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STUDY OF ANTIDIABETIC AND IMMUNOMODULATOR ACTIVITY OF PHARMACOLOGICAL PREPARATION - BRAMHRASAYANA

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Abstract

Nature always stands as a golden mark to exemplify the outstanding phenomena of symbiosis. Today about 80% of people in developing countries still relay on traditional medicine based largely on the different species of plants for their primary health care. About 500 of plants with medicinal uses are mentioned in ancient literature and 800 plants have been used in indigenous system of medicine. Ayurvedic medicines are the combinations of selected herbal drugs and are manufactured under different pharmaceutical processes to result in various dosage forms such as churnas, bhasmas, liquid, lehas, pill, tablet etc. In recent years, the usage of herbal drugs for the treatment of various diseases has increased all over the world. The herbal drugs are believed to be safe and free from serious adverse reactions, as they are obtained from nature. Also, the limited therapeutic success of modern medicine has steered the increase in the usage of alternative medicine including herbal preparations. Written records of the use of herbal medicine date back more than 5,000years. In fact, for most of history, herbal medicine was the only medicine. Although many herbs are primarily of historical interest, thousands of herbal products are available over the counter and commonly used by patients in the United States. The current review focuses on to find out the antidiabetic, Immunomodulatory response of the formulated bramhrasayana (*in vivo*).

Keywords: Herbal drug, Antidiabetic, Immunomodulatory action, Bramhrasayana, *Emblica officinalis.*

Introduction

Herbal medicine sometimes referred to as Herbalism or Botanical Medicine, is the use of herbs for their therapeutic or medicinal value. An herb is a plant or plant part valued for its medicinal, aromatic or savory qualities. Herb plants produce and contain a variety of chemical substances that act upon the body. Herbal system and greater parts of its medicaments are based on indigenous herbals. Knowledge about the medicinal plant is mandatory for all who is working in the field of ayurveda, in order to identify and select the appropriate plant for a specific disease. A number of scientific investigations have highlighted the importance and the contribution of many plant families i.e. Asteraceae, Liliaceae, Apocynaceae, Solanaceae, Caesalpinaceae, Rutaceae, Piperaceae, Sapotaceaeused as medicinal plants. An estimate of the EXIM Bank puts the international market of medicinal plants related trade at US$ 60 billion per year growing at the rate of 7% only. Bramhrasayana is a traditional polyherbal formulation, which widely used as tonic, rejuvenator, anabolic, immunomodulator and memory enhancer. Bramhrasayana contains the pulp of *Emblica officinalis* as the prime ingredient, along with powders and extracts of several other herbs. Diabetes mellitus is a widespread disorder, which has been long recognized in the history of medicine. Before theadvent of insulin and oral hypoglycemic drugs, the major form of treatment involved the use of plants. More than 400 plants are known to have been recommended, and recent investigations have affirmed the potential value of some of them treatment. Herbs are used to manage Type 1 and Type II diabetes ,The hypoglycemic and anti-hypoglycemic effect of several plants used as anti-diabetic studied Hepatotoxicity, Immunomodulator and

their complications. For this, therapies developed along the principles of western medicine (allopathic) are often limited in efficacy, carry the risk of adverse effects, and are often too costly, especially for the developing world.

Aim and Objective

The aim of the research is to find out the antidiabetic, hepatoprotective and immunomodulatory response of the formulated bramhrasayana (*in vivo*).

Therefore the object of the present study is carried out:-

- Acute toxicity study for formulated bramhrasayana.
- Antidiabetic activity of bramhrasayana in alloxan induced rats.
- Estimation of biochemical parameters (SGOT, SGPT, SU, SC, S Cho, ST, HDL, VDL) in normal, diabetic and bramhrasayana treated rats.
- To study the histopathological changes in pancreas of normal and alloxan induced diabetic and formulated bramhrasayana treated rats.
- Hepatoprotective activity of bramhrasayana in paracetamol induced liver toxicity in rats.
- Estimation of biochemical parameters (SGOT, SGPT, ALP, TB, TC TP) in paracetamoland bramhrasayana treated rats.
- To study the histopathological changes in liver of paracetamol induced and formulated bramhrasayana treated rats.
- Estimation of immunomodulator activity of bramhrasayana on rats.

Materials and Methods

S .No	Common name	Botanical name	Quantity
1	Amla	*Emblicaofficinalis*	600pieces
2	Pippali	*Piper longum*	6g
3	Punaranava	*Boerhaaviadiffusa*	6g
4	Cardamom	*Ellatariacardamomom*	6g
5	Shatavri	*Asparagus racemosus*	6g
6	Bala	*Sidacordifolia*	6g
7	Mandukparin	*Centellaasiatica*	6g
8	Kalaagra	*Aquilariaagallocha*	6g
9	Malabra nut	*Justiciaadhatoda*	6g
10	Bhumyamalaki	*Phyllanthusamarus*	6g
11	Sarivan	*Desmodiumgangticum*	6g
12	Madhuca	*Madhucalongifolia*	6g
13	Jivanti	*Leptaderia reticulate*	6g
14	Shankhpuship	*Canscora decussate*	6g
15	Mulathi	*Glycyrrhizaglabar*	6g
16	Red chandan	*Santalum album*	6g
17	Kamalkeshar	*Nelumbonucifera*	6g
18	Gokharu	*Tribulusterrestris*	6g
19	Neelkamal	*Nymphoeastellata*	6g
20	Bilva	*Agelemarmelos*	6g
21	Pankaja	*Nelunbiumspeciosum*	6g
22	Nagbala	*Grewishirseta*	6g
23	Honey	*Apisdorsata*	750g
24	Ghee	--	750g

Table.1 Ingredients used in Bramhrasayana

S.No	Name of bhasma	Quantity
1	Swarnabhasma	125mg
2	Raupyabhasma	1g
3	Lohbhasma	5g
4	Tamrabhasma	5g

Table.2 List of Bhasma

Procedure according to Characksamhita.

- 600 amla were taken.
- Out of 600, 300 amla were taken and steam boiled with milk. The distance maintained between amla and milk was maintained 3-4 inch's.
- Then seeds were separated from amla fruit dried in shade and powdered.
- Remaining 300 amlawere taken for preparation of juice, the extracted juice mixed with previous prepared amorphous amla powder.
- Pippali, Honey, Punaranava, Shatavri, Bala, Mandukparin, Kalaagra, Malabra nut, Bhumyamalaki, Sarivan, Madhuca, Jivanti, Shankhpushpi, Mulathi, Red chandan, Kamalkeshar, Gokharu, Neelkamal, Bilva, Pankaja and Cardamom were taken in equal proportion, grinded and mixed with amla powder.
- The amount of all drug powder should be 8^{th} part of total amla powder.
- The total mixture of drug powder along with alma powder was mixed with juice of *Grewishieseta* and then dried in shade.
- Then, honey and ghee was added to above mixture. The quantity has double of amla powder.
- The whole mixture was placed in a clay pot which was internally coated with ghee and covered with a clay dish.
- Then the pot was buried in soil and surrounded by ash for 15 days.
- After 15 days the pot was removed from soil and then Swarnabhasma, Lohbhasma, Raupyabhasma and Tamrabhasma were added to 20 gm of above mixture according to the given proportion and mixed.
- The prepared bramhrasayana was stored in a well closed container

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Physiochemical Evaluation:

- Physiochemical studies
- Loss on drying
- pH values
- Total solids
- Total soluble solids
- Total alkaloids
- Total fat
- Total sugar

Pharmacological activities

Swiss albino mice (20-25 g) and wistar rats (125-200 g) of either sex and of approximate 9-12 week old, used in the present studies were procured from Institutional of Health and Biological, Mhow (M.P.). The experimental protocol approved by Institutional animal ethics committee (reg. no. TIT/IAEC/831/P'cog/2010/08*).*

Anti diabetic Activity

Requirement:

- Alloxan (150 mg/kg i.p).
- Lower dose of bramhrasayana (100 mg/kg)
- High dose of bramhrasayana (200 mg/kg)
- Glibenclamide (10 mg/kg)

Experimental Design

The animals were divided into five groups of six rats.

Group I (Normal): treated with 0.3% CMC solution (0.5 ml/100 g), orally.
Group II (Control): treated with alloxan (150 mg/kg, i.p).
Group III (Standard); treated with alloxan (150 mg/kg, i.p.) + glibenclamide (10 mg/kg, p.o.).
Group IV (Test group A): treated with alloxan (150 mg/kg, i.p.) + bramhrasayana (100 mg/kg, p.o.).
Group V (Test group B): treated with alloxan (150 mg/kg, i.p.) + bramhrasayana (200 mg/kg, p.o.).
Blood samples were collected for the measurement of blood glucose level from the tail vein on initial, 1h, 2h, 4h, 5h and 7^{th} day. The blood glucose level was determined by digital glucometer (ON CALL EZ).The values were compared with the control group.

Hepatoprotective Activity
Requirement:

- Paracetamol 500 mg/kg.
- Lower dose of bramhrasayana (100 mg/kg)
- High dose of bramhrasayana (200 mg/kg)
- Liv-52 (standard drug 2 ml/100gm)

Experimental Design

The rats were randomly divided into five groups of six rats;

Group I (Normal); rats were treated with 0.3% CMC
Group II(Control); rats were treated with paracetamol ones (500 mg /kg).
Group III (Standard); rats were treated with paracetamol (500 mg/kg) + liv-52 (2 ml/100gm)
Group IV (Test group A); rats were treated with paracetamol (500 mg/kg) orally +. lower dose of bramhrasayana (100 mg/kg)
Group V (Test group B); rats were treated with paracetamol (500 mg/kg) orally + higher dose of bramhrasayana,(200 mg/kg)

On the 5th day animal were anaesthetized with light ether anaesthesia. Blood was withdrawn directly from heart with sterile syringe and collected in sterilized vial for serum separation and analysis. After collecting the blood, vials were kept at room temperature for 2h for blood coagulation and serum was separated out by centrifugation at 2500 rpm.

Immunomodulatory Activity Requirement:

- Carbon suspension (0.1 ml)
- Low dose of bramhrasayana (100 mg/kg)
- High dose of bramhrasayana (200 mg/kg)
- Acetic acid (0.1% 2 ml)

Experimental design

The rats were divided into three groups comprising of six rats.

Grouping and treatment protocol

- **Group I (Control);** rats were treated with 0.3% CMC.
- **Group II (Test group A)**; rats were treated with lower dose of bramhrasayana,
- (100 mg/kg) orally.
- **Group.III (Test group-B);** rats were treated with higher dose of bramhrasayana,
  (200 mg/kg) orally.

Results and Discussion

Physicochemical studies

Physicochemical parameters were determined for bramhrasayana and results showed total loss on drying 15%, total solid 45%, total soluble solid 28.66%, total alkaloid 3.5%, pH value 6.5, total fat 75% and sugar 55%..

Acute toxicity study

The bramhrasayana were screened for acute toxicity study by OECD guideline for the determination the LD_{50} value. Hence, we selected the effective dose 100 and 200 mg/kg for antidiabetic, hepatoprotective and immunomodulatory activity.

Group	No. of Animal	Dose (mg/kg)	Result
1.	5 Animal	2000	4 death
2.	4 Animal	300	2 death
3.	3 Animal	300	0 death

Table.3 Acute toxicity study

Anti diabetic activity:

Effect of bramhrasayana on blood glucose level of alloxan induced diabetes rats after 7 days of treatment in rat mg/dl

Group	Treatments	Blood glucose level (mg/dl)					
		0h	1h	2h	4h	5h	7th day
Normal	0.3% CMC solution	71.16 ±1.53	77.16 ±1.62	79.26± 1.511	80.10± 1.52	78.0±1. 52	76.22±1.56
Control	Alloxan (150mg/kg)	172.0 ±2.67	190.8 3±2.7 2	191.33 ±2.74	192.0± 2.47	200.16 ±2.52	205.33±2.27
Standar d	Glibenclamide (10mg/kg)	184.5 ±3.19	180.0 ±3.02* *	182.33 ±2.95**	155.16 ±2.73**	142.33 ±2.66**	135.66±2.98**
Test A	Bramhrasayan a (100 mg/kg)	183.1 6±2.9	173.1 6±2.9*	171.66 ±2.98*	170.16 ±3.1*	168.16 ±3.2*	159.16±3.0*
Test B	Bramhrasayan a (200 mg/kg)	187.6 6±3.2	177.8 ±2.9**	161.83 ±1.42**	159.33 ±1.0**	156.33 ±0.76**	153.16±1.74**

Table.4 Anti diabetic activity

Effects of bramhrasayana on SU, S. critinine, SC, ST, HDL and LDL in alloxan induced diabetic rats

Group	Treatments	SU	S.critinine	SC	ST	HDL	LDL
Normal	0.3% CMC solution	21.4 ± 0.34	0.80 ±0.006	71.90 ±0.17	31.40 ± 0.21	27.50± 0.22	20.2 ±0.15
Control	Alloxan (150mg/kg)	130.5 ±0.18	1.73 ±0.16	121.6 ±0.21	164.3 ± 0.43	11.10 ±0.22	56.6 ±0.23
Standard	Glibenclamide (10mg/kg)	23.33 ±0.12**	0.83 ±0.08**	71.33 ±0.38**	32.50 ± 0.69**	15.33 ±0.15**	23.16 ± 0.32**
Test A	Bramhrasayana (100 mg/kg)	32.16 ±0.19**	1.53 ±0.05**	86.50 ±0.11**	80.50 ±0.42**	15.16 ±0.31**	32.33 ±0.26**
Test B	Bramh rasayana (200 mg/kg)	31.36 ±0.14**	1.40 ±0.04*	82.25 ±0.12**	78.67 ±0.2**	14.25 ±0.25**	25.14 ±0.14**

Table.5 Effects of bramhrasayana on SU, S. critinine, SC, ST, HDL and LDL inAlloxan induced diabetic rats:

Group I (Normal): Islets with normal round and elongated structural intactness with their nucleus.
Group II (Control): Theislets damaged and shrunken in size with infiltration of lymphocytes.
Group III (Standard): The islet shows depletion of cells. There is milled infiltrate of lymphocyte at some foci.
Group IV(Test A 100 mg/kg): The architecture is partially effaced, the islet and acinar cells are normal..
Group V(Test B 200 mg/kg): The islets shows depletion of the acinar cells and the acinar cells shows moderate cytoplasm, round to oval nuclei. There is no evidence of inflammation.

Hepatoprotective study:

Effect of bramhrasayana on the serum transminase (SGOT and SGPT), ALP, TB, TC and TP in paracetamol induced hepatic damage in rats

Group	Treatments	SGOT	SGPT	ALP	TB	TC	TP
1	0.5% CMC (0.2 ml/100g)	32.34 ± 0.26	28.84 ± 0.23	70.60 ± 0.23	0.93 ± 0.02	72.89 ± 0.31	5.37 ± 0.12
2	Paracetamol (500 mg/kg, i.p.)	115.86 ± 0.18	113.26 ± 0.24	130.50 ± 0.31	6.12 ± 0.08	160. ± 0.30	3.6 ± 0.03
3	Paracetamol (500 mg/kg, i.p.) + LIV 52 (2ml/kg)	40.33 ± 0.16**	38.26 ± 0.26**	90.43 ± 0.27**	4.16 ± 0.06**	99.75 ± 0.19**	4.8 ± 0.17**
4	Paracetamol (500 mg/kg, i.p.) + Bramhrasayana (100 mg/kg)	59.89 ± 0.18**	62.33 ± 0.27**	103.5 ± 0.22**	5.86 ± 0.12**	109.85 ± 0.23**	3.50 ± 0.08**
5	Paracetamol (500 mg/kg, i.p.) + Bramhrasayana (200 mg/kg)	51.89 ± 0.20**	52.19 ± 0.27**	95.33 ± 0.19**	4.60 ± 0.09**	105.5 ± 0.13**	3.0 ± 0.11**

Table.6 Hepatoprotective study

Group I (Normal): liver from rat treated with saline shows normal cellular architecture with distinct hepatic cells, sinusoidal space and a central vein.

Group II (Control): liver from rat treated with paracetamol exhibited severe hepatocyte degeneration and necrosis.

Group III (Standard): liver treated with Liv-52 shows normal architecture with mild hepatocyte degeneration.

Group IV (Test A 100 mg/kg): Liver treated with Bramhrasayana (100 mg/kg) showed normal hepatocytes with mild inflammation of portal triad.

Group V (Test B 200 mg/kg): Liver treated with Bramhrasayana (200 mg/kg) showed micro fatty changes with a dense collection of lymphoid cells suggesting evidence of very little necrosis or degeneration.

Immunomodulatory activity:

Effect of bramhrasayana on nonspecific and specific immune system

Time interval	Control	Test A (100 mg/kg)	Test B (200 mg/kg)
0 min	1.45±0.14	1.55±0.10	1.40±0.11
4 min	1.58±0.20	1.61±0.14**	1.60±.010**
8 min	1.62±0.16	1.72±0.14**	1.70±0.12**
12 min	1.80±0.12	1.89±0.11**	1.91±0.10**
16 min	1.64±0.17	1.71±0.09**	1.83±0.10**

Table.7 Immunomodulatory activity

Phagocytic index:

Time interval	Control	Test A (100 mg/kg)	Test B (200 mg/kg)
0 min	1	1.01±.0.01	1.05±0.04
4 min	1	1.00±0.01*	1.01±0.01**
8 min	1	1.00±0.01*	1.02±0.01**
12 min	1	1.02±0.02*	1.04±0.04**
16 min	1	1.00±0.01*	1.02±0.02**

Table.8 Phagocytic index

HPLC Study:

No of Sample	Name of plants	RT	Prepared bramhrasayana	Marketed chyawanprash
Sample no. 1	Pippali	1.52	P	P
Sample no. 2	Shatavri	1.61	P	P
Sample no. 3	Punaranava	1.41	P	A
Sample no. 4	Kalaagara	1.62	A	A
Sample no. 5	Bala	1.44	P	P
Sample no. 6	Mandukparin	1.51	P	A
Sample no. 7	Malabra nut	1.62	A	P
Sample no. 8	Bhumyamalaki	1.53	P	A
Sample no. 9	Sarivan	1.61	P	P
Sample no. 10	Madhuca	1.61	A	A
Sample no. 11	Jivanti	1.61	A	A
Sample no. 12	Shankhpuship	1.55	P	P

Sample no. 13	**Mulathi**	**1.61**	**P**	**P**
Sample no. 14	Red chandan	1.62	P	A
Sample no. 15	Kamalkeshar	1.45	P	A
Sample no. 16	Gokharu	1.45	P	P
Sample no. 17	Neelkamal	1.44	P	A
Sample no. 18	Bilva	1.44	P	P
Sample no. 19	Pankaja	1.45	P	P
Sample no. 20	Cardamom	1.40	P	P
Sample no. 21	Swarna bhasma	-	P	A
Sample no. 22	Raupya bhasma	-	P	P
Sample no. 23	Loh bhasma	-	P	P
Sample no. 24	Tamra bhasma	-	P	A

Table 9- Validation of formulated bramhrasayana with respect to marketed chyawanprash by HPLC (P= Present, A=Absent)

Conclusion

Bramhrasayana traditional polyherbal formulation, which widely used as tonic, rejuvenator, anabolic, immunomodulator and memory enhancer.Bramhrasayana manifests the entire human quest for immortality, freedom from disease and prevention of aging. In conclusion, we can confirm that the formulation Bramhrasayana showed the better antidiabetes and antihepatotoxicity and act better immunomodulator and memory enhancer as compared to the chyawanprash. On the basis of study data, it can be concluded that Polyherbal formulation Bramhrasayana has promising antidiabetes and antihepatotoxicity and act better. immunomodulator and memory enhancer. It can be employed as safe and effective treatment for hepato-toxicity or liver damage.

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