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Evaluation of Hepato-Renal Protective potential of Aloe Vera Gel and Bitter Melon Seed Powder in Sprague Dawley Rats

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[MS]Conception and design, Collection and assembly of data, [RS]Analysis and interpretation of the data, Statistical expertise, [KJ] Final approval and guarantor of the article

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A B S T R A C T

Background. Worldwide, for the management of numerous diseases Complementary and alternative medicine (CAM) are used. Aloe barbadensis Miller (aloe vera) and Momordica charantia (bitter melon) both are known for their medicinal and therapeutic potential including anti-inflammatory, anti-diabetic, and antibacterial.

Objective. To probe bitter melon seed (BMS) powder and aloe vera gel (AVG) powder effect on liver and kidney functions in diabetic Sprague Dawley Rats.

Methodology. For this purpose, BMS powder and AVG powder were prepared and used in two different concentrations to check its action through efficacy studies in Sprague Dawley Rats during 30 days' trial. Blood samples were collected fortnightly and assessed for change liver function tests and kidney function tests. All the parameters were analyzed statistically to determine the level of significance.

Results. Results revealed that AVG and BMS powder in combination was very effective in controlling diabetes-related dysfunction of the liver and kidney. The group receiving 50% AVG and 50% BMS powder showed significantly reduce blood urea nitrogen (13.17 mg/dL) and uric acid (6.03 mg/dL) over the period of 30 days, whereas the group treated with and 25% AVG and 75% BMS powder showed improvement in alanine transferase (56.50 U/L), aspartate aminotransferase (48.67 U/L), alkaline phosphatase (170.17 U/L), and creatinine (0.61 mg/dL). Conclusion. Supplementation of BMS and AVG has no bad effect on the liver and kidney, moreover, it was effective in controlling elevated liver and kidney function parameters.

Keywords: Bitter melon seed, aloe vera gel, alanine aminotransferase, aspartate aminotransferase, Sprague Dawley Rats.

Introduction

In the last few decades, because of several reasons, the reputation and acceptance of complementary medicine had been increased. In Ayurveda and other indigenous systems of medicine, the prescription of dietary recommendations along with the traditional plant use as therapy had been raised and is commonly used in Asia. Surveys accompanied in the US and Australia revealed that nearly 48.5% of participants had been used at least one form of unconventional therapy including herbal medicine.[1] In 1980, World Health Organization recommended evaluating the effectiveness of plant-based alternatives, where we dearth the safe pharmaceuticals. There is plenty of archaeological proof that showed, medicinal plants had been regularly used since prehistoric times. The botanical products were consumed for their psychotherapeutic and biomedical purposes. Evidence advocates that the prompt homeopaths were well aware of the mind-body interconnection and knew that the patient's relaxation plays an important role in medical treatment and health restoration and rehabilitation. The ancient Egyptians had been credited for developing an elaborative pharmacological collection derived from numerous natural products such as plants. The Egyptian doctors prescribed remedies for analgesics, common cold, gastrointestinal disorders, and urinary tract diseases. Extracts of the plant had been prepared to use topically or ingested

orally or inhaled by fumigation. The Egyptians are also attributed to finding medicinal use of barley, castor oil, marijuana, mints, opium, and wine.[2] Greece healthcare practitioners also continued the plant-based treatments. In the first century A.D., an authority on herbs "Dioscorides", assembled the 24 books on nearly 600 potentially therapeutic plants under the title of "De Materia Medica".[3]

Herbal medicines are very famous in many countries, including the developed-, developing and underdeveloped nations. The reason for the widespread use of plant-based remedies is very complex as well as very simple. The assumption that "natural is safe" is certainly an important factor behind this. However, this impression is dangerously misleading as sometimes herbal medicines contained pharmacologically active ingredients that are found to be associated with adverse effects.[4] In Pakistan, research on medicinal plants is on very initial steps of documentation. The research is mainly conducted in research institutes as well as universities. Indigenous knowledge about traditional uses of the plants as medicine had been transferred from generation to generation in some areas of Pakistan. These plants had been used to treat or to manage almost every type of disease from a simpler one such as headache to a complex one as cancer.[5] Few plants are commercially cultivated and harvested for the extraction of different kinds of bioactive compounds. There are very few educational institutes that are providing education practical implications of plants as medicine. The major reason for use of plants as medicines is that they have synergistic combinations of side effects and their neutralization. Forever, medicinal plants are also income sources for as well as for herbal dealers and exporters. It is also a very cheap source for people with poor income, little resources, and limited access to medical health care facilities.[6]

Aloe vera is well known for its spectacular therapeutic characteristics since ancient times and had been used widely by the Assyrian civilians, the Mediterranean, Egyptians and in times of Biblical. The reliable information of aloe vera is a healing plant that is credited to the "Mesopotamian clay tablet" dated 2100 B.C. However, the first thorough aloe's medicinal value depiction was found in an Egyptian document, "Papyrus Ebers", dated 1550 B.C. that described numerous aloe vera products for the management of internal and external diseases. In 1820, USA pharmacopeia declared the aloe vera as a cleansing and skin protectant [7] in the 1930s start using it clinically for the handling of burnt skin and mucosal membranes because of radiotherapy. These ornamental plants have been grown widely and their different parts are used in several remedial products for the treatment of various health ailments. Still today, aloe vera is a vital medicinal plant in many countries, together with China, Japan, India, the West Indies, and South Africa. It has been used to treat different diseases in different traditions such as in India, it has been used for colic, constipation, infections, worm infestation, and skin diseases, in Tobago and Trinidad has been used for management of hypertension [8] in Mexican Americans aloe vera used to treat insulin-dependent Diabetes Mellitus [9] and in China aloe, vera recommended as a medicine for fungal infections. In Western civilization, it has common usage in herbal medicines and is found extensively in the cosmetic, functional foods industries and pharmaceutical.[10]

About 80% of developing countries' population used traditional medicine of plants origin in the number of medical complications. Therefore, in the last few decades, researchers had been started to focus on the scientific basis of traditional remedies. Bitter melon is an example of such vegetable plants that are commonly used for medicinal functions. In Asian cuisine, It is a traditional food. Bitter melon is a tropical vegetable and is also known with other names like karela or balsam pear. All parts of bitter melon are very bitter, due to the presence of a bioactive component known as momordicin, which is famous for having an anti-diabetic and slight stomachic effect.[11] Bitter melon is used as food or for medicinal purposes all over the world. It is an important part of folk medicine and is used as a remedy widely to control and manage Diabetes Mellitus. Seeds and fruits had therapeutic potential against ulcer, HIV, inflammation, leukemia, some microbial diseases, cancer, diabetes, and many others.[12] In Ayurveda, each part of bitter melon is recommended for different diseases like the fruit had been used sexual tonic, laxative, anti-bilious, emetic, stomachic, and stimulant. It's also used to cure anemia, blood diseases, bronchitis, cholera, and gonorrhea. Like other bitter foods, bitter melon improved digestion problems. It may help people with constipation, diarrhea, dysentery, and dyspepsia. Sometimes side effects may develop if consumed so abruptly like heartburn and ulcers. It also acted as a demulcent and had inflammation modulatory effect.[13]

Keeping in view the current situation and increasing trend of consumption of CAM therapies present study has been planned to evaluate the safety aspect of bother ingredients. The main objectives of the project are development of AVG and BMS powder and the impact of prepared powders on the liver and renal function test.

Methodology

Aloe vera was purchased from the Vegetable Research Area of the Institute of Horticultural Sciences in the University of Agriculture, Faisalabad and bitter melon dried seeds from Ayub Agricultural Research Institute (AARI),

Faisalabad. Aloe vera leaves of homogenous color, freshness, ripeness, and size are then washed to make them dirt-free. To ensure the purity of gel, aloe latex was completely removed by cutting the base of leaves and placed vertically for one hour. Leaf base, leaf top, side spines, and the epidermis were removed to extract the gel. The extracted gel cut into small pieces and placed into the food dehydrator at sixty°C for twentyfour hours. Dried material in the form of gel flakes were crushed and converted into fine gel powder. Seeds were examined for damage and dirt followed by drying cleaning with a cotton cloth to remove dust. Then seeds were covered into fine powder by the electrical mill of mesh size 50. Both powders are stored in airtight glass jars at room temperature for further

Animal trail: For safety evaluation of AVG and BMS powder efficacy trail was performed on 18 Sprang Dawley Rats (180-200 g) were keep in the rat room facility of the FFNHS, NIFST, UAF. The guideline of the Institutional Biosafety Committee (IBC) were used for the conduct of experiment. Before the start of the study, rats were acclimatized for one week. Rats were randomly divided into 3 groups and fed on a high sucrose diet for 15 days to induce hyperglycemic condition and when rats attain fasting blood glucose >250mg/dl are considered for further study. After that period animals were fed on experimental powder along with a normal diet. For biochemical analysis, blood samples were collected thrice (initiation, mid, and end of the study), including liver function tests (alanine transferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP)) and kidney function tests (blood urea nitrogen (BUN), uric acid, and creatinine) through the modified method of Virdi *et al.*[14] The following treatment plan was followed for theefficacy trail. G_0= Diabetic control, G_1= 50% AVG powder and 50% BMS powder, G_2= 25% AVG powder and 75% BMS powder.

To determine the level of significance, the recorded data for each parameter was analyzed statistically through Completely Randomize Design and Tukey HDS Test.[15]

Results

Rats were randomly divided into 3 groups and fed on a high sucrose diet for 15 days to induce hyperglycemic condition and when rats attain fasting blood glucose >250mg/dl are considered for further study. After that period animals were fed on experimental powder along with a normal diet and biochemical analysis were performed.

Serum Alanine Transferase (ALT): Means values for the effect of AVG and BMS powder on ALT revealed highest ALT levels (49.50U/L) in control group rats fed on regular diet with no supplementation followed by 45.56U/L and 43.38U/L G1 and G2 correspondingly. At the initiation of the experiment, the mean values of ALT were 34.11U/L which progressively increased to 36.89U/L and then to 66.25U/L by consuming AVG and BMS powder (Table I).

Serum Aspartate Aminotransferase (AST): Means values for the effect of AVG and BMS powder on serum AST revealed that highest AST levels (38.78U/L) was found in control group followed by 35.28U/L and 35.83U/L in groups fed on 25% AVG and 75% BMS powder (G2) and 50% AVG and 50% BMS powder (G1) correspondingly. At the initiation of the experiment, the mean values of AST were 20.06U/L which progressively increased to 34.38U/L and then to 55.44U/L by consuming AVG and BMS powder (Table II).

Table I: Effect of various treatments on ALT (U/L) concentration.

Groups	ALT			
	Study intervals (Days)			
	0	15	30	Mean±SD
G0	33.67f	35.00ef	79.83a	49.50± 26.28a
G1	34.00f	36.67de	66.00b	45.56±17.75b
G2	34.67ef	39.00d	56.50c	43.38±11.56c
Means	34.11 ±0.51c	36.89±2.01b	67.444±11.73a	

SD= ±, Means sharing same letters within a row are statistically non-significant, G_0= Diabetic control, G_1= 50% AVG powder and 50% BMS powder, G_2= 25% AVG powder and 75% BMS powder

Table II: Effect of various treatments on AST (U/L) concentration.

Groups	AST			
	Study intervals (Days)			
	0	15	30	Mean±SD
G0	19.83e	36.33c	60.17a	38.78± 20.27a
G1	19.67e	30.33d	57.50a	35.83±19.51b
G2	20.67e	36.50c	48.67b	35.28±14.04b
Means	20.06±0.54c	34.38±3.51b	55.44±6.02a	

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Table III: Effect of various treatments on ALP (U/L) concentration.

	ALP			
Groups	**Study intervals (Days)**			
	0	**15**	**30**	**Mean±SD**
G0	217.50a	219.83a	218.50a	218.61±1.170628a
G1	217.33a	155.67d	193.67b	188.89±31.10b
G2	218.33a	141.83e	170.17c	176.78±38.68c
Means	217.72±0.54a	172.44±41.62c	194.11±24.17b	

Table IV: Effect of various treatments on BUN (mg/dL) concentration.

	BUN			
Groups	**Study intervals (Days)**			
	0	**15**	**30**	**Mean±SD**
G0	29.17b	30.17ab	31.50a	30.28±1.17a
G1	28.50b	19.67c	13.17d	20.44±7.69c
G2	30.17ab	19.50c	18.33c	22.67b±6.52b
Means	29.28±0.84a	23.11±6.11b	21.00±9.45c	

Table V: Effect of various treatments on Creatinine (mg/dL) centration

	Creatinine			
	Study intervals (Days)			
	0	**15**	**30**	**Mean±SD**
G0	0.78cd	0.85b	0.91a	0.85±0.06a
G1	0.83bcd	0.82bcd	0.78d	0.81±0.03b
G2	0.84bc	0.62e	0.61e	0.68±0.13c
Means	0.82±0.03a	0.76±0.13b	0.76±0.15b	

Table VI: Effect of various treatments on Uric acid (mg/dL) concentration

	Uric Acid			
	Study intervals (Days)			
	0	**15**	**30**	**Mean±SD**
G0	4.96de	5.33cd	7.56a	5.95±1.40a
G1	4.93e	4.61e	6.03b	5.19±0.75c
G2	4.98cde	5.37c	6.10b	5.48±0.57b
Means	4.96±0.03b	5.10± 0.43b	6.56±0.86a	

SD= ±, Means sharing same letters within a row are statistically non-significant, G_0= Diabetic control, G_1= 50% AVG powder and 50% BMS powder, G_2= 25% AVG powder and 75% BMS powder

Serum Alkaline Phosphatase (ALP): Means values for the effect of AVG and BMS powder on serum ALP revealed that highest ALP levels (218.61U/L) was found in control group followed by 176.78U/L and 188.89U/L in groups fed on 25% AVG and 75% BMS powder (G2) and 50% AVG and 50% BMS powder (G1) correspondingly. At the initiation of the experiment, the mean values of ALP were 217.72U/L which slowly reduced to 172.44U/L and then again increased to 194.11U/L by consuming AVG and BMS powder (Table III).

Blood Urea Nitrogen (BUN): Means values for the effect of AVG and BMS powder on BUN revealed that highest BUN concentration (30.28mg/dL) was found in control followed by 22.67mg/dL and 20.44mg/dL in groups fed on 25% AVG and 75% BMS powder (G2) and 50% AVG and powder 50% BMS (G1) correspondingly. At the initiation of the experiment, the mean values of BUN were 29.28mg/dL which gradually decreased to 23.11mg/dL and then to 21.00 mg/dL by consuming AVG and BMS powder (Table IV).

Creatinine: Means values for the effect of AVG and BMS powder on blood creatinine revealed that highest creatinine level (0.85mg/dL) was found in control group followed by 0.81mg/dL and 0.68mg/dL in G1 and G2 correspondingly. At the initiation of the experiment, the mean values of creatinine were 0.82mg/dL which gradually decreased to 0.76mg/dL by consuming AVG and BMS powder (Table V).

Uric Acid: Means values for the effect of AVG and BMS powder on blood uric acid revealed that highest uric acid levels (5.95mg/dL) was found in control group followed by 5.19mg/dL and 5.48mg/dL G1 and G2 correspondingly. At the initiation of the experiment, the mean values of uric acid were 4.96mg/dL which gradually increased to 5.10mg/dL and 6.56mg/dL by consuming AVG and BMS powder (Table VI).

Discussion

The rats consuming 25% AVG and 75% of BMS showed a minimum increase in ALT concentration of 38.64% followed by

rats consuming 50% AVG and 50% BMS powder resulted in a 48.48% rise in ALT concentration. Whereas, there was 57.83% rise in ALT level of rat that was not given any supplementary powder. It is evident from the present findings, that ingesting of AVG and BMS may have hepatoprotective potential which helps to reduce the liver damage that is caused by hyperglycemia in diabetics' patients. Findings of the current study were quite related to the results of Virdi *et al*. [14], that aqueous extract of bitter melon's seeds reduced the raised level of SGPT by 69% in diabetics. Oral intake of extracts' AVG and pulp caused a significant reduction in ALT and ALP. Reduction in the hepatocytes damage to was observed in histological examination of liver tissues.[16]

There were 67.31% rise in AST level of rat that was not given any AVG or BMS powder. It is evident from the present study's results, that consumption of BMS and AVG in combination may have hepatoprotective potential which helps to reduce the live damage that is caused by hyperglycemia in diabetics' individuals. Findings of the current study are closely related to the conclusions of Virdi *et al*. [14], that aqueous extract of bitter melon seed reduced the raised levels of SGOT 21% in diabetics. Aloe-Emodin, an active compound, showed protective potential for hepatocytes in the liver against liver damaging diseases.[17]

Rats consuming 50% AVG and 50% BMS powder induced a 10.89% reduction in ALP concentration. Whereas ALP level remained elevated in rat that was not given any BMS or AVG powder. It is clear from the current results, that utilization of BMS and AVG may have hepatoprotective potential which helps to reduce liver damage or prevent any further damage to the liver that is caused by hyperglycemia in diabetics' individuals. Findings of the this study were thoroughly related to the results of Virdi *et al*., [14], Can *et al*. [16], that oral intake of extracts' AVG and BMS and pulp caused a significant reduction in enzymes like ALP and ALT. Reduction in the hepatocytes damage to was observed in histological examination of liver tissues.

The rats consuming different concentration of AVG and BMS powder induced a 39.23% reduction in BUN concentration. Whereas BUN level remained elevated in rat that was not given any AVG or BMS powder. It is clear from the current results, that utilization of BMS and AVG may have renal-protective potential which helps to reduce the kidney damage or prevent any further damage to the kidney that is caused by hyperglycemia in diabetics' patients. Findings of the this study were thoroughly related to the results of Virdi *et al*., [14], that oral administration of an aqueous extract of bitter melon maintained the blood urea nitrogen and inhibits its further increase. On oral intake of AVG, there was a significant rise in glutathione-S-transferase, glutathione catalase, reduced superoxide dismutase, and glutathione peroxidase in the kidney, which protects the kidney from damage.[18]

Creatinine concentration remained elevated in rat's blood that was not given any BMS or AVG powder. It is clear from the current results, that utilization of BMS and AVG in combination may have renal-protective potential which helps to reduce the kidney damage or prevent any further damage to the kidney that is caused by hyperglycemia in diabetics' patients. Findings of the this study were thoroughly related to the results of Virdi *et al*. [14], that consumption of bitter melon extract potentially inhibited the elevation of creatinine. Histopathological analysis of the kidney showed that the AVG's extract significantly changed the composition of the fatty acid of the kidney and was restored in both liver and kidney of diabetic rats following treatment with the gel extract.[19] Whereas uric acid level increased in the blood of rats by 34.33% that were not given any supplemental powder. The consumption of AVG and BMS in combination may have renal-protective potential which helps to reduce the kidney damage or prevent any further damage to the kidney that is caused by hyperglycemia in diabetics' individuals. the findings of Virdi *et al*., [14], showed that consumption of bitter melon extract potentially inhibited the elevation of uric acid. Consumption of aloe vera's gel extract showed improvement in biochemical parameters as there was a significant reduction in serum creatinine and urea level.[20]

Conclusion

Vegetables and other herbaceous plants can be used as medicine to prevent and treat various health ailments. As these vegetables are part of daily cuisine in most of the countries that why no difficulty will be faced to increase their consumption and utilization to derive specific health benefits. Because of the unique composition of bioactive components responsible for blood glucose control, increased utilization of bitter melon should be appreciated. CAM may be used as an alternative and or as a complementary approach for treating or preventing certain health disorders. But scientific evidence is required to save the use of CAM. Aloe vera and bitter melon are effective in treating diabetes-related liver and kidney dysfunction.

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