mixer and a blender. At the final stage, BAM is vacuumed, Vitamin C added and stored in a glass container containing the total dosage of 7 days. Potential daily dosage of BAM was set at 250cc in volume.

Testing *in vitro* cytotoxicity

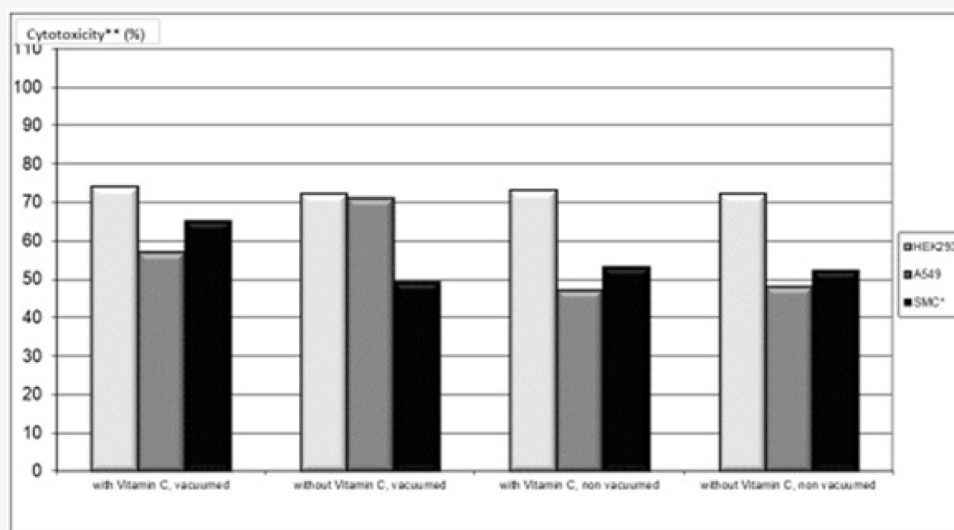
To test the *in vitro* cytotoxic effects of BAM, 3 cell lines were used. Smooth muscle cell culture isolated from rat arteries was used to test the effect of BAM on the atherosclerotic process. HEK293 kidney cells, and A549 lung cancer cell lines were utilized to test cytotoxicity on cancer cells, and primitive, embryonic cells. We used MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrasodium bromide) assays to explore growth inhibition in cell lines. MTT testing was conducted as used in the literature previously [13,14].

Briefly, cancer cells were seeded into 96-well microtiter plates at appropriate densities. Cells were exposed for 72 hours to a standard concentration of BAM prepared with or without Vitamin C, or air, and tests were repeated for a total of 3 times. After 72 hours, 20µL of 5mg/ml MTT was added to each well, and all plates were incubated at 37 °C for 4 hours. Finally, DMSO (150µL) was introduced to each well and optical absorbance was read at 570nm. Cytotoxicity was formulated as the following: $100 \times (1 - ((\text{absorbance of treated cells}) / (\text{absorbance of control cells})))$.

Statistical methods

Percent inhibition rate by BAM in cell lines was expressed as the mean inhibition value of 3 repeated tests as compared to control (no BAM).

Results and Discussion



*: Rat smooth muscle cells. **: Cytotoxicity is defined as $100 \times (1 - ((\text{absorbance of treated cells}) / (\text{absorbance of control cells})))$.

Figure 1: In vitro cytotoxicity of biologically active mixture (BAM).

The inhibition rates by BAM, compared to control (no BAM), in the cell cultures utilized were as follows: 65% in rat artery smooth muscle cell culture, 74% in HEK293 kidney cell line, and 71% in A549 lung cancer cell line. Addition of Vitamin C or vacuuming did not seem to have major impact on the cytotoxicity profile. Please refer to Figure 1 for details. Additionally, after 1 month of storage, BAM did not appear to lose cytotoxic potential as compared to no storage (freshly prepared BAM), data not shown here.

We demonstrated in this study that BAM clearly exerted cytotoxicity in all cell lines tested. Our data clearly shows the potential of BAM to inhibit at least the smooth muscle cell proliferation state in atherosclerosis, as well as cellular proliferation in cancer cells. This dual effect makes this natural mixture of 8 super food items a candidate of complementary treatment for prevention and prophylaxis of cancer and atherosclerosis. There is an also hypothetical prospective that BAM may be helpful in the treatment of these states as a complementary modality. Because components of BAM were selected and dosed on their published effects on cancer and atherosclerosis models and cell cultures, the resultant mixture, BAM, may be more effective as a sum than individual components.

Previous reports show that cancerogenesis and atherogenesis share common pathways, inflammation being one of them and some common molecules like Alarmins, macrophage derived Wnt5a and inflammasomes take part in the pathogenesis of both of these disease states [15-17]. A possible anti-inflammatory effect of BAM additionally opens doors to the probability of prevention and treatment of other chronic inflammatory conditions such as Diabetes Mellitus, metabolic syndrome, neurological diseases, and osteoporosis. Although the anti-inflammatory effect of BAM as a mixture was not quantified in this study, it is likely, as the constituents of BAM have been separately shown to possess anti-tumoral, anti-atherosclerotic and anti-inflammatory effects.

Our findings are encouraging to advise chronic use of a natural mixture, such as BAM, which is non-toxic and may well be used as a complementary treatment modality. One of the components of BAM; component 6, is also known to contain the ingredient Catechin which facilitates lipolysis [18-20]. Thus, BAM may also potentially be used to prevent or impede obesity, and usage during childhood and adolescence also makes sense, as weight control earlier in life has been shown to augment protection against cancer.

In short, in this study we propose a natural and non-toxic food supplement that has anti-cancer and anti-atherosclerotic effects. Our in vitro findings necessitate prospective clinical trials, to test the proposed effects alone or when used in conjunction with standard treatment methods.

Conclusion

We show in this study that a natural food supplement exerts anti-tumoral and anti-atherosclerotic effects in vitro. This finding has implications for use of this supplement (BAM) as a complementary treatment modality.

Conflict of Interest

- No financial support was received for this study.
- Patent pending for BAM.

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DOI: [10.19080/JCMAH.2018.04.555655](https://doi.org/10.19080/JCMAH.2018.04.555655)

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