

THERAPEUTIC PROSPECT OF PUNARNAVA KWATH: ANTI-INFLAMMATORY ACTIVITY BY EGG ALBUMIN DENATURATION TECHNIQUE

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ABSTRACT:

The science of healthy living is at the heart of Ayurveda, which is equally interested in the normal as it is in the abnormal or unhealthy. *Boerhaavia diffusa* Linn, also known as Punarnava, is a well-known herb in Ayurveda. The entire plant is used as medicine in traditional medicine. The herb has shown its importance in a number of formulations and as a single medicine. Most of the Ayurveda *Granthas* have described *Pancha- Kashaya Kalpana*, which are main preparation procedures. While reviewing this traditional knowledge of Ayurvedic medicine, one comes across a very important herb *Punarnava* (*Boerhavia diffusa*). It is an herb which is found throughout India of these *Kwatha* is in the middle i.e it is neither *Laghu* nor *guru*. In other words, it is *Madhyama* to digest or any patient can digest it readily due to its preparation method or *Agnimatra* given to it. *Kwatha* is prepared by not keeping lid on it otherwise it will become difficult to digest or heavy. All eight ingredients were used in equal amounts to make Punarnavashtaka Kwatha, whereas water was used sixteen times their combined amount. Heat was given until the solution remained 1/4th of the initial one. Considering these facts, *Punarnavashtaka Kwatha* was prepared. It is typically used to treat gout, wounds, skin conditions, kidney stones, joint inflammation, bronchitis, liver illness, hepatitis, and respiratory system disorders. Although this plant was highly helpful in the past, it is currently solely used as a medication of choice, but there is still room for research into this medication because it may be valuable in the future.

KEYWORDS:

Punarnavadi Kwath, *Boerhaavia diffusa*, Diclofenac, Protein Denaturation, Inflammatory.

1. INTRODUCTION:

As an alternative to single plant extracts, plant formulations of several plant components and their extracts are utilized as drugs in the traditional Indian medical system. These herbal remedies are used to treat a wide variety of illnesses. [1] A series of actions that take place in

reaction to harmful stimuli, infections, trauma, or injury to living tissues can be referred to as the inflammatory process.[2] Medicinal plants have been utilized to treat a wide range of illnesses, according to thousands of doctors' knowledge and traditional medical practices from different ethnic groups.[3] Punarnava can aid to revitalize the body's dying organs and restore dying cells.[4] According to Acharya Charka, a drug's effectiveness is dependent on its nature, habitat, administration, maintenance, and other factors. [5] Traditionally, medicinal plants have been utilized to treat pain-related conditions. [6] Enzyme activation, fluid extravasations, mediator release, cell migration, tissue breakdown, and healing mechanisms are some of the events that start inflammation.[7] The plant-based Indian medical system (Ayurveda) is very helpful in managing and controlling a variety of medical disorders. [8] Herbal medicines have been used to treat a wide range of ailments since ancient times. Medicinal plant use has been essential to world health. Even though modern medicine has advanced significantly in recent decades, plants still have a big impact on healthcare. Punarnava roughly translates to "renewer" or "bring back to life." This creeper dries out in the summer yet grows wild all year long in Brazil and India. These Kwatha are in the midway, meaning they are neither guru nor Laghu. In other words, because of the way it is prepared or the Agnimatra that is provided to it, it is Madhyama to digest or any patient may digest it easily. [10] The World Health Organization (WHO) defines herbal medicines as completed, labeled pharmaceutical products that comprise active compounds, aerial or underground plant parts, or other plant material or combinations.[11] India has very renal care facilities with little infrastructure and a few numbers of centers, all located in metropolitan cities. Patients are expected to visit the primary health centers run by the Indian government, which spends very little on health each year. Patients choose not to come because doing so costs them a day's pay. In India, the average person cannot afford this expensive medication, especially for CKD, an incurable illness. These expenses must be paid for the rest of one's life if one is still able to do so. The patient and his or her family face an intolerable burden as a result. The primary cause of the 2–3% treatment rate for kidney failure patients in India is this. To lessen the financial strain on their family, the rest would like to pass away early. [12] *Boerhavia diffusa* (BD), also known by its Punarnava Latin name, is a member of the *Nyctaginaceae* family and is commonly referred to as a spreading hogweed.[13] It is distinguished by its low-lying growth, perennial habit, pink blooms, and sticky fruits. The Sanskrit word for literary renewal or rejuvenation is punarnava, which is often used. Its well-known anti-aging properties are what give it this moniker. The punarnava plant is rasayana in traditional medicine because its various sections are rich in a

variety of bioactive chemicals. It is highly valued and used for its remarkable qualities, which include immune-boosting powers, youthfulness restoration, and improvement of physical and mental health [14]. The meaning of Punarnava (*Boerhaavia diffusa* Linn.) is "Punar jivati eti," or "one with rejuvenative property." The top of the plant dries up in the summer and regrows in the rainy season [15,16][17].

2. MATERIAL AND METHODS:

Collection of Raw Material:

The plant of Punarnava was procured from Local market shop near the Ichalkaranji. Punarnava prepared powder was used for decoction preparation.

Preparation of kwath:

Eight herbs Punarnava, Haritaki, Nimb, Daruharidra, Katuki, Patol, Guduchi, and Sunthi make up Punarnavastak Kwath. It was made according to the Sharangdhara Samhita, Madhyam Khand's description of kwath nirman vidhi. After mixing all the components in equal amounts, about 10 grams (1 tola) of each herb was taken to form a decoction in 160 milliliters (16 tola) of water, which was then cooked over moderate heat until it was reduced to 1/4 of that amount, or 40 milliliters [18,19].

Table 1: Ingredient of Punarnava kwath

Sr. No	Drugs	Scientific Name	Part	Quantity
1	Punarnava	<i>Boerhavia diffusa</i> .	1	20 gm
2	Nimb	<i>Azadirachta indica</i>	1	10gm
3	Patol	<i>Tricosanthes dioica</i> Roxb.	1	10gm
4	Shunthi	<i>Zingiber officinale</i>	1	10gm
5	Kutaki	<i>Picrorhiza kurroa</i>	1	10gm
6	Guduchi	<i>Tinospora cordifolia</i>	1	10gm
7	Deodar	<i>Cedrus deodara</i>	1	10gm
8	Haritaki	<i>Terminalia chebula</i>	1	10gm

Organoleptic Characteristics:

The color, taste, smell, and look of kwatha were noted in Table No. 2. [20]

Physicochemical Test:

Preliminary phytochemical analyses of the kwath were performed as described by Mekala P. And Kokate, C.K Seeking to highlight the major groups of secondary metabolites. It evaluated the presence of Carbohydrate, Alkaloids, Glycosides, steroids and sterols, phenols, Amino acid and proteins, flavonoids, Saponins, and Tannis.

Determination of pH:

The pH value was ascertained using a calibrated pH meter. One by one, the 10% solution of samples I through VII was added, and the reading was recorded. The average reading was reported after the test was conducted three times [21, 22].

Determination of Loss on Drying:

In a 100 ml beaker that had been previously weighed, 4 g of the material was heated for 5 hours at 105 °C. After chilling in a desiccator, the beaker was weighed. Up until two consistent observations, the process was repeated [21,22, 23]. The following formula was used to determine the sample's weight loss percentage.

$$\text{Percentage of loss on drying at } 105^{\circ}\text{C} = \frac{\text{ss in weight of the sample}}{\text{weight of the sample by taken}} \times 100$$

Determination of Total ash:

Burn two to three grams of the ground drug, precisely weighed, at a temperature of no more than 4500 degrees until it is carbon-free in a tared platinum or silica dish. Once it is cool, weigh it. Use hot water to put out the charred matter, collect the residue on ashless filter paper, burn the residue and filter paper together, add the filtrate, evaporate until dry, and ignite at a temperature no higher than 4500 if this procedure is unable to create carbon-free ash. Calculate the proportion of ash in relation to the air-dried medicine.[24]

$$\text{Percentage of Total ash} = \frac{\text{Weight of ash}}{\text{Weight of sample}} \times 100$$

Determination of Acid- insoluble Ash:

The dish containing the whole ash was heated slowly for five minutes before being filtered after 45 ml of 1:5 hydrochloric acid was added in three increments of 15 ml each. The residues were washed with distilled water until they were acid-free after the insoluble material was collected on Whatman no. 41 ash-less filter paper. After being returned to the original dish, the filter paper holding the insoluble stuff was dried and burned until its weight stayed constant [21, 22]. After cooling the dish in a desiccator, the percentage of acid-insoluble ash was calculated as follows:

$$\text{Percentage of Acid-insoluble ash} = \frac{\text{Weight of the Acid - insoluble residue}}{\text{Weight of sample}} \times 100$$

Weight of the sample

Determination of Water- soluble Extractive:

A glass stopper flask was filled with 4 g of the sample. After adding 100 millilitres of distilled water and shaking it periodically for six hours, it was let to stand for eighteen hours. The extract was quickly filtered so as not to lose any solvent. In a 100 ml beaker that had been previously weighed, 25 ml of the filtrate was pipetted out and dried by evaporation on a water bath. Additionally, the beaker was cooled in a desiccator, weighed, and heated to 105 °C for six hours in a hot air oven. Two iterations of the experiment produced an average value [21,22, 23]. The following formula was used to perform the computation.

$$\text{Percentage of water-soluble extractive} = \frac{\text{Weight of residue}}{\text{Weight of sample}} \times 100$$

Determination of Alcohol - soluble Extractive:

The process used to determine the water-soluble extractive value was repeated. Absolute alcohol (about 95%) was used in place of water to make the transformation [21,22,23].

$$\text{Alcohol Soluble Extractive} = \frac{\text{Weight of residue}}{\text{Weight of Sample}} \times 100$$

IN VITRO METHOD

Assessment of Anti-Inflammatory Activity *in Vitro* Using the Egg Albumin Denaturation Technique

Principle:

Inflammation is associated with protein denaturation [25]. A substance's potential anti-inflammatory action is demonstrated by its capacity to prevent protein denaturation. The *in-vitro* egg albumin denaturation method is used to screen for anti-inflammatory qualities using a fresh hen's egg. Egg whites contain the proteins ovalbumin, clustering, ovoinhibitor, overmastering, and prostaglandin D2 synthase. This method evaluates the capacity to inhibit the denaturation of the egg protein (albumin) using the NSAID diclofenac sodium. Furthermore, the anti-inflammatory strength of the plant extract is assessed and assumed to be an NSAID. Protein denaturation through heating is used to screen for the decrease in prostaglandin activity. The percentage inhibition of egg albumin denaturation [26] shows this. The capacity to reduce inflammation increases with the percentage of denaturation inhibition [27].

Method:

Standard concentrations of 1000, 500 and 250 µg/mL were generated. All diclofenac sodium concentration series were made identically, and diclofenac sodium was used as a positive control. Phosphate buffer saline (PBS, pH 6.4) was made by dissolving 28.80 g of the disodium salt of hydrogen phosphate and 11.45 g of the dipotassium salt of hydrogen phosphate in 1000 mL of distilled water [28]. The identified test samples were prepared by adding 2.8 millilitres of PBS, 0.2 millilitres of egg albumin, and 0.2 millilitres of plant extract. Test samples for the reference medication diclofenac sodium were made with 2.8 millilitres of PBS, 0.2 millilitres of egg albumin, and 0.2 millilitres of diclofenac sodium. Two millilitres of distilled water, 2.8 millilitres of PBS, and 0.2 millilitres of egg albumin were used to create a control sample. All of the produced samples were heated to 70 °C for five minutes after being incubated for 15 to 20 minutes at 37 °C in a water bath using a laboratory shaking water bath. Let the samples cool at room temperature for ten minutes. At 660 nm, absorbances were measured with the Double Beam UV-Visible spectrophotometer in comparison to a blank. The entire test process was carried out three times. The following calculation was used to calculate the percentage inhibition of egg albumin denaturation [28].

$$\% \text{ Inhibition of egg albumin denaturation} = \frac{\text{Absorbance of the control} - \text{Absorbance of the Standard}}{\text{Absorbance of the control}} \times 100$$

Preparation of 1% of egg albumin solution:

A 1% solution of egg albumin can be made from fresh hen eggs or easily purchased egg albumin powder. A fresh hen's egg should be carefully cracked before 1 mL of the translucent portion is added to 100 mL of w/v distilled water and thoroughly stirred to create an egg-albumin solution. Egg albumin is the transparent part of the egg. When creating the solution, the water should be cold. When water is brought to a boil, it will coagulate.

Evaluation of *In-Vivo* Anti-Arthritic Study

Initially weighing between 150 and 210g, adult Wistar albino rats of either sex were taken after the approval of IAEC committee. All of the animals were kept in a group of six in a temperature (22±2°C) and humidity (50-60%) controlled room. 12-hour light/dark cycle maintained, and

standard pellet food and water was given. They were divided into six groups after a week of acclimatisation.

RESULTS:

Evaluation Test:

- **Organoleptic test:**

Table 2: Organoleptic Characteristics

Sr. No	Parameters	Result
1	Appearance	Coarse powder
2	Colour	Dust with a dark Brownwood
3	Odour	Qualities
4	Taste	Bitter
5	Touch	Gentle

- **Physicochemical Test:**

Table 3: Physicochemical Test

Sr. No		Test	Result
1		pH	3-4
2		Loss on drying	3
3		Total Ash	21.66
	i)	Acid insoluble ash	70.7
	ii)	Water soluble ash	68
4		Water soluble extract	28
5		Alcohol soluble extract	16.5

- **Phytochemical Test:**

Table 4: Phytochemical Test

Sr.no	Test	Present/Absent (+/-)
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1	Carbohydrate	-
2	Protein	-
3	Alkaloid	+
4	Cardiac glycoside	+
5	Flavonoids	+
6	Tannins	+
7	Steroids	-

IN VITRO METHOD:

Egg Albumin Denaturation Technique:

Table 5: Result of Protein Denaturation Assay

Sr. No	Sample	Concentration (µg/ml)	Absorbance (Mean ±SD)	% Inhibition
	control		-	-
1	Standard Drug (Diclofenac)	250	0.515±0.016	54.01
		500	0.473±0.032	57.76
		1000	0.414±0.007	63
2	Test (Punarnava Kwath)	250	0.653±0.001	41.70
		500	0.578±0.011	48.39
		1000	0.482±0.002	56.96

Data are expressed in Mean±SD (N=3) using ANOVA followed by T – Test comparison between Diclofenac Sodium and Punarnava Kwath. $P < 0.0001$ the % inhibition of Punarnava kwath was lower than the standard drug.

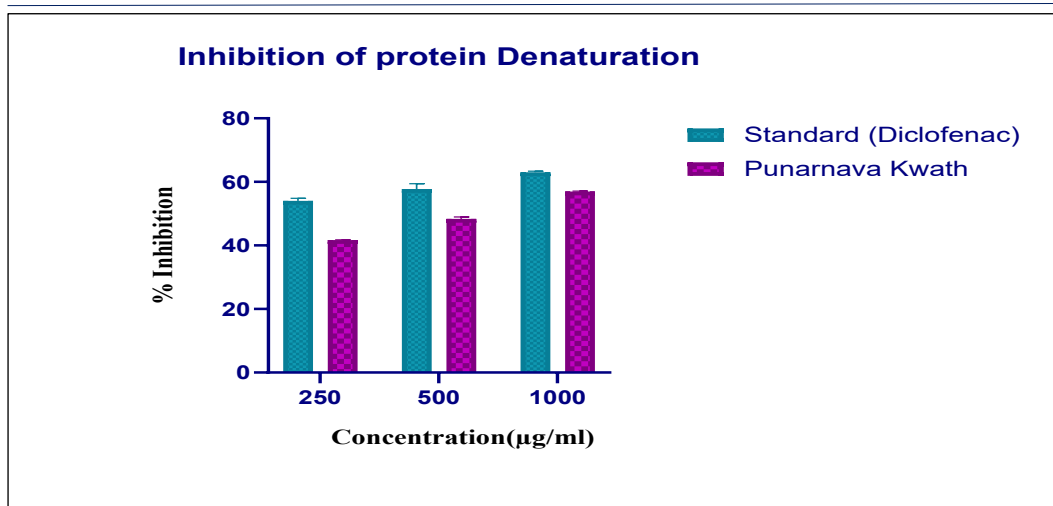


Fig.1: Graphical representation of Protein Denaturation Assay

DISCUSSION:

Inflammation is a complicated biological reaction of vascular tissues to potentially damaging stimuli. It is also the organism's protective attempt to eliminate harmful stimuli and start the healing process [29]. Punarnava kwath is an Ayurvedic herbal decoction made from the Punarnava plant. It is beneficial for Diuretic activity, Anti-Inflammatory activity and Anti-oxidant activity. The principal objective behind the egg albumin denaturation assay is to determine whether agents or compounds can stop or hinder egg albumin from becoming denatured under particular circumstances. Denaturation is the term used to describe how a protein changes in structure and loses its biological activity [30]. The method of Preparation of kwath is by Decoction. The Phytochemical constituent is present such as Flavonoid and Phenolic. In this present study Punarnava kwath inhibit Protein Denaturation by using Egg Albumin in a dose dependent manner and showed maximum inhibitory effect at 1000 µg/ml. The % inhibition Diclofenac Sodium which was used as standard drug for reference purpose showed 63% inhibition of Protein Denaturation where as kwath shows 56.96 % of Protein Denaturation at the same highest concentration taken in *In Vitro* study. The presence of Flavonoid and Phenolic content in Punarnava kwath might be the reason for Protein Denaturation inhibition to prevent a damage and destruction of Cartilage.

CONCLUSION

On the basis of present investigation, we conclude that Punarnava kwath Significantly decreased Protein Denaturation of Inflammatory condition. The Phytochemical such as Flavonoid and phenolic are major component in the kwath they are might to be responsible behind the Anti-Inflammatory effect.

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