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Formulation and Pharmacological Evaluation of Bramhrasayana



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ABSTRACT

Herbal medicine sometimes referred to as Botanical Medicine, is the use of herbs for their therapeutic or medicinal value. Nearly 1 in 5 adults in the United States report taking an herbal product. Written records of the use of herbal medicine date back more than 5,000 years. In fact, for most of history, herbal medicine was the only medicine. Even from 1890, 59% of the listings in the US Pharmacopeia were from herbal products, and it has been estimated that as many as one third to one half of currently used drugs were originally derived from plant. 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. An herb is a plant or plant part valued for its medicinal, aromatic or savory qualities. 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. The use of traditional medicine and medicinal plants in most developing countries, as a normative basis for the maintenance of good health, has been widely observed (UNESCO, 1996). Bramhrasayana is a traditional polyherbal formulation, which widely used as tonic, antidiabetes, hepatotoxicity, immunomodulator and memory enhancer. Bramhrasayana contains the pulp of *Emblica officinalis* as the prime ingredient, along with powders and extracts of several other herbs. In the present study the research is to find out the antidiabetic, hepatoprotective, immunomodulatory response of the formulated bramhrasayana (*in vivo*).

INTRODUCTION

A number of medicinal plants, traditionally used for over 1000 years named Rasayana are present in herbal preparations of Indian traditional health care systems. Herbalists treat many conditions such as asthma, eczema, premenstrual syndrome, rheumatoid arthritis, migraine, menopausal symptoms, chronic fatigue, and irritable bowel syndrome, among others. Herbal preparations are best taken under the guidance of a trained professional. Be sure to consult with your doctor or an herbalist before self-treating¹.

Some common herbs and their uses are discussed below. Please see our monographs on individual herbs for detailed descriptions of uses as well as risks, side effects, and potential interactions. Herbal therapy for diabetes, hepatotoxicity, immunomodulator been followed all over the World successfully. Herbs are used to manage Type 1 and Type II diabetes, 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^{2,3}.

Scientific validation of several Indian plant species has proved the efficacy of the botanicals in reducing the toxic level could be considered as of possible therapeutic value. Thus many different plants have been used individually or in formulations for treatment of diabetes, Hepatotoxicity etc. Chyavanaprasha has potent restorative and tonic activity and used in weakness, and emaciation. Various trials have shown its rejuvenating, adaptogenic, cardioprotective and neuroprotective activities. It also delays the sickness and maintains well-being after various radiation exposures. In *Ayurveda*, a number of herbal formulations and dietary schedules have been especially advocated for the elderly in the hope of rejuvenating them and preventing the many problems that come with advancing age^{4,5}.

The last few years have witnessed a general revival of interest in such herbal drugs. Bramhrasayana, a polyherbal formulation is an ideal tonic to solve the problems associated with diabetes, Hepatotoxicity and act as immunomodulator and memory enhancer. Being a nonhormonal preparation, Bramhrasayana can be used safely. Its efficacy can be attributed to the synergistic actions of the individual herbs. Bramhrasayana is a traditional polyherbal formulation, which widely used as tonic, antidiabetes, Hepatotoxicity, immunomodulator and

memory enhancer. Bramhrasayana contains the pulp of *Emblica officinalis* as the prime ingredient, along with powders and extracts of several other herbs⁶.

Aim and Objective:

The aim of the research is to find out the antidiabetic,hepatoprotective,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.
- Hepatoprotective activity of bramhrasayana in paracetamol induced liver toxicity in rats.
- Estimation of biochemical parameters (SGOT, SGPT, ALP, TB, TC TP) in paracetamol and bramhrasayana treated rats.
- To study the histopathological changes in liver of paracetamol induced and formulated bramhrasayana treated rats.
- To study the histopathological changes in pancreas of normal and alloxan induced diabetic and formulated bramhrasayana treated rats.
- Estimation of immunomodulator activity of bramhrasayana on rats.

MATERIALS AND METHODS

Table.1 Ingredients used in Bramhrasayana

S.No	Common name	Quantity
1	Amla	600 pieces
2	Pippali	6g
3	Honey	750g
4	Punaranava	6g
5	Shatavri	6g

6	Bala	6g
7	Mandukparin	6g
8	Kalaagra	6g
9	Malabar nut	6g
10	Bhumyamalaki	6g
11	Sarivan	6g
12	Madhuca	6g
13	Jivanti	6g
14	Shabkhpuship	6g
15	Mulathi	6g
16	Red chandan	6g
17	Kamalkeshar	6g
18	Gokharu	6g
19	Neelkamal	6g
20	Bilva	6g
21	Pankaja	6g
22	Cardamom	6g

List of Bhasma:

Table.2 List of Bhasma

S.No.	Name of bhasma	Quantity
1	Swarna bhasma	125mg
2	Raupya bhasma	1g
3	Loh bhasma	5g
4	Tamra bhasma	5g

Method:

600 amla were taken.



Out 600,300 alma were taken for stem boil with milk. The distance between amla and milk was maintained 3-4 inches.



Remaining part of amla was dried in shade and then grinded in mixer to obtained amorphous form.



Then seeds were separated from amla fruit dried in shade and powdered.



Remaining 300 amla were 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 Cardemom were taken in equal proportion, grinded and mixed with amla powder.



The amount of all drug powder should be 8th part of total amla powder.



The total mixture of drug powder along with amla powder was mixed with juice of *Grewia hieseta* 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 inner 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 Swarna bhasma, Loh bhasma, Raupya bhasma and Tamra bhasma 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.

Phytochemical Evaluation:

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

Pharmacological activities:

• **Procurement of experimental animals;** Swiss albino mice (20-25 g) and 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.).

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, 1 h, 2 h, 4 h, 5 h and 7th 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)

Collection of blood

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.

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

- **Physicochemical studies;** Physicochemical parameters were determined for bramhrasayana and results showed total loss on drying 18%, total solid 40%, total soluble solid 26.66%, total alkaloid 2%, pH value 6.5, total fat 79% and sugar 60%.
- **Acute toxicity study;** The bramhrasayana were screened for acute toxicity study by OECD guideline for the determination the LD₅₀ value, the effective dose 100 and 200 mg/kg for antidiabetic, hepatoprotective and immunomodulatory activity.

Table.3 Acute toxicity study

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

Anti diabetic activity:

Effect of bramhrasayana on blood glucose level.

Table.4 Anti diabetic activity

Group	Treatments	0h	1h	2h	4h	5h	7 th day
Normal	0.3% CMC solution	75.16±1.53	75.16±1.62	76.26±1.51	76.10±1.52	77.0±1.52	73.22±1.56
Control	Alloxan (150mg/kg)	177.0±2.67	185.83±2.72	186.33±2.7	193.0±2.47	199.16±2.52	204.33±2.27
Standard	Glibenclamide (10mg/kg)	188.5±3.19	182.0±3.02**	162.33±2.95**	155.16±2.73**	141.33±2.66**	130.66±2.98**

Test A	Bramhrasayana (100 mg/kg)	180.16±2.9	177.16± 2.9*	173.66± 2.98*	168.16± 3.1*	166.16± 3.2*	157.16± 3.0*
Test B	Bramhrasayana (200 mg/kg)	182.66±3.2	173.8± 2.9**	164.83± 1.42**	160.33± 1.0**	157.33± 0.76**	146.16± 1.74**

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

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

Group	Treatment	SU	S. critinine	SC	ST	HDL	LDL
Normal	0.3% CMC solution	23.4 ± 0.34	0.85 ±0.006	73.90 ±0.17	34.40± 0.21	28.50 ±0.22	22.2 ±0.15
Control	Alloxan (150mg /kg)	132.5 ±0.18	1.76 ±0.16	124.6 ±0.21	168.3± 0.43	10.10 ±0.22	58.6 ±0.23
Standard	Glibenclami de (10mg/kg)	26.33 ±0.12**	0.88 ±0.08**	75.33 ±0.38**	34.50± 0.69**	17.33 ±0.15**	26.1± 0.32**
Test A	Bramhrasay ana (100 mg/kg)	34.16 ±0.19**	1.56 ±0.05**	89.50 ±0.11**	82.50 ±0.42**	17.16 ±0.31**	30.33 ±0.26**
Test B	Bramhrasay ana (200 mg/kg)	33.36 ±0.14**	1.43 ±0.04*	84.25 ±0.12**	80.67 ±0.2**	16.25 ±0.25**	28.14 ±0.14**

- Group I (Normal):** Islets with normal round and elongated structural intactness with their nucleus.
- Group II (Control):** The islets damaged and shrunken in size with infiltration of lymphocytes.

3. **Group III (Standard):** The islet shows depletion of cells. There is milled infiltrate of lymphocyte at some foci.

4. **Group IV (Test A 100 mg/kg):** The architecture is partially effaced, the islet and acinar cells are normal..

5. **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 transaminase (SGOT and SGPT), ALP, TB, TC and TP in paracetamol induced hepatic damage in rats.

Table.6 Hepatoprotective study

Grou p	Treatment	SGOT	SGPT	ALP	TB	TC	TP
1.	0.5% CMC (0.2 ml/100g)	30.34 ± 0.26	29.84 ± 0.23	68.60 ± 0.23	0.89 ± 0.02	61.89 ± 0.31	6.37 ± 0.12
2.	Paracetamol (500 mg/kg, i.p.)	113.86 ± 0.18	118.26 ± 0.24	134.50 ± 0.31	5.12 ± 0.08	156. ± 0.30	2.6 ± 0.03
3.	Paracetamol(5 00 mg/kg, i.p.)+ LIV 52 (2ml/kg)	41.33 ± 0.16**	40.26 ± 0.26**	89.43 ± 0.27**	3.16 ± 0.06**	96.75 ± 0.19**	4.8 ± 0.17**
4.	Paracetamol(5 00 mg/kg, i.p.)+ Bramhrasayan a(100 mg/kg)	57.89 ± 0.18**	59.33 ± 0.27**	101.5 ± 0.22**	4.86 ± 0.12**	107.85 ± 0.23**	3.72 ± 0.08**
5.	Paracetamol(5 00 mg/kg, i.p.)+ Bramhrasayan a (200 mg/kg)	53.89 ± 0.20**	54.19 ± 0.27**	97.33 ± 0.19**	4.77 ± 0.09**	107.5 ± 0.13**	3.2 ± 0.11**

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

2. **Group II (Control):** liver from rat treated with paracetamol exhibited severe hepatocyte degeneration and necrosis.
3. **Group III (Standard):** liver treated with Liv-52 shows normal architecture with mild hepatocyte degeneration.
4. **Group IV (Test A 100 mg/kg):** Liver treated with Bramhrasayana (100 mg/kg) showed normal hepatocytes with mild inflammation of portal triad.
5. **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

Table.7 Immunomodulatory activity

Time interval	Control	Test A (100 mg/kg)	Test B (200 mg/kg)
0 min	1.40±0.14	1.50±0.10	1.45±0.11
4 min	1.61±0.20	1.64±0.14**	1.62±0.10**
8 min	1.68±0.16	1.74±0.14**	1.72±0.12**
12 min	1.82±0.12	1.93±0.11**	1.90±0.10**
16 min	1.67±0.17	1.75±0.09**	1.80±0.10**

- **Phagocytic index:**

Table.8 Phagocytic index

Time interval	Control	Test A (100 mg/kg)	Test B (200 mg/kg)
0 min	1	1.03±0.01	1.07±0.04
4 min	1	1.01±0.01*	1.03±0.01**
8 min	1	1.02±0.01*	1.03±0.01**
12 min	1	1.04±0.02*	1.06±0.04**
16 min	1	1.01±0.01*	1.04±0.02**

• HPLC:

Validation of formulated bramhrasayana with respect to marketed chyawanprash by HPLC.

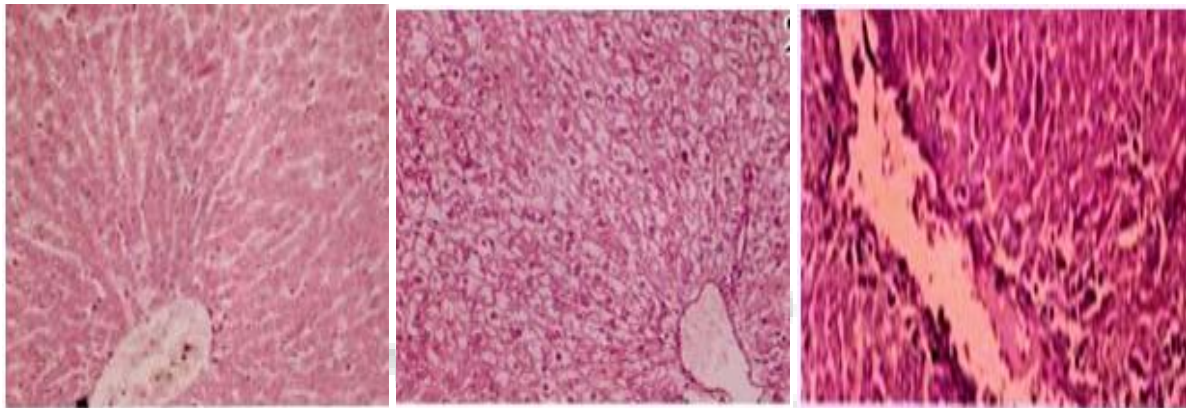
Table 9. HPLC

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

(P- present, A- Absent)

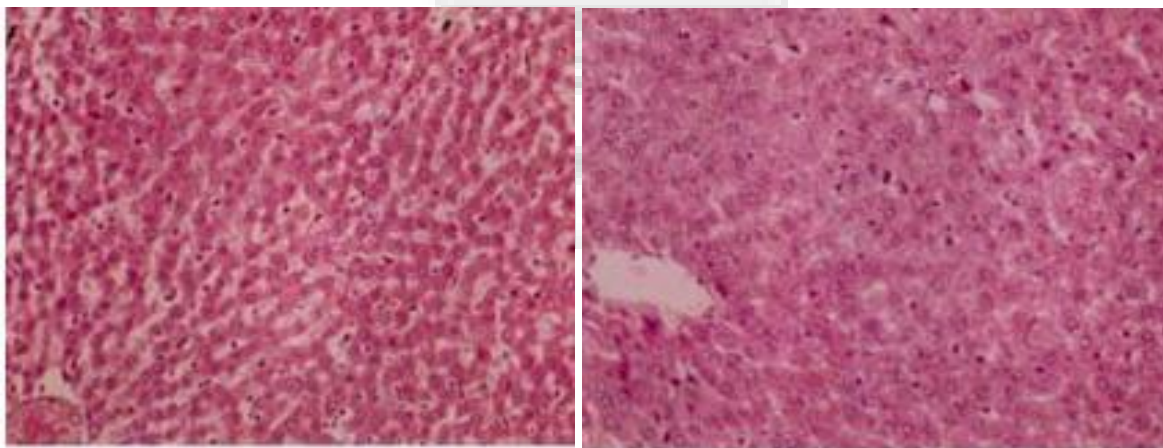
Histopathology of liver:



Normal

Control

Standard



Test A

Test B

- **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.

CONCLUSION

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 antidiabetic and hepatotoxicity 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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