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SAFETY AND EFFICACY STUDIES OF INFLEE TABLETS IN GOUTY ARTHRITIS

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Abstract

Inflee Tablet is the combination Rasnaerandadi Quath Extract, Punernavadi Quath Extract, Kaishoreguggul etc. Anti-oxidant, Xanthine oxidase inhibitory activity, Albumin denaturation and acute toxicity studies were conducted to understand the mechanism of action and safety of Inflee tablets in Gouty arthritis. Inflee Tablets was found to be completely safe upto 5000mg/kg of body weight. It showed powerful antioxidant activity with IC50 value of 247.76mcg. It significantly inhibited Xanthine oxidase enzyme and albumin denaturation showing promising results for the management of gouty arthritis. Thus Inflee tablets can be used safely in the treatment of gouty arthritis.

Keywords: Antioxidant, Gouty, Safety, Inflee, Rasna, Guggul, Ayurchem.

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INTRODUCTION

Inflammation in any part of the body results either due to physical injury or due to internal faulty metabolism of the body. Faulty metabolism of the body may originate from improper diet, irregular food timings, anger, stress. As per Ayurveda, faulty metabolism of body produces aam (toxins or Reactive Oxygen Species). These aam or Reactive Oxygen Speices (ROS)are highly unstable compounds and are key signaling molecules that play an important role in the progression of inflammatory disorders. As per Ayurveda ROS leads to imbalance in the three main pillars of the body that is vata-pitta-kapha. Under normal conditions, ROS levels are controlled by the body's complex antioxidant defense system and there is an equilibrium between ROS formation and degradation. Overproduction of ROS and/or inadequate antioxidant defense disturbs this equilibrium in favor of a ROS upsurge that results in oxidative stress. A deficiency in the body's natural antioxidant defense mechanisms has been implicated as the etiological or pathological factor in several clinical disorders [1]. Various research studies have indicated the role of ROS in Arthritis. ROS affects the micro-circulation of blood vessels near the site of inflammation. The microcirculation is the main playground where the process of inflammatory cascade was evaluated and analyzed [1]. Inflammation includes a long chain of molecular reactions and cellular activity, which are designed to restore a tissue from simple skin cut or to cure several burn injuries. in inflammatory process, it is important to understand the role of chemical mediators. These mediators come from plasma proteins or cells including mast cells, platelets, neutrophils and monocytes.They are triggered by bacterial products or host proteins. Chemical mediators bind to specific receptors vascular permeability, neutrophil, chemotaxins, stimulate smooth muscle contraction, have direct enzymatic activity, induce pain or Protein denaturation and mediate oxidative damage, causing the protein to lose its molecular conformation and functions or become denatured [2-5]. It is therefore deduced that, compounds which are able to prevent these changes and inhibit thermally or heat induced protein denaturation, have potential therapeutic value as anti-inflammatory agents [4]. Various ayurvedic classical text mentions the use of aqueous extract RasnaerandadiQuath Extract, Punernavadi Quath Extract and Kaishoreguggul. Till date no scientific literature is available which reports the anti-inflammatory and antigout activity of the above mentioned ayurvedic formulation. Ours is the first scientific study to report the same. Inflee tablets, a polyherbal combination of Rasnaerandadiquath extract, punernavadiquath extract, kaishoregugguletc from Ayurchem

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Products was evaluated for the Toxicity and above mentioned activity. Study will also reflect the synergistic activity of above individual extracts and in combination [5-8].

EXPERIMENTAL

MATERIAL AND METHODS

Chemicals - 2,2-diphenyl-1-picrylhydrazyl Powder (DPPH), Bovine serum albumin Fraction-V(BSA) and Aspirin powder from Hi Media. Solvent Methanol(analytical grade) obtained from Merck, Inflee Tablets from Ayurchem Products, Mumbai.

Studies

Acute Toxicity Studies of Inflee Tablets

Acute Toxicity studies on Inflee tablets were conducted as per the OECD guilines 420. The study was conducted at Bombay college of Pharmacy with CPCSEA registration no. 242/PO/RE/S/2000/CPCSEA; 01/08/2000 vide protocol approval no. CPCSEA-BCP-/2003-03. Briefly Swiss Albino mice were grouped into following groups as shown in table no 1. After giving drug all animals were observed for first 30 mins and then every 24 hours till 14 days. Observation such as change in Skin fur, Locomotor activity, autonomic signs and weight, as per OECD guidelines.

Table 1: Grouping of animals for acute toxicity studies:

Group No	Medicine	Dose
I	Vehicle Control	-
II	Inflee Tablets	300 mg / kg
III	Inflee Tablets	900 mg /kg
IV	Inflee tablets	3000 mg / kg

Antioxidant activity of Inflee tablets using Determination of DPPH free radical scavenging Activity

Free radical scavenging Capacity of Inflee tablets was determined using based on 2,2-diphenyl-1-picrylhydrazyl. The ability of the antioxidants present in the Inflee tablet as well as its individual ingredients Rasnaerandadi Quath Extract, Punernavadi Quath Extract and kaishoreguggul. The formulation use to decolorize the DPPH radical. DPPH radicals absorbed maximum at 514 nm, which disappears with reduction by an antioxidant compound. Briefly about 0.5 gm Inflee tablet powder, Rasnaerandadi Quath Extract, PunernavadiQuath Extract and Kaishoreguggulwas measured using an Analytical balance (Citizen CY 220) and was added to 5 ml of distilled water separately. The solution was mixed well using a vortex. Boil on water bath for 10 min.Serial

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dilution from 100 to 1000 µg/ml was performed using methanol for Inflee tablet, Rasnaerandadi Quath Extract, Punernavadi Quath Extract and Kaishoreguggul. Test solution (0.5 ml) of different concentrations (100 to 1000mcg/ml)and Control solution were mixed with 2.5ml DPPH solution (100µM). Then the samples were incubated at Room temperature in dark for 20min.Absorbance of reaction mixture was measured for each concentration at 514 nm using UV-Visible spectrophotometer (Shimadzu UV-1800) Each test was repeated thrice and the mean absorbance was recorded. The percentage of inhibition of Radical scavenging activity was determined on a percentage basis with respect to control using the following formula:Inhibition of DPPH (%) = (AC - AT/ AC) X 100Where,AC - Absorbance of control solution and AT - Absorbance of Test solution [9-11].

Anti-inflammatory activity of Inflee tablets using Bovine serum albumin (BSA) denaturation method

About 0.2gm of Aspirin, Inflee tablet, RasnaerandadiQuath Extract, PunernavadiQuath Extract and Kaishoreguggulwas measured using an Analytical balance (Citizen CY 220) and was added to 20 ml of distilled water Separately. The solution was mixed well using a vortex.Serial dilution from 1000µg/ml to 0.01µg/ml was performed for Inflee tablet, RasnaerandadiQuath Extract, PunernavadiQuath Extract and Kaishoreguggulandfor reference Drug (Aspirin).Test solution (0.05ml) of different concentrations from 0.01 microgram per ml – 1000 microgram per ml and standard drug Aspirin (0.05ml) of different concentrations 0.01 ,0.1,1,10,100,1000 µg/ml) were mixed with 0.5% w/v aqueous solution of BSA (0.45ml). Then the samples were incubated at 37ºC for 20min followed by incubation at 57ºC for 3min. 2.5ml of phosphate buffer (pH 6.4) was added to all the above samples after cooling. Absorbance of reaction mixture was measured for each concentration at 255nm using UV-Visible spectrophotometer (Shimadzu UV-1800) Each test was repeated thrice and the mean absorbance was recorded. The percentage of inhibition of protein was determined on a percentage basis with respect to control using the following formula:Percentage inhibition (%) = 100- [(ATS - APC)/ ATC] x100 whereas: ATS - absorbance of the test solution, APC - absorbance of the Product control ATC - absorbance of the test control solution [12-19]

Anti-gouty activity of Inflee tablets using Xanthine oxidase method

Xanthine oxidase inhibition assay was measured spectrophotometrically at λ 295 nm under aerobic condition (Unno *et al.*, 2004; Umamaheswari *et al.*, 2007). The reaction mixture contained solution of xanthine as a substrate, a solution of enzyme (xanthine oxidase) and the

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test solutions (50, 100, 250 and 500 µg mL^{-1}) prepared in Di-Methyl Sulfoxide (DMSO). Concentrations of allopurinol Viz., 10, 25, 50 and 100 µg mL^{-1} were prepared in phosphate buffer (ph 7.5). To each 1 mL sample, added 2.9 mL phosphate buffer and 2 mL xanthine (0.15 Mm) solution. The sample mixture was pre-incubated for 10 min at 30°C. 0.1 mL of xanthine oxidase (0.1 units mL^{-1}) was added to each test tube and incubated at 30°C for 30 min. After the incubation period, 1 mL of 1 N HCL was added to stop the reaction and the contents homogenized. After then absorbance was measured at 284 nm to see the formation of uric acid that occurs in the test solution and percentage inhibition was determined according to the following equation:Percentage inhibition (%) = 100- [(ATS - APC)/ ATC] x100. IC_{50} values were obtained through linear regression analysis the plot of concentration against percent inhibition [20-25].

Statistical analysis

All data were analyzed statistically using UV spectrophotometer (Shimadzu UV-1800). The descriptive data were expressed as mean ± standard error of mean. Linear regression analysis was performed to find out correlation coefficient. Thepercentage of inhibition rate between different groups were analyzed by independent sample t-test.

RESULTS

Acute Toxicity of Inflee Tablets:

Acute toxicity studies gives knowledge about the potential of a chemical, mixture or formulation to be hazardous to health. The maximum dose tried in the experimental study was 5 times higher than the normal human dose. Inflee Tablets did not show any signs of locomotors, autonomic signs like salivation, urination, convulsions, tremors was observed. There were no signs of pain was observed. All the animals which were weighed before and after the study were normal and did not show any signs of toxicity, mortality. Infact natural and normal increase in the weight was observed in all animals.

Antioxidant activity of Inflee tablet , RasnaerandadiQuath Extract, PunernavadiQuath Extract and Kaishoreguggul:

Free radical scavenging potential (DPPH) of the polyherbal Formulation Inflee tablet, Rasnaerandadi Quath Extract, Punernavadi Quath Extract and Kaishoreguggulat different concentrations is represented below. The free radical scavenging activity increases with increase in the concentration of the sample which was reflected at the decrease in the absorbance. The

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ability of Inflee tablet, RasnaerandadiQuath Extract, PunernavadiQuath Extract and Kaishoreguggulto scavenge DPPH free radical was calculated as percentage inhibition and inhibitory concentration at 50%. TheIC_{50} value ofInflee tablet, RasnaerandadiQuath Extract, PunernavadiQuath Extract and Kaishoreguggulwas found to be 247.76 µg/ml, 655.83 µg/ml, 216.52 µg/mland 116.13 µg/mlrespectively as shown in Table no. 2.

Table 2: Percentage inhibition of Free radicals using DPPH

Conc(µg/ml)	Percentage inhibition of Free radicals			
	Inflee Tablets	Rasnaerandadi Quath Extract	PunernavadiQuath Extract	Kaishoreguggul
100	33.25	30.27	38.56	48.27
200	49.25	30.54	41.27	52.52
300	53.97	37.94	52.59	54.82
400	54.54	47.39	57.19	73.79
500	69.16	48.49	84.08	73.90
600	84.04	48.90	95.51	85.40
700	97.00	50.13	95.75	96.78
800	97.23	54.52	96.34	97.58
900	97.46	54.79	96.46	97.70
1000	97.58	61.91	97.52	98.50
IC_{50}	247.76	655.83	216.52	116.13

Anti-inflammatory Activity of Inflee tablet, RasnaerandadiQuath Extract, PunernavadiQuath Extract and Kaishoreguggul using bovine serum denaturation:

Anti-inflammatory activity of Inflee tablet , RasnaerandadiQuath Extract, PunernavadiQuath Extract and Kaishoreguggulwas evaluated against BSA denaturation method. Inhibition of protein denaturation increased with increase in the concentration. The value of IC 50 of Inflee tablet , aqueous extract of RasnaerandadiQuath Extract, aqueous extract of PunernavadiQuath Extract and Kaishoreguggulwas 3.35 mg/ml, 10.62 mg/ml, 20.41 mg/ml and 4.80mg/ml, respectively. In addition to above the of IC 50 of Standard Aspirin was 8.87 mg/ml.

Table 3: Anti-inflammatory activity using Albumin denaturation:

Conc (µg/ml)	Inflee Tablets	Rasnaerandadi Quath Extract	Punernavadi Quath Extract	Kaishoreguggul	Aspirin
0.01	10.67 ± 2.18	8.06 ± 1.04	1.48 ± 0.22	7.76 ± 0.90	12.02 ± 2.80
0.1	14.49 ±1.68	8.56 ± 1.36	2.43 ± 0.39	8.88 ± 1.64	12.19 ± 3.00
1	19.34 ±2.66	9.60 ± 0.80	3.25 ± 0.38	11.78 ± 1.62	12.50 ± 0.01
10	24.00 ± 4.12	10.51 ± 1.40	3.65 ± 0.66	14.11 ± 1.38	15.70 ± 1.71
100	25.55 ± 4.46	12.03 ± 0.95	4.74 ± 0.59	16.48 ± 1.31	16.30 ± 0.93
1000	27.24 ± 3.85	13.20 ± 0.46	5.15 ± 0.21	19.13 ± 2.10	17.43 ± 0.02
IC 50	3.35 mg/ml	10.62 mg/ml	20.41 mg/ml	4.80 mg/ml	8.87 mg/ml

Results are shown as mean ± SEM. SEM:Standard error of the mean.

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Anti-Gout Activity of Inflee tablet, RasnaerandadiQuath Extract, PunernavadiQuath Extract and Kaishoreguggul using Xanthine Oxidase inhibition method:

Anti-Gout activity of Inflee tablet , RasnaerandadiQuath Extract, PunernavadiQuath Extract and Kaishoreguggul was evaluated against Xanthine oxidase inhibition method. Inhibition of xanthine oxidase enzyme increased with increase in the concentration. The value of IC 50 of Inflee tablet , aqueous extract of RasnaerandadiQuath Extract, aqueous extract of PunernavadiQuath Extract and Kaishoreguggul was 1.01 mg/ml, 1.30 mg/ml, 0.80 mg/ml and 2.48mg/ml, respectively. In addition to above the of IC 50 of Standard Allopurinol was 0.66 mg/ml.

Table 4: Anti-gout activity using Xanthine Oxidase inhibition method:

Conc **(µg/ml)**	Inflee Tablets	Rasnaerandadi Quath Extract	PunernavadiQuath Extract	Kaishoreguggul	Allopurinol
25	8.67 ± 2.78	5.06 ± 1.95	9.48 ± 1.22	2.76 ± 0.75	12.62 ± 1.66
50	15.49 ± 3.66	8.56 ± 2.36	18.43 ± 1.39	6.88 ± 2.66	22.75 ± 3.15
100	22.34 ± 2.12	10.60 ± 1.80	28.25 ± 3.38	8.78 ± 2.38	35.70 ± 3.51
250	35.00 ± 5.12	18.51 ± 2.40	39.65 ± 2.66	11.11 ± 1.62	48.40 ± 4.71
500	42.55 ± 4.12	28.03 ± 2.95	48.74 ± 3.59	16.48 ± 1.31	64.50 ± 2.93
1000	49.24 ± 3.66	38.20 ± 3.46	62.15 ± 3.21	20.13 ± 2.10	75.23 ± 1.02
IC 50	**1.01mg/ml**	**1.30 mg/ml**	**0.80 mg/ml**	**2.48 mg/ml**	**0.66mg/ml**

Results are shown as mean ± SEM. SEM:Standard error of the mean.

DISCUSSION

Ayurvedic text has more than 9000 formulations for different ailments. As per Ayurveda gouty arthritis is coomonly known as Vatarakt. as the main dosha involved is vata and it affects raktdhatu. Vitiation (impairment) of vatadosha and raktdhatu is caused by increased intake of spicy, sour, salty, alkaline, heavy and penetrating foods. The impaired doshas travel through the channels of the body and get accumulated at the smaller joints causing damage to the tissues and bones of the joint resulting in vatarakt. Rasnaerandadi Quath Extract is being widely used in southern state of India mainly kerala. It is believed to alleviate symptoms by relieving pain, swelling and inflammation. PunernavadiQuath extract is classical formulation used mainly for pain inflammation and kidney disorder. It is believed to reduce uric acid levels via increase in urination through kidney. Kaishoreguggul is a remedy based on purified guggulipid in Ayurvedic medicine. Kaishoreguggulu is believed to be used to support healthy joints (in gout), muscles (in fibromylegia), in back pain and connective tissue. Inflee Tablets a polyherbal formulation is the combination of Standardized aqueous extract of RasnaerandadiQuath Extract, Punernavadi Quath Extract and Kaishoreguggul etc. Inflee Tablet was found to be completely safe for human

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consumption as observed in toxicity studies. LD50 value of Inflee Tablet is more than 5000 mg/kg and can be classified as Class 5 category as per the OECD guidelines. It is well known reported fact that free radicals are the major cause for majority of the autoimmune disorder. In Ayurveda free radicals are termed as 'aam' (toxins). Free radicals causes increased reaction of uric acid with free radicals, such as hydroxyl radical and hypochlorous acid (HOCl) results in allantoin production. In many studies serum allantoin levels have found to be increased which suggests the oxidation of uric acid after reacting with free radicals. Hence scavenging free radicals is of prime importance for the treatment of gouty arthritis. Ingredients of Inflee tablets have shown very good free radical scavenging activity. When combination of all ingredients is taken IC_{50} value of inflee tablets is quite lower as compared to individual ingredients. This shows that when in combination synergistic activity is seen which helps to combat the diseases more powerfully. Hence when used in combination it shows better activity as compared to individual ingredients. Protein Denaturation is a process in which proteins lose their tertiary structure and secondary structure by application of external stress or compound, such as strong acid or base, a concentrated inorganic salt, an organic solvent or heat. Most biological proteins lose their biological function when denatured. Denaturation of proteins is a well-documented cause of inflammation. As part of the investigation on the mechanism of the anti-inflammation activity, ability of plant extract to inhibit protein denaturation was studied.Nonsteroidal anti-inflammatory drugs (NSAIDs) are commonly prescribed medications in the world because of their verified effectiveness in reducing pain and inflammation. NSAIDs has accounted for prevention of the protein denaturation, which acts as antigens and prompts autoimmune diseases. These drugs contain several adverse effects, particularly gastric irritation prompting the development of gastric ulcers. Gout is also an inflammatory joint disease, associated with an elevated uric acid level in blood which further leads to the deposition of urate crystals in the joints and kidneys followed by painful inflammation, gouty arthritis, and uric acid nephrolithiasis. Xanthine oxidase (XO) is responsible for oxidation of hypoxanthine to xanthine and finally xanthine to uric acid. Over activity of this enzyme, impair renal excretion of uric acid and result in hyperuricemia and gout.

Various ayurvedic text mentions the role of Rasnaerandadi, Punernavadi and kaishoreguggul in pain and inflammation. No scientific report is available till date to study the anti-inflammatory and anti-gout activity of Rasnaerandadi, Punernavadi and kaishoreguggul. Ours is the first study to report the same. It was observed that all the three ingredients showed good anti-inflammatory and anti-gout activity. The results are highly significant and comparable to the standard drug used

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to assess the activity. Since standard drugs like Aspirin has many side effects whereas the use of Rasnaerandadi, Punernavadi and kaishoreguggul makes it more beneficial. Similarly results were obtained when Inflee Tablets (polyherbal combination of Rasnaerandadi, Punernavadi and kaishoreguggul) was used in inhibition of protein denaturation and xanthine oxidase enzyme. Infact IC_{50} value was lesser than the individual ingredients indicating the synergistic effect when used in combination rather than used alone. This indicates Inflee tablet in the combination of Rasnaerandadi, Punernavadi, kaishoregugguletc shows very anti-inflammatory and anti gout activity. The mechanism may be attributed due to following factors like antioxidant activity, while scavenging aami.e toxins generated in the body due to faulty metabolism and incompatible food habits, anti-inflammatory activity there by reducing the protein damage and reduction in serum uric acid levels by inhibiting xanthine oxidase enzyme activity..

CONCLUSION

Inflee tablets shows very good antioxidant, anti-inflammatory and anti-gout activity.

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