Evaluation of anti-inflammatory and anti oxidant activity of a poly herbal formulation - *Chyavana* drink

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Abstract :

The study was designed to evaluate the anti-inflammatory and antioxidant activity of a poly herbal formulation-*Chyavana* drink. The inflammation was induced in albino rats by carrageenan. The formulation was effective when compared with a known anti-inflammatory drug. The antioxidant activity was studied by invitro methods. The DPPH radical scavenging activity was found to be showing higher percentage of inhibition and reducing power at high concentrations. The phenolic content of Chyavana drink was observed to be high showing a good antioxidant effect.

Key words : Carrageenan, Anti-inflammation, Chyavana drink, DPPH., PMNL.

Introduction :

India harbors a rich diversity of valuable medicinal plants[1]. Use of plant based drugs and chemicals for curing various ailments and personal adornment is as old as human civilization. The WHO estimates that 80% of the world population presently uses herbal medicine for some aspect of primary healthcare. Herbal formulations play a major role in the treatment of various diseases than a single herb, because other herbs can act as a booster to the main herb as well as enhancing bioavailability[2]. The use of natural remedies for the treatment of inflammatory disease has a long history. Plant drugs are known to play a vital role in the management of inflammatory diseases. Rheumatoid arthritis is a chronic progressive inflammatory disease affecting the synovium, cartilage and bone. The immune complexes in the synovial fluid activate the complete system, attracting polymorphonuclear leucocytes into the joint space. Phagocytosis of the complex triggers the release of PMNL, lysosomal enzymes and free radicals into the joint space, which may cause damage[3]. Free radicals and oxidant can trigger per oxidation as well as the oxidation of protein and DNA causing extensive damage to the body cells[4]. Antioxidants protect cells against the damaging effect of ROS[5, 6]. The plant extract acts as antioxidant by scavenging reactive oxygen species and to effectively prevent all cellular damage[7].The present study involves the use of a herbal formulation "Chyavana drink" for the evaluation of anti-inflammatory and anti-oxidant activity.

Materials and Methods :

The present study was undertaken to evaluate the anti-inflammatory activity, antioxidant activity and phytochemical

analysis of an herbal formulation- *Chyavana drink.*

Selection of animal :

Adult female albino rats weighing between 180-210 gm were obtained from the small animals breeding station, Kerala Agricultural University, Mannuthy. They were acclimatized to laboratory conditions for 10 days before commencement of experiment.

Induction of inflammation:

Inflammation was induced by the sub carrageenan into the right hind paw of the experimental rats.

Preparation of *Chyavana drink*:

C*hyavana drink*, an herbal formulation consisting of 39 different medicinal plants, used for this study was obtained from *Lok Swasthya Parampura Samvardhan Samithi* (LSPSS), Ramanat hapuram, Coimbatore.

The herbal powder (2g) decoction was prepared by continuous stirring. The decoction was administered orally to the rats.

Preparation of different extracts:

The petroleum ether, chloroform, ethyl acetate and ethanol extracts of *Chyavana drink* were prepared.

Anti-inflammatory study:

Experimental rats were divided into four groups of four animals each. The treatment schedule is described as follows:

Group 1 : Control group: (0.2 ml of 3% carrageenan induced in the right hind paw).

Group II : Reference group: 0.2ml of 3% carrageenan induced in the right hind paw and after 24 hours a single dose of 1ml of Diclofenac sodium given orally for 9 days.

Group III : Treatment group: 0.2ml of 3% carrageenan injected in the right hind paw After 24 hours a double dose of 2mg of Chyavana drink administered for 9 days.

Group IV: Normal control group: Animals received a double dose of 2mg of Chyavana drink for 9 days.

The hind paw thickness was measured by using vernier calipers after carrageenan injection daily for 9 days.

Anti-oxidant assay :

The sample was subjected to the following antioxidant assays,

1) Test for DPPH Radical Scavenging activity and IC50 calculated.
2) Test for reducing power
3) Phenolic estimation.

Phyto chemical screening test :

The following phytochemical screening tests such as test for alkaloids, steroids, sterols, flavanoids, saponins, tannins, phenolic compounds, terpenoids, glycosides, carbohydrates were done.

Results and Discussion :

Anti inflammatory activity (in vivo method) :

In the present study, *Chyavana drink* showed a potent anti-inflammatory activity as shown in graph (1). From the graph it is evident that the carrageenan significantly developed the formation of edema in-group I, II and III animals compared to normal

control (group IV) indicated inflammation. In the present study, the simultaneous treatment (group III) of poly herbal formulation (*Chyavana drink*) with the phlogistic agent (0.435± 0.020) showed a significant decline in the edema formed when compared with carrageenan control group (0.66± 0.0081). On the other hand, there was a similar action of treatment group (0.435±0.020) is compared with the standard reference group (0.44±0.010).

Anti oxidant activity :

The evaluation of antioxidant activity of the poly herbal formulation – *Chyavana drink* was done by using invitro methods.

1) DPPH Radical scavenging activity:

Reduction of the DPPH radicals can be observed by the decrease in absorbance at 517nm is shown in table 2. From table 2, it is clear that DPPH radical is scavenged at a high percent by the poly herbal formulation. The scavenging capacity of the herbal formulation *Chyavana drink* was found to be 94% at 1000µg /ml (table 2) with the IC50 being 38.83±1.258. It shows that the poly herbal formulation has higher antioxidant activity.

2) Reducing power:

The reducing power of poly herbal formulation *Chyavana drink* is shown in table3. The table shows that at a concentration of 1000µg/ml relatively high reducing power was observed. The reducing power of poly herbal extract was found to be 0.0836±0.0057 at low concentration of 31.25µg/ml and 11.33±0.057 at high concentration 1000µg/ml. It reveals that the reducing power increases by increasing the concentration of herbal formulation.

3) Phenolic estimation:

Total soluble phenolic constituents of the ethanol extract of herbal formulation chyavana drink are shown in table 4. Presence of phenolics scavenges free radical formation and prevents age related diseases.

Phytochemical studies :

The results of phytochemical screening are tabulated in table 5.

The petroleum ether extract of herbal formulation shows the presence of steroids, cardio glycosides, saponins, and oil. All the other compounds show negative result.

The chloroform extract shows the presence of Alkaloids, steroids, aminoacids, cardio glycosides, and oil.

The ethyl acetate extract shows positive results for steroids, flavonoids, carbohydrates, cardio glycosides and oil.

The ethanol extract shows the presence of Alkaloids, steroids, aminoacids, carbohydrates, tannins and phenolic compounds, oil and terpenoids.

Summary and Conclusion:

The summary and conclusion arising out of the research findings of the study are summarized below:

1) The poly herbal formulation *Chyavana drink* has exhibited consistent and moderate anti-inflammatory activity in the study. It shows the higher anti inflammatory activity is compared with the standard reference group. The anti inflammatory profile of the *Chyavana drink* observed in this study tends support to the claim made regarding its medicinal value in the treatment of

Rheumatoid Arthritis especially in the endogenous system of medicine.

2) The present findings also revealed the anti oxidant activity by using *in vitro* methods. The DPPH radical scavenging activity of the poly herbal formulation was found to be high percentage of inhibition and high reducing power was observed at high concentrations. Anti oxidant activity also depends on the phenolic content. The phenolic content of the poly herbal formulation *Chyavana drink* is relatively high.

3) In this study the phytochemical analysis revealed the presence of secondary metabolites. The screening reveals the presence of steroids and oils in all the extracts of *Chyavana drink.* Cardiac glycosides are present in all the extracts except ethanol. It also shows the presence of more than one active compound in this formulation.

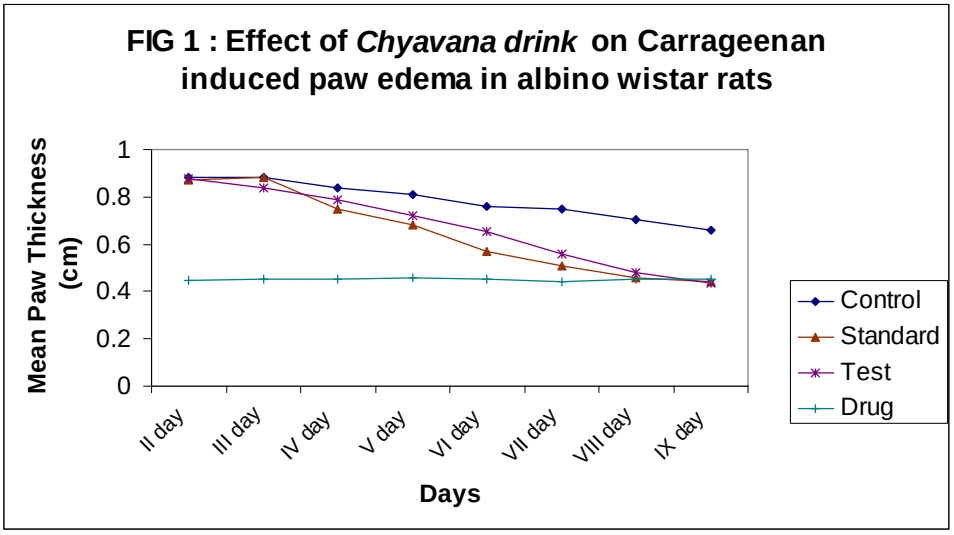

Table 2: Free radical scavenging activity of *Chyavana drink* by DPPH reduction

Concentration (µg/ml)	Inhibition (%) (Mean std)	IC_{50} (µl/ml)
1000	93.6±0.782	
500	91.29±0.775	
250	89.47±0.8140	38.83±1.258
125	88.55±0.82	
62.5	83.976±0.248	
31.25	40.03±1.585	
Ascorbic acid		11.24±0.022

Table 3: Reducing power activity of *Chyavana drink*

Concentration (µg/ml)	Concentration at 700nm
1000	1.33±0.057
500	0.76±0.0577
250	0.43±0.058
125	0.23±0.057
62.5	0.18 ±0.01
31.25	0.086±0.0057
Control	0.076

Table 4: Phenolic Estimation

Sample	Total phenolic content mg/100g of extract
Chyavana drink	6200

Table 5: Results for phytochemical screening

Herbal formulation	Extract Name	AL	ST	FL	TA	AA	CH	CG	SA	OIL	TER
Chyavana drink	PE	-	+	-	-	-	-	+	+	+	-
	CH	+	+	-	-	+	-	+	-	+	-
	EA	-	+	+	-	-	+	+	-	+	-
	ET	+	+	-	+	+	+	-	-	+	+

PE-Petroleum ether, CH-chloroform, EA- ethyl acetate, ET -ethanol

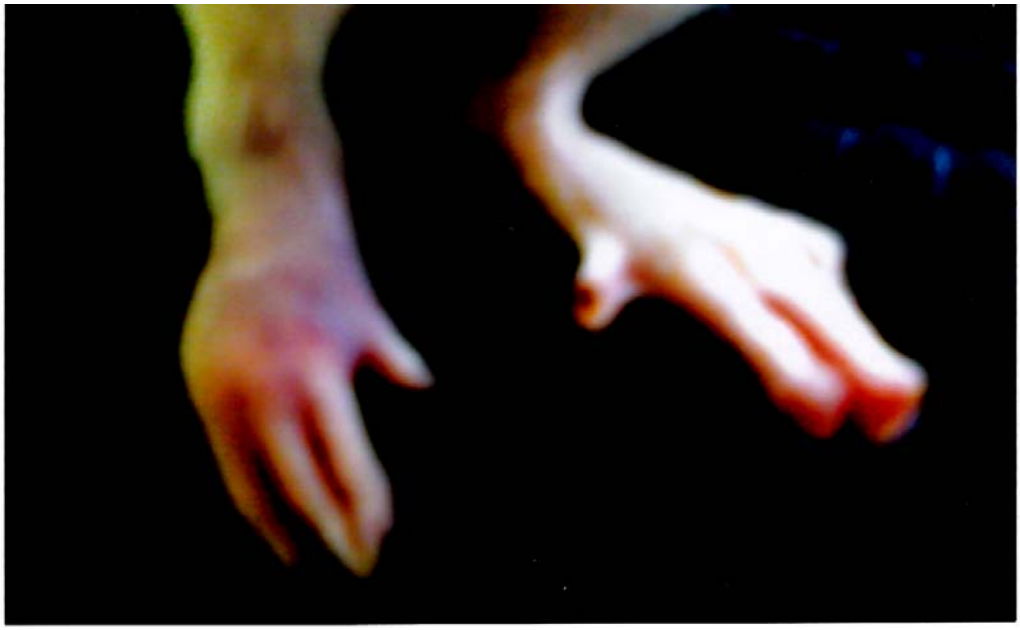

Fig. 1: After injecting Carrageenan into the hind paw

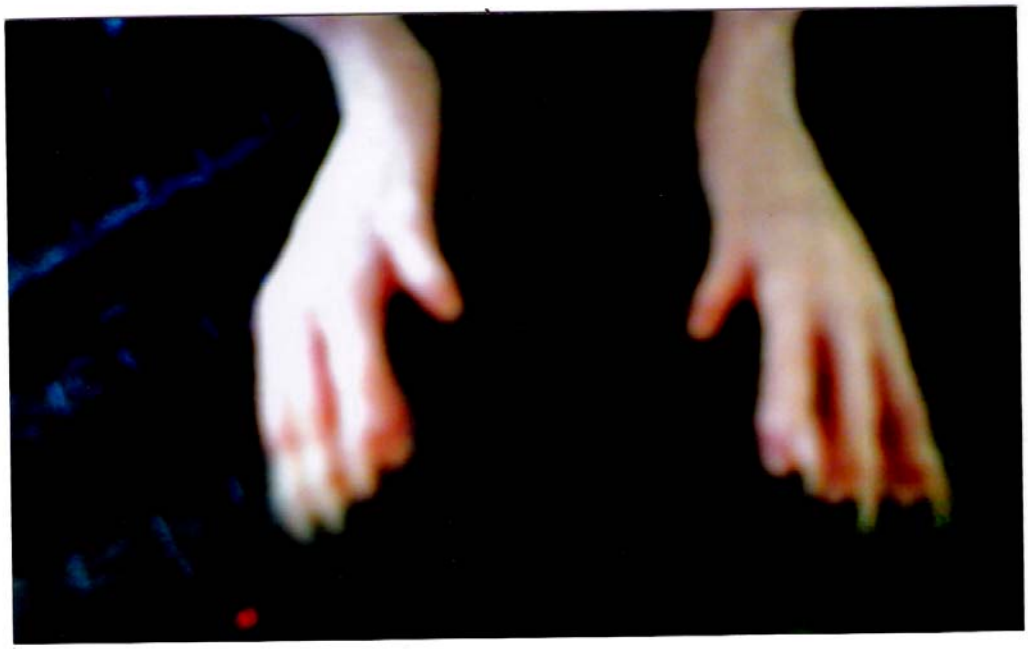

Fig. 2: After treating with *Chyavana drink*

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