In vitro xanthine oxidase inhibitory and *in vivo* hypouricemic activity of herbal coded formulation (Gouticin)

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Abstract: Currently, natural products have been used in treating gouty arthritis and are recognized as xanthine oxidase inhibitors. Current study was designed to evaluate *in vitro* xanthine oxidase inhibitory potential of Gouticin and its ingredients extracts and *in vivo* hypouricemic activity of gouticin tablet 500 mg twice daily. Ethanol extracts of Gouticin and its ingredients were evaluated *in vitro*, at 200, 100, 50, 25 µg/ml concentrations for xanthine oxidase inhibitory activity. IC_{50} values of Gouticin and its ingredients were estimated. Further, *in vivo* therapeutic effect of Gouticin was investigated in comparison with allopathic medicine (Allopurinol) to treat gout. Total patients were 200 that were divided into test and control group. Herbal coded medicine (Gouticin) was given to test group and allopathic medicine allopurinol was administered to control group. *In vitro*, Gouticin has the highest percent inhibition at 96% followed by Allopurinol with 93% inhibition. *In vivo* study, mean serum uric acid level of patients was 4.62 mg/dl and 5.21mg/dl by use of Gouticin and Allopurinol at end of therapy. The study showed that herbal coded formulation gouticin and its ingredients are potential sources of natural xanthine oxidase inhibitors. Gouticin 500 mg twice daily is more effective than the allopurinol 300mg once daily in the management of gout.

Keywords: Gouty arthritis, herbal medicine, Allopurinol, medicinal plants, plant extracts, xanthine oxidase, clinical efficacy, hypouricemic activity.

INTRODUCTION

Herbal remedies obtained from traditional herbs and medicinal plants are commonly used in Unani system of medicine. In rural areas, health and healing are usually in the alternative form of a hand-me-down herbal concoction. Even in the developed countries, herbal vendors trade fresh plants and preparations for various conditions ranging from fever to abortifacients. There are thousands of herbal plants that folklore had attributed medicinal benefits to treat gout (Zhang & Liu, 2010). However, a considerable number of plants still need to be scientifically validated; hence, much work is still needed to investigate the bioactivity of these plant. Excessive production of uric acid leads to deposition of urate crystals in soft tissues and joints which is linked to gout (Harris & Siegel, 1999). Gout is metabolic disorder in which deposition of uric acid occurs in joints. All opurinol is commonly prescribed to manage gout. Thus, screening of the Gouticin and its ingredients extracts for the xanthine oxidase inhibitor activity may play an important role in management of gout. In the present study, Gouticin and its ingredients (*Apium graveolens, Colchicum autumnale, Tribulus terrestris, Withania somnifera, Zingiber officinale*) were screened for their xanthine oxidase inhibitory activity and further clinical trial were conducted to investigate clinical efficacy in hyperuricemia and gouty arthritis.

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MATERIALS AND METHODS

Chemicals and reagents

Inhibitor (Allopurinol), enzyme (xanthine oxidase from cow milk) and substrate (xanthine) were obtained from Sigma. All other reagents were purchased from Merck.

Plant material

Medicinal plants *(Apium graveolens, Colchicum autumnale, Withania somnifera, Smilax chinensis, Tribulus terretris* and *Zingiber officinale* were purchased from Judia market Karachi and submitted to Dr Iqbal Azhar, Department of Pharmacognosy, University of Karachi, Karachi for authentication. Voucher specimens for each plant were also deposited that are *Apium graveolens* UK-FPH-DP-AG-103, *Colchicum autumnale* UK-FPH-DP-CA-104, *Withania somnifera* UK-FPH-DP-WS-105, *Smilax chinensis* UK-FPH-DP-SC-106, *Tribulus terretris* UK-FPH-D P-TT-107 and *Zingiber officinale* UK-FPH-DP-ZO-108.

Plant extraction

All plants were extracted with ethanol. Approximately, 200 g dry weight of plant material was macerated at room

temperature for 15 days with solvent and filtered. The solvents were removed by rotary evaporation under reduced pressure and the resulting extracts were tested for inhibition of xanthine oxidase at Pakistan council of scientific and industrial research (PCSIR) Labs Complex, Karachi

Assay of xanthine oxidase activity

The xanthine oxidase inhibitory activity was measured using a spectrometric procedure according to *Noro et al*. (Noro *et al*., 1983). The assay mixture contains test solution (50µL), 0.1 mM phosphate buffer (35µL) having pH of 7.5 and enzyme solution (30µL). Assay mixture was prepared immediately prior to experiment. This assay was pre-incubated for 15 minutes at temperature of 25 centigrade. Substrate solution (60µL) was added and reaction was started. Then assay mixture was incubated for 30 minutes at temperature of 25 centigrade. Measurement of absorbance was done at 295 nm by using double beam spectrophotometer (Jasco). Preparation of blank was done in the same way. Formula for percentage inhibition of xanthine oxidase was (1-B/A) x100. A means enzyme activity without test material and B means enzyme activity with test material. Values of IC_{50} were calculated. Dimethyl sulfoxide (DMSO) was used for dissolution of crude extracts and later on phosphate buffer was used for dilution. DMSO concentration in final solution was less than 0.25%. In our study we tried gouticin and its ingredients extracts to observe their xanthine oxidase inhibitory activity. The mixture (Gouticin) and ethanol extract of *Apium graveolens, Colchicum autumnale, Withania somnifera, Tribulus terretris and Zingiber officinale* at a concentration of 200, 100, 50 and 25µg/ml were used for experiment. Positive control was Allopurinol. Percent xanthine oxidase inhibition effect of the assayed samples was determined through the slope of the plot of absorbance against time (seconds). The obtained results show the percentage of enzyme inhibition

Clinical efficacy of herbal coded formulation (Gouticin)
Materials and Methods

Study design

Clinical trials were conducted in Hamdard University Hospital for Eastern Medicine and Memona Clinic Korangi Crossing, Bhittai Colony, Karachi. Clinical trial protocol approval number is 23. The study was carried out in the period 2010-2011. Study period was of 18 weeks with a window for the follow up visit of 6 weeks accounting for variable duration of hyperuricemia treatment.

Patients

Total patients were 200 that completed the clinical trial Hamdard University Hospital for Eastern Medicine and Memona Clinic Korangi Crossing, Bhittai Colony, Karachi. Mean age of male patients was 50.55 and mean age of female was 60.23. 154 were male and 56 were female. 100 patients received Allopurinol (300 mg one time per day orally from Sigma Chemical Co. St. Louis, MO) and other one hundred patient received Gouticin Tab. 500 mg (twice a day orally). Gouticin is an herbal coded formulation of compound drugs with their synergistic action of herbal drugs design and calculated according to herbal pharmacopoeia, monographs of Unani medicine on scientific basis. Each 500 mg Gouticin tablet contains *Apium graveolens* 100 mg, *Colchicum autumnale* 50 mg, *Withania somnifera* 75 mg, *Smilax chinensis* 75 mg, *Tribulus terrestris* 100 mg, *Zingiber officinale* 100 mg.

STATISTICAL ANALYSIS

Statistical analysis was carried out using SPSS, Excel software, Wilcoxine test and the Chi Square test.

Inclusion criteria

1. The patients with serum uric acid > 8 mg/dL
2. Male and female patients between 20 to 80 years of age.
3. Subjects with history of gout, initiating or currently on urate lowering therapy who are at risk of gout flare
4. All socioeconomic classes including lower middle and higher.

Exclusion criteria

The major exclusion criteria for this trial were:

1. Patients with acute gouty arthritis.
2. Hyperuricemia due to cancer chemotherapy
3. Patients belonging to the distant area outside Karachi will be excluded because of inherent difficulty in follow up.
4. Unstable angina
5. Uncompensated CHF
6. Poorly controlled arrhythmia; or
7. Uncontrolled hypertension (>150/95 mm Hg)
8. Dialysis
9. History of solid organ transplantation
10. G6PD deficiency
11. Hyperuricemia due to enzyme defects
12. Patient having renal will not be included in the study.

RESULTS

In vitro study

All the 07 extracts assayed, demonstrated more than 50% xanthine oxidase inhibitory activity at 100µg/ml as shown in table 1. IC_{50} values were obtained through linear regression analysis the plot of concentration (200, 100, 50, 25µg/mL) against percent inhibition. Gouticin and its ingredients have the IC50 values include *Apium graveolens* (180µg/ml), *Withania somnifera* (95µg/ml), *Colchicum autumnale* (42µg/ml), *Zingiber officinale* (23.6µg/ml), *Tribulus terrestris* (19.8µg/ml), and Gouticin

Table 1: Percent inhibition of the Gouticin and its ingredients extracts

Plant extract	Code	200 µg/ml Percent inhibition	100 µg/ml Percent inhibition	50 µg/ml Percent inhibition	25 µg/ml Percent inhibition
Apium graveolens	A.G.	69	54	31	11
Colchicum autumnale	C.A.	79	61	42	30
Gouticin	G.C.	96	88	69	40
Tribulus terrestris	T.T.	91	78	49	31
Withania somnifera	W.S.	76	69	48	26
Zingiber officinale	Z.O.	92	80	51	36
Allopurinol	A.P.	93	86	70	41

(17.3µg/ml), The IC50 for allopurinol was given as 6.1µg/ml (table 2).

Table 2: IC_{50} values of all plant Extracts with Allopurinol

Plant Extract	Code	IC_{50} µg/ml
Apium graveolens	A.G.	180
Colchicum autumnale	C.A.	42
Gouticin	G.C.	17.3
Tribulus terrestris	T.T.	19.8
Withania somnifera	W.S.	95
Zingiber officinale	Z.O.	23.6
Allopurinol	A.P.	6.1

In vivo study

The test drug Gouticin was prescribed to 100 patients with mean serum uric level 10.12mg/dl at base line as shown in table 3. Mean serum uric acid level of 100 patients prescribed Gouticin was 7.56mg/dl at 1st follow up of 6 weeks as shown in table 4. Mean serum uric acid level of 100 patients prescribed Gouticin was 6.41 mg/dl at 2nd follow up as shown in table 5. Mean serum uric acid level of 100 patients prescribed Gouticin was 4.62 mg/dl at 3rd follow up as shown in table 6. The control drug allopurinol was prescribed to 100 patients with mean serum uric level 10.01mg/dl at base line as shown in table 3. Mean serum uric acid level of 100 patients prescribed allopurinol was 7.87mg/dl at 1st follow up as shown in table 4. Mean serum uric acid level of 100 patients prescribed allopurinol was 6.33mg/dl at 2nd follow up as shown in table 5. Mean serum uric acid level of 100 patients prescribed allopurinol was 5.21mg/dl at 3rd follow up as shown in table 6. The mean serum uric acid of 100 patients both male and females prescribed Gouticin was 10.12mg/dl and the mean serum uric acid level of 100 patients both male and female prescribed Allopurinol was 10.01mg/dl at baseline. Hyperuricemia may be due to high purine diet including organ meat, beans, peas, spinach and cauliflowers. The mean serum uric acid of total patients prescribed Gouticin was 7.56 mg/dl while the mean serum uric acid level of total patients prescribed Allopurinol was 7.81mg/dl at 1st follow up. The mean serum uric acid level of patients prescribed Gouticin was 6.41mg/dl while the mean serum uric acid level of patients prescribed Allopurinol was 6.33mg/dl at 2nd follow up. The mean serum uric acid level of patients prescribed Gouticin was 4.62mg/dl while the mean serum uric acid of patients prescribed Allopurinol was 5.21mg/dl at 3rd follow up.

Table 3: Mean of serum uric acid at base line

Treatment Group	Sex	Mean	Number (n)	Standard Deviation
Test drug (Gouticin)	Male	10.88	81	1.7
	Female	9.21	22	1.71
	Total	10.12	100	1.69
Control drug (Allopurinol)	Male	10.67	79	1.72
	Female	9.17	24	1.82
	Total	10.01	100	1.74

Table 4: Mean of serum uric acid at 1st follow up

Treatment Group	Sex	Mean	Number (n)	Standard Deviation
Test drug (Gouticin)	Male	7.59	78	1.89
	Female	7.53	22	2.24
	Total	7.56	100	2.00
Control drug (Allopurinol)	Male	8.57	76	2.09
	Female	7.78	24	2.25
	Total	7.81	100	2.13

Table 5: Mean of serum uric acid at 2nd follow up

Treatment Group	Sex	Mean	Number (n)	Standard Deviation
Test Drug (Gouticin)	Male	6.45	64	1.19
	Female	6.37	36	2.14
	Total	6.41	100	1.80
Control Drug (Allopurinol)	Male	6.58	68	1.96
	Female	6.22	32	2.13
	Total	6.33	100	2

Comparative analysis of intensity of symptoms between treatment groups by Wilcoxone signed rank test

In this study, we recorded the intensity of symptoms at baseline (T0), 6th week (T1), 12 weeks (T2) and 18 weeks (T3) of treatment. Median values, inter-quartile

ranges were determined and p values were calculated by using Wilcoxon signed-rank test to record the indirect effects of Gouticin in the reduction of sign and symptoms of gouty arthritis. There was a statistically significant reduction in sign and symptoms of gouty arthritis. There was a statistically significant decrease in the overall symptom score from baseline (T0: median 13, range 6-18) to 6th weekk (T2: median 10, range 6-16), 12week (T2: median 7, range 0-11) and 18th week (T3: median 3, range 0-6) as given in table 7

Table 6: Mean of serum uric acid at 3rd follow up

Treatment Group	Sex	Mean	Number (n)	Standard Deviation
Test Drug (Gouticin)	Male	4.66	64	1.43
	Female	4.58	36	1.86
	Total	4.62	100	1.58
Control drug (Allopurinol)	Male	5.31	68	1.98
	Female	5.17	32	2.08
	Total	5.21	100	2

Improvement in intensity of symptoms in test group (Gouticin)

Joint pain (T0: median 2, range 1-3; T1: median 1.5, range 1-3; T2, median 1, range 0-1 T3 median 0.5, range 0-1, Tenderness of joints (T0: 2, range 1-3; T1: median 1.5, range 1-2; T2: median 1, range 0-2, median 0.5, range 0-1), Swelling of joint(T0: 2, range 1-3; T1: median 1.5, range 1-3; T2: median 1, range 0-2, T3, median 0.5, range 0-1), stiffness of joints (T0: median 2.5, range 1-3; T1: median 2, range 1-3; T2: median 1.5, range 0-2, T3: median 0.5, range 0-1), pain on movement of joints (T0: median 2.5, range 1-3; T1: median 2, range 1-3; T2: median 1.5, range 0-2, T3: median 0.5, range 0-1), and tophi size (T0: median 2, range 1-3; T1: median 1.5, range 1-2; T2: median 1, range 0-2, T3 median 0.5, range 0-1) all showed statistically significant improvement after treatment with Gouticin. All symptom scores were showed in table 8.

Improvement profile with Allopurinol

Allopurinol also exhibited a decrease in the overall symptom score from baseline (T0: median 12, range 6-18) to 6th week (T1: median 10.5, range 6-17), 12th week (T2: median 9.5, range 6-13 and 18th week (T3: median 9, range 6-12) as shown in table 9.

Improvement in Intensity of symptoms with Allopurinol

Joint pain (T0: median 2, range 1-3; T1, median 2, range 1-3; T2, median 1.5, range 1-2, T3 median , range 0-2, Tenderness of joints (T0: 2, range 1-3; T1: median 1.5, range 1-2; T2: median 1.5, range 1-2, T3: median 1, range 0-2), swelling of joints (T0: median 2, range 1-3; T1: median 1.5, range 1-3; T2: median 1.5, range 1-2, T3: median 1, range 0-2), stiffness of joint (T0: 2, range 1-3; T1: median 2, range 1-3; T2: median 2, range 1-3, T3: median 1, range 0-2), pain on movement of joints (T0: median 2, range 1-3; T1: median 1.5, range 1-3; T2: median 1.5, range 1-2, T3: median 1, range 0-2), and tophi size (T0: median 2, range 1-3; T1: median 2, range 1-3; T2: median 1.5, range 1-2, T3: median 1, range 0-2) all showed improvement after treatment with Allopurinol. All symptom scores were showed in table 10.

Comparative analysis of intensity of symptoms between treatment groups

A comparative analysis was done in the level of intensity of symptoms between two treated groups i.e., test and control groups before and after the treatment. Wilcoxone signed rank test was applied to see the statistical difference after calculating the median values and inter-quartile ranges. It was concluded from this statistical analysis that Gouticin (Test) possesses greater value to lower down the intensity of symptoms as compared to Allopurinol (Control) as shown in table 11.

Table 7: Overall improvement in severity of symptoms in Test group by Wilcoxone Signed Rank Test

Overall severity of symptoms										
Baseline (T0)		6th week			12th week			18th week		
Median	IQR	Median	IQR	p-value	Median	IQR	p-value	Median	IQR	p-value
13	6-18	10	6-16	0.12	7	0-11	0.05	3	0-6	0.01

Table 8: Improvement in Intensity of symptoms in test group(Gouticin) by Wilcoxone Signed Rank Test

Intensity of symptoms											
Symptoms	Baseline (T0)		After 6 weeks (T2)			After 12 weeks			After 18 weeks		
	Median	IQR	Median	IQR	P-value	Median	IQR	P-value	Median	IQR	P-Value
Joint pain	2	1-3	1.5	1-3	0.07	1	0-1	0.04	0.5	0-1	0.01
Tenderness of joints	2	1-3	1.5	1-2	0.07	1	0-2	0.06	0.5	0-1	0.01
Swelling of joints	2	1-3	1.5	1-3	0.07	1	0-2	0.06	0.5	0-1	0.01
Stiffness of joints	2.5	1-3	2	1-3	0.08	1.5	0-2	0.06	0.5	0-1	0.01
Pain on movement of joints	2.5	1-3	2	1-3	0.08	1.5	0-2	0.06	0.5	0-1	0.01
Tophi	2	1-3	1.5	1-2	0.07	1	0-2	0.04	0.5	0-1	0.01

Comparative analysis of serum uric acid between test and control group by using Wilcoxone signed rank test

In this study, we recorded the level of serum uric acid at baseline (T0), 6th week (T1), 12 weeks (T2) and 18 weeks (T3) of treatment. Median values, inter-quartile ranges were determined and p values were calculated by using Wilcoxon signed-rank test to record the indirect effects of Gouticin in the reduction of serum uric acid level. There was a statistically significant decrease in the serum uric acid level from baseline (T0: median 9.5, range 7-10) to 6^{th} week of treatment (T1: median 8.1, range 7-9), 12^{th} week of treatment (T2: median 6.5, range 6-8) and 18^{th} weeks of treatment (T3: median 5.5, range 5-7) as shown in table 12.

Reduction in serum Uric Acid level in control group by Allopurinol

In this study, we recorded the level of serum uric acid at baseline (T0), 6th week (T1), 12 weeks (T2) and 18 weeks (T3) of treatment. Median values, inter-quartile ranges were determined and p values were calculated by using Wilcoxon signed-rank test to record the indirect effects of Allopurinol in the reduction of serum uric acid level. There was decrease in the serum uric acid level from baseline (T0: median 9.5, range 7-11) to 6^{th} week of treatment (T1: median 8, range 7-10), 12^{th} week of treatment (T2: median 7.5, range 6-9) and 18^{th} weeks of treatment (T3: median 6.5, range 5-8) as shown in table 13.

Overall reduction in serum uric level in Test group and Control group by Wilcoxone Signed Rank Test is shown in table 14.

DISCUSSION

In vitro study

All the 07 extracts assayed, demonstrated more than 50% xanthine oxidase inhibitory activity at 100 µg/ml. Gouticin, *Zingiber officinale*, *Tribulus terrestris*, *Withania somnifera*, *Colchicum autumnale*, *Apium graveolens*, exhibited 88, 80, 78, 69, 61 and 54% inhibitory activity at 100µg/ml concentration respectively (table 1). It had been reported previously that extracts causing>50% enzyme inhibitory activity at 50 µg/ml concentration warranted further investigation. The IC_{50} value of Gouticin and its ingredients was determined. Highest inhibition of 88% was exhibited by Gouticin. Percentage inhibition exhibited by Gouticin was more than allopurinol that was 88 and 86% respectively. Gouticin is herbal coded formulation approved by the Faculty of Eastern Medicine and Surgery, and used as a antihyperuricemic. *Zingiber officinale* extracts exhibited 80% inhibitory activity at 100µg/ml. *Zingiber officinale* has IC_{50} value of 23.6 ug/ml. *Zingiber officinale* has been prescribed to treat gouty arthritis. Gingerol is found in *Zingiber officinale* and has analgesic effect. It helps in preventing acute gouty flares induced by initiation of urate lowering therapy (Junji *et al*., 2003). *Zingiber officinale* is widely prescribed for treatment of rheumatoid arthritis and gout. *Zingiber officinale* is traditionally prescribed to treat gouty arthritis, peptic ulcer, nausea, vomiting, abdominal pain, sciatica and rheumatoid arthritis (Connor *et al*., 2009). *Tribulus terrestris* has IC_{50} value of 19.8 ug/ml. *Tribulus terrestris* is commonly prescribed as anti-inflammatory drug to alleviate pain and arthritis and other joint related disorders. It is prescribed in kidney stones, diabetes mellitus, gouty arthritis and sexual weakness. It is uricosuric, lithotriptic, diuretic and aphrodisiac (Heidari *et al*., 2007, Al-Ali *et al*., 2003). *Apium graveolens* exhibited 54% inhibitory activity at 100µg/ml. *Apium graveolens* has IC_{50} value of 180 ug/ml. In one study, *Apium graveolens* has shown significant reduction in serum uric acid level in rats (Doha, 2008). Its use as anti-inflammatory, uricosuric and hypouricemic is well known. This plant contains falconoid aliening that has xanthine oxidase inhibitory activity. *Apium graveolens* is used to treat fungal infections and tumors. *Apium graveolens* contains furocoumarins that are typically prescribed for their stomachic, carminative, diuretic and emmenagogue properties (Nehal & Abd, 2011). *Colchicum autumnale* extracts exhibited 61% inhibitory activity at 100 µg/ml. In this study, *Colchicum autumanle* showed a significant XO inhibitory activity with an IC_{50} value of 42 µg/ml. Such activity was not reported before and this is the first report on *Colchicum autumnale.* In Unani system of medicine, *Colchicum autumnale* has been used in gouty arthritis. Colchicine is effective in crystal induced inflammation. Colchicin helps in prevention of acute gouty flares induced by initiation of urate lowering therapy (Katzung, 2004). *Tribulus terrestris* extracts exhibited 78% activity at 100 µg/ml. Efficacy of *Tribulus terrestris* in reducing urate stone has been reported. It contains flavonoids (Quercetin and kaemferol), that has xanthine oxidase inhibitory activity (Su *et al*., 2009). *Withania somnifera* extracts exhibited 69% activity at 100µg/ml. *Withania somnifera* roots have long been used to treat rheumatism and immune dysfunctions. It contains quercetin that is Xanthine oxidase inhibitor (Miean & Mohamed, 2001). *Withania somnifera* is prescribed as an anti-inflammatory agent in gouty arthritis (Sehgal *et al*., 2012). Allopurinol exhibited 86% inhibition and showed the lowest IC_{50} of 6.1 µg/ml. It is possible that the inhibitory activities and IC_{50} values would improve once the compounds responsible for the activity are identified. This work has scientifically validated the use of the Gouticin in gouty arthritis. Allopurinol extracts exhibited 86% at 100µg/ml. Gouticin, *Tribulus terrestris*, *Zingiber officinale*, *Colchicum autumnale*, *Withania somnifera*and *Apium graveolens* showed the lowest IC_{50} of 17.3, 19.8, 23.6, 42, 95, 180µg/ml respectively. In this study, allopurinol showed lower IC_{50} value (6.1µg/ml) as compared to the Gouticin (17.3µg/ml). Our results indicate that gouticin

and its ingredients inhibited xanthine oxidase enzyme significantly. As in traditional medicine they were used for management of gout it is thought that their anti-gout activity, at least by part, was related to this xanthine oxidase inhibitory activity. Gouty arthritis is metablic disorder that is characterized by deposition of uric acid into joints (Terkeltaub, 2009). Now there is new trend to use herbal medicine because of their potential to cure and fewer or no side effects. Medicinal plants having anti-inflammatory, uricosuric and xanthine oxidase inhibitory activities are used in gouty arthritis. Herbal medicines are comparably safe, and are useful in the management of gout. Although allopurinol is commonly used in the treatment of gouty arthritis but this has side effects such as skin rashes, nauseas and vomiting.

In vivo study

Randomized controlled clinical trial comparing Gouticin and Allopurinol has been conducted in patients with gouty arthritis. Study duration was 18 weeks study with a follow up period of 6 weeks. Primary outcome measure was to reduce serum uric acid less than 6 mg/dl. In eighteen weeks clinical trial, Gouticin exhibited significantly reduction in serum urate levels in 91% patients. In contrast, allopurinol exhibited reduction in serum urate level in 78% patients. The findings presented here demonstrate that Gouticin is more effective than Allopurinol in the treatment of gouty arthritis. Urate-lowering response to Gouticin 500 mg twice a day was better than allopurinol (300mg once daily). In our study, there was significant reduction in occurrence of gout flare by lowering of serum uric acid level below 6mg/dl. This observation is similar to a study by Shoji *et al*., (Shoji *et al*, 2004) that decrease of uric acid level below 6.0 mg/dl decreases the gouty attack. In one clinical trial, febuxostat exhibited nearly complete abolition of gout flares in patients completing the study (Schumacher *et al*, 2009). In a randomized clinical trial of colchicine for acute gouty arthritis prophylaxis at the beginning of allopurinol, less flare occurred in patients treated with colchicine when compared with patients with placebo (Borstad *et al*, 2004). In our study, we found that Gouticin 500 mg twice a day was effective for treatment of gouty arthritis. Outcome measures in our study were to lower serum uric level and decrease associated sign and symptoms of gouty arthritis. The outcome measures and conclusion of previous studies are summarized in table 15**.** In one cohort study, physical disability in gout was assessed in which, it was concluded that tophi formation and cardiovascular disorders lead to physical disability (Alvarez *et al*, 2005). Our observations are compatible with findings from other studies that proper treatment of gouty arthritis leads reversal of physical disability that was evident from radiological findings (Fernando *et al*, 2009). In developing countries, self-medication is common that is major cause of gouty arthritis in patients using aspirin at low dose (1-2mg/day). In our study, many patients told that they were using aspirin at lose dose as self medication and pain was timely relieved without control of hyperuricemia because they were not using hypouricemic agents. This study is related to observation by Darmawan *et al*, where exposure to long term intermittent or continuous prednisone as part of the self-medication in patient with gout have increased existing or induced hyper-triglyceridemia and hypercholesterolemia (Darmawan *et al*, 2003). Our study indicates that by reducing serum uric acid level with Gouticin 500mg twice daily, symptoms of gouty arthritis are reduced and comorbidity and early death is prevented. In chronic from of gouty arthritis, tophus formation is common. There are various methods for measurement of tophus formation. Physical and ultrasonic measurement is usually practiced (Nicola *et al*, 2011, Schumacher *et al*, 2005, Dalbeth *et al*, 2006). Perez-Ruiz and colleagues (Perez *et al*, 2002) stated the use of calipers for measurement of tophi formation. In our study, physical measurement has been done that demonstrates that Gouticin 500 mg twice daily is more effective than Allopurinol 300mg once a day in reducing tophus size. This observation is similar to a study where febuxostat therapy was found more effective in reducing tophus size as compared to allopurinol (Schumacher *et al*, 2006). Recently, Schumacher and colleagues (Schumacher *et al*, 2005) stated that the use of tape for measurement of tophus size. Biochemical measurement of serum uric acid levels is also indicative of disease progression. In our study, 91% of subjects whose serum uric acid was decreased less than 6 mg/dl exhibited significant reduction in tophus size in comparison with 9% of patients whose serum uric acid was not decreased to normal. This observation is similar to a study where seventy six percent of patients whose serum uric acid was reduced less than 6 mg/dl exhibited significant reduction in tophus formation in comparison with twenty two percent of patients whose serum uric acid was not reduced to normal (Perez & Martin, 2006). A decrease in serum urate after administering the Gouticin 500 mg twice a day indicated that it could be used as a uric acid lowering agent. These values supported the need for further research to evaluate the xanthine oxidase inhibitory activity. This activity has been carried out which proved that Gouticin has xanthine oxidase inhibitory activity. Allopurinol extracts exhibited 86% inhibitory activity at 100µm/ml. In contrast, Gouticin exhibited 88% inhibitory activity at 100µm/ml. Most of the patients experienced improvement in symptomatology of gouty arthritis within study period. This observation validate the anti-inflammatory effect of ingredients of Gouticin such as *Smilax chinensis* (Khan *et al.*, 2009). In our study, when serum uric acid was reduced to <6 mg/ml, the symptoms of gouty arthritis were significantly reduced. Our observations are compatible with findings from other studies (Grayson *et al*, 2011). Gouticin is an effective alternative when allopurinol cannot be tolerated or when gouty arthritis becomes resistant to allopurinol.

Table 9: Overall severity of symptoms in control group by Wilcoxone signed rank test

		Overall severity of symptoms								
Baseline (T0)		6th week			12th week			18th week		
Median	IQR	Median	IQR	P-value	Median	IQR	P-value	Median	IQR	P-value
12	6-18	10.5	6-17	0.006	9.5	6-13	0.06	6	0-12	0.05

Table 10: Improvement in Intensity of symptoms with Allopurinol

Symptoms	Intensity of symptoms										
	Baseline (T0)		After 6 weeks (T2)			After 12 week			After 18 week		
	Median	IQR	Median	IQR	P-value	Median	IQR	P-value	Median	IQR	P-Value
Joint pain	2	1-3	2	1-3	0.1	1.5	1-2	0.07	1	0-2	0.032
Tenderness of joints	2	1-3	1.5	1-2	0.07	1.5	1-2	0.07	1	0-2	0.032
Swelling of joints	2	1-3	1.5	1-3	0.07	1.5	1-2	0.07	1	0-2	0.032
Stiffness of joints	2	1-3	2	1-3	0.08	2	1-3	0.1	1	0-2	0.032
Joint Pain on movement	2	1-3	1.5	1-3	0.07	1.5	1-2	0.07	1	0-2	0.032
Tophi	2	1-3	2	1-3	0.08	1.5	1-2	0.07	1	0-2	0.032

Table 11: Comparison in intensity of symptoms between two treatment groups by Wilcoxone signed rank test

Gouticin					Allopurinol				
Before treatment		After 18th week of treatment			Before treatment		After 18 week of treatment		
Median	IQR	Median	IQR	P-value	Median	IQR	Median	IQR	P-value
13	6-18	3	0-6	0.003	12	6-18	6	0-12	0.05

Table 12: Serum Uric Acid level in test group by Gouticin

Serum Uric Acid level by Gouticin										
Baseline (T0)		After 6 weeks (T1)			At 12th week of treatment (T2)			At 18th week of treatment (T3)		
Median	IQR	Median	IQR	P-value	Median	IQR	P-value	Median	IQR	P-value
9.5	7-10	8.1	7-9	0.125	6.5	6-8	0.02	5.5	5-7	0.01

Table 13: Reduction in serum Uric Acid level in control group by Allopurinol

Serum Uric Acid level by Allopurinol										
Baseline (T0)		After 6 weeks (T1)			At 12th week of treatment (T2)			At 18th week of treatment (T3)		
Median	IQR	Median	IQR	P-value	Median	IQR	P-value	Median	IQR	P-value
9.5	7-11	8	7-10	0.125	7.5	6-9	0.03	6.5	5-8	0.02

Table 14: Overall reduction in serum uric level in Test group and Control group by Wilcoxone Signed Rank Test

Gouticin					Allopuroinol				
Before treatment		After treatment			Before treatment		After treatment		
Median	IQR	Median	IQR	p value	Median	IQR	Median	IQR	p value
9.5	7-10	5.5	5-7	0.01	9.5	7-11	6.5	5-8	0.02

Gouticin is also effective in gouty arthritis resistant to systemic therapy. The outcome of the present study showed that Gouticin at dose of 500 mg twice daily has more potential to treat gout as compared to allopurinol (300 mg once daily).

CONCLUSION

Results of our study indicate that *Apium graveolens, Colchicum autumnale, Zingiber officinale, Tribulus terrestris* and *Withania somnifera* inhibits xanthine oxidase enzyme significantly. All the 07 extracts assayed, demonstrated more than 50% xanthine oxidase inhibitory activity at 100µg/ml. Gouticin, *Zingiber officinale, Tribulus terrestris, Withania somnifera, Colchicum autumnale, Apium graveolen* exhibited 88, 80, 78, 69, 61, 54% inhibition respectively. Gouticin, *Tribulus terrestris, Zingiber officinale, Colchicum autumnale, Withania somnifera* and *Apium graveolens* showed the lowest IC_{50} of 17.3, 19.8, 23.6, 42, 95, 180 µg/ml respectively. In this study, allopurinol showed lower IC_{50} value (6.1µg/ml) as compared to the Gouticin (17.3µg/ml). These *in vitro*

Table 15: Randomized controlled trials of treatment for gouty arthritis

Study (Ref.)	Intervention	Outcome measures and conclusion
Cheng *et al* 2004	Rofecoxib, diclofenac, meloxicam	Pain (10-point scale combining tenderness, function, swelling scores) In this study, rofecoxib was found more effective than diclofenac sodium and meloxicam. Rofecoxib is safe and well tolerated (Cheng *et al*, 2004)
Rubin *et al*. 2004	Etoricoxib, indomethacin	Pain, joint tenderness and swelling, patient global assessment of response. In this study, Etoricoxib was found effective for treatment of acute gouty arthritis. Etoricoxib was comparable in efficacy to indomethacin and it was generally safe and well tolerated (Rubin *et al*, 2004).
Schumacher *et al* 2002	Etoricoxib, indomethacin	Pain, joint tenderness and swelling In this study, Etoricoxib was found effective for treatment of acute gouty arthritis. It is comparable to indometacin. Etoricoxib was generally safe and well tolerated in this study (Schumacher *et al*, 2002).
Shresta *et al*. 1995	Ketorolac, indomethacin	Pain (5-point Wong–Baker faces rating scale) In this study, IM ketorolac and oral indomethacin are similar in the relief of the pain of acute gouty arthritis (Shresta *et al*, 1995).
Siegel *et al*. 1994	ACTH, tiamcinolone acetonide	Number of reinjections required, time to complete resolution of symptoms In this study, Triamcinolone acetonide resulted in fewer rebound attacks and treatment failures than ACTH and required fewer reinjections (Siegel *et al*, 1994).
Maccagno *et al* 1991.	Edodolac, naproxen	Pain, joint tenderness, erythema, warmth, range of motion, patient global assessment, physician global assessment. In this study, etodolac was found more effective than naproxen (Maccagno *et al*, 1991).
Altman *et al*. 1988	Ketoprofen, indomethacin	Pain In this study, Ketoprofen compared favorably for efficacy and safety with indomethacin in the treatment of gouty arthritis (Altman *et al*, 1988).
Ahern *et al*. 1987	Colchicine, placebo	Pain In this study, it was concluded that colchicine is more effective than placebo (Ahern *et al*, 1987).
Butler *et al*. 1985	Flurbiprofen, phenylbutazone	Pain In this study, it was concluded that flurbiprofen is satisfactory alternative to phenylbutazone in the management of acute gouty arthritis (Butler *et al*, 1985).
Sundy *et al*. 2005	PEG-uricase	Serum urate In this study, it was concluded that PEG-uricase is effective antihyperuricemic treatment for gout patients with refractory disease (Sundy *et al*, 2005).
Becker *et al*. 2005	Febuxostat, allopurinol	Serum urate, index tophus area, tophi number, treatment events for gout flare In this study, febuxostat was found more effective than allopurinol in lowering serum uric acid level. Similar reductions in gout flares and tophus area occurred in all treatment groups (Becker *et al*, 2005).
Becker *et al*. 2005	Febuxostat, placebo	Serum urate, gout flare In this study, treatment with febuxostat resulted in a significant reduction of serum uric acid levels at all dosages. Febuxostat therapy was safe and well tolerated (Becker *et al*, 2005).

results, moreover, suggest that the Gouticin and its ingredients are effective in gout. In clinical trials, the evaluation of treatment is significantly improved in the test group compared with control group at the end of therapy. Hypouricemic activity of Gouticin in clinical trial may be due to its uricosuric effect. So it can be concluded that the efficacy of the Gouticin is significant due to its xanthine oxidase inhibitory activity and as well as uricosuric activity as compared as allopurinol that has only xanthine oxidase inhibitory activity. Gouticin is safe alternative because it shows more potential in reducing serum uric acid levels with minimum side effects as compared to allopurinol in selected dose. Gouticin provides anti-inflammatory profile in the treatment of gouty arthritis, while allopurinol lacks this type of activity.

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