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RESEARCH ARTICLE

EFFECTIVENESS OF *CISSUS QUADRANGULARIS, MICHELIA CHAMPACA, CASSIA AURICULATA* AGAINST HYPERURICEMIA IN ALBINO WISTAR RATS

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ABSTRACT

The herbal plants are widely used as therapeutic agents. In this present study we evaluated the efficiency of *Cissus quadrangularis*, *Michelia champaca* and *Cassia auriculata against* hyperuricemia also called *metabolic arthritis*) is a disease due to a congenital disorder of uric acid metabolism. These three plant extract was tested with potassium oxonate and allopurinol to check activity against the gout disease. Then finally it was confirmed that given plant extract with those two chemicals reduced uric acid level in the blood and showed significant changes biochemical assays such as Creatinine, Urea, Total protein, Albumin, Globulin, and Albumin Globulin Ratio. The tests for rheumatoid arthritis such as RA test, C-Reactive protein test, and Antistreptolysin O test showed negative result after treatment.

INTRODUCTION

Since ancient ages plants have served human beings as a natural source of treatments and therapies, among them medicinal herbs have gain attention because of its wide use and less side effects. In current scenario focus on plant research has increased throughout the world and a huge amount of evidences have been collected to show immense potential of medicinal plants used in various traditional systems. More than 15000 plants have been studied during the last 5 year period. Recently scientists are using these renewable resources to produce a new generation of therapeutic solutions. Many of the plant extracts have proven to posses pharmacological actions. The photochemical and pharmacological aspects of *Cissus quadrangularis* Linn. *Cissus quadrangularis,* a perennial climber widely used in traditional medicinal systems of India has been reported to posses bone fracture healing, antibacterial, antifungal, antioxidant, anthelminthic, antihemorrhoidal and analgesic activities. *Cissus quadrangularis* Linn. Has been recognized as a rich source of carotenoids, triterpenoids and ascorbic acid and is proved to have potential for medical effects.

It has been prescribed in Ayurveda as an alternative, anthelmintic, dyspeptic, digestive, tonic, analgesic in eye and ear diseases, and in the treatment of irregular menstruation and asthma. In some parts of world, the whole plant is used in oral re-hydration, while the leaf, stem, and root extracts of this plant are important in the management of various ailments. Some other reports on *Cissus quadrangularis* justifies its effectiveness in management of obesity and complications associated with metabolic disorders **[3]**, as well as its antioxidant and free radical scavenging activity *in vitro*. **(**Mallika and Shyamala, 2005**;** Mehta *et al*., 2001)In current scenario formulations now contain extracts of *Cissus quadrangularis* in combination with other active ingredients, used for the purpose of management of overweight and obesity, as well as complications resulting from these conditions, specifically metabolic syndrome (syndrome X). Phytochemical screening of *Cissus quadrangularis* revealed high contents of ascorbic acid, carotene, anabolic steroidal substances, and calcium. The stem contains two asymmetric tetracyclic triterpenoids, and two steroidal principles. The presence of β-sitosterol, δ-amyrin, δ-amyrone, and flavanoids (quercetin) having different potential metabolic and physiological effects have also been reported. Jakikasem *et al*., 2000**,** Jainu *et al.*, 2004**)**

Current research is now directed towards finding naturally occurring antioxidants and antibacterial agents of plant origin. In this context the flower extracts of *Michelia champaca* were screened for antibacterial and antioxidant activity. *Michelia champaca* belonging to family Magnoliaceae locally known as 'swarnachampaca' or 'sampangi' is a tree with golden-yellow fragrant flowers and aggregate fruits. It is native to India and grows wild in humid tropical evergreen forests. Literature survey reveals that it possesses anti-inflammatory, antimicrobial and leishmanicidal activity.(Vimala *et al*., 1997;Khan *et al*., 2002;Takahashi .,2004)And previously antidiabetic activity of flower buds of *M. champaca*](Jarald *et al*.,2000)and antioxidant, analgesic and cytotoxic activities for methanolic extracts of *M.champaca* had been reported.(Kumar and Saminathan, 1995).Literature survey also reveals that *M. champaca* flowers were effective agents for healing wounds in immune-compromised patients.

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Cassia auriculata L. commonly known as tanner's cassia, also known as "avaram" in Tamil language is a shrub belongs to the Caesalpiniaceae family. The shrub is especially famous for its attractive yellow flowers which are used in the treatment of skin disorders and body odour. It is widely used in traditional medicine for rheumatism, conjunctivitis and diabetes. It has many medicinal properties. Itsbark is used as an astringent, leaves and fruits anthelminthic, seeds used to treat in eye troubles and root employed in skin diseases (Siva and Krishnamurthy, 2005). It is also used for the treatment of ulcers, leprosy, hypouricemia and liver disease (Kumar *et al*., 2002). The antidiabetic, hypolipidemic (Umadevi *et al*., 2006) and antioxidant (Kumaran and Joel, 2007) and hepatoprotective (Kumar *et al*., 2003) effect of *Cassia auriculata* have been reported. It was also observed that flower and leaf extract of *Cassia auriculata* shown to have antipyretic activity (Vedavathy and Rao, 1991). The aim of the present study was to determine the antibacterial activity of various extracts of *Cassia auriculata* flowers which is having traditional claims for several diseases.

MATERIALS AND METHODS

Animal Used

Animals were divided into six groups, each group containing 6 animals. Adult male albino rat of Wister strain weighing around 250 to 300 g were procured from Tamilnadu veterinary and animal sciences university, Chennai. The animals were kept in polypropylene cage (four in each cage) at an ambient temperature of 25+20°C and 55-65% relative humidity. A 12+ 1 hour light and dark cycle was maintained in the animal house till the animal were acclimatized to the laboratory conditions, and were fed with commercially available rat pellet (Hindustan Lever Ltd., Bangalore India) and had free access to water. The experiments were designed and conducted in accordance with the institutional animal ethics committee.

Animals were marked using picric acid solution for identification. The control animals had the marking on tail (control). The test animals-I were induced a gout using immobilization method which marking on right forelimb. The test animals-II were induced a gout by Potassium oxonate, which has marking on left forelimb. The test animals-III were induced gout and treated with *Cissus quadrangularis* which has marking on right hind limb. The test animals-IV was treated with *Michelia champaca* and V with *Cassia auriculata* L. The experimental animals are captioned here as control, Test animal I, II, III, IV, V and VI.

Preparation of plant extracts

Extraction of Cissus quandrangularis

The coarse powder 150 grams of the given samples (*Cissus quadrangularis L* and *Solanum torvum)* were extracted using 600 ml of hydroalcohol (20:80) by continuous hot percolation with the help of Soxhlet apparatus until the extraction procedure is complete. The successive extractions were done separately for each solvent namely, ethanol and water. The powder solvent ratio employed for the present study was 1:4. On completion, the extracts were filtered and the solvents were removed by distillation and dried under reduced pressure and controlled temperature 50º – 60ºC and refrigerated until use. The samples of the two extracts were subjected to various analyses such as organoleptic characters (Wallis, 1985) fluorescence studies (Chase and Pratt, 1949) physico-chemical properties (Anonymous, 1965) and preliminary phytochemical screening (Tyler *et al*., 1985; Kokate *et al*., 1997)

Extraction of Michelia champaca

The leaves of *M. champaca* were dried under shade and then powdered with a mechanical grinder. The powder was passed through sieve No. 40 and stored in an airtight container for further studies. Hydro-alcoholic extract of *M. champaca* was obtained by cold maceration method. The extracts were concentrated by vacuum distillation to reduce the volume to 1/10; the concentrated extracts were transferred to 100ml beaker and the remaining solvents were evaporated on a water bath. Then they were cooled and placed in desiccators to remove the excessive moisture. The dried extracts were packed in airtight containers and used for further (Trease ***et al.,*** 2003).

Extraction of Cassia auriculata

Methanol extract was prepared by taking 50 g of *Cassia auriculata* dried flower powder in a separate container, to this 200mL of methanol was added and kept for 24 h in a shaker. Filtered through eight layers of muslin cloth and extract was collected, the extraction process was repeated twice. The collected extracts were pooled. Ethanol extract was prepared like methanol extract. Water extract wasprepared by taking 50 g of *Cassia auriculata* flower powder in a separate container, to this 200mL of water was added and boiled for 2 h in a mild heat and kept for 24 h. Then filtered and extract was collected. The extraction process was repeated twice. Then the collected filtrates were pooled (Akpulu *et al*., 1994).

Experimental design

For control I: Water with routine food.
For Induce animals II: Pottasium oxonate for 3months
For test animal III: potassium oxonate + *Cissus quadrangularis* for 3months
For test animal IV: potassium oxonate + *Michelia champaca* L for 3months
For test animal V: potassium oxonate + *Cassia auriculata* L for 3months
For test and treatment with allopathy : potassium oxonate + Allopurinol for 3months

Specimen collection

After 21 days the animals were sacrificed by cervical decapitation, for the analysis of biochemical estimation, by applying local anesthesia using chloroform. The blood sample were collected and then transferred in to tubes. The tubes of the blood sample and the vials of serum sample were marked similarly for identification.

1. The blood samples were centrifuged at 1500 rpm for 10 minutes.
2. Serum sample collected was transferred into the vials.
3. The specimens collected were used for the biochemical estimations.
4. Biochemical estimations were follows.

The chemicals and reagents needed for the above experiments were obtained from Span Diagnostics Ltd, Cochin.

Estimation of Test for Serum Uric Acid by Caraway Method

Principle

Uric acid is determined colorimetrically by reduction of alkaline phosphotungastate to tungsten blue. The intensity of the final colour was measured at 630 nm or with a red filter. Phosphotungstic acid was used as the protein precipitant, this eliminates the use of tungstate and sulphate anions, which are frequent of turbidity, interfering non –uratechromosmes are eliminated by tri sodium phosphate, the carbonate urea reagent helps to stabilize the colour in addition to giving the alkaline medium necessary for colour development.

Reagents

1. Sulphuric acid , 2/3 N
2. Sodium tungstate 10% w/v
3. Sodium carbonate 14% w/v
4. Phosphotungstate
5. Stock uric acid standard, 100 mg%.

Depolarization of the samples

1 ml of the sample was taken for the test 8 ml of distilled water, 0.5 ml of 2/3 N sulphuric acids, 0.5 ml of 10% sodium tungstate added to the test and mixed well and centrifuged for 10 minutes.

3 ml of supernatant is taken for the test tube. In blank 3 ml distilled water and for 3 ml of working standard solution are used and 1 ml of sodium carbonate, Phosphotungstate was added to all tubes. All the tubes were mixed well and stand in dark place for 15 minutes and measured the O.D. of Blank (B), Standard (S), and Test (T) against distilled water on the colorimeter used red filter.

Calculation

$$\text{Serum uric acid in mg/dl} = \frac{\text{O.D. Sample} \times \text{standard concentration}}{\text{O.D. Standard.}}$$

Normal Values

Men = 2.5 – 7.0 mg / dL
Women = 1.5 – 6.0 mg/dL.

Test for Albumin by BCG Method

Principle

Colorimetric determination of serum albumin using bromocresol green (BCG) at pH 4.2 resulting in the formation of a green color, which shows maximum absorbance at 630nm.

Reagents

R1 = bromocresol green, succinate buffer (pH 4.2), brij 35, sodium azide,
R2 = bovine albumin standard 5 g/ dL.

Procedure

0.5 ml of sample was taken in the clean test tube. Added the 2.5 ml of R1 in test tube. Only 2.5 ml of R2 was taken in the blank tube. 2.5 ml of R2 was taken in the standard tube, mixed well the all the tubes and incubated at 20 to 25 C after 5 minutes Read the optical density in colorimetrically at 639nm.

Calculation

$$\text{Albumin in g /dL} = \text{O.D. Sample} \times \frac{n \text{ [Where n= 5.0 g/ dL]}}{\text{O.D. standard}}$$

Normal Values

Serum albumin = 3.8 – 5.4 g / dL.

Test for Creatinine by Jaffe's Method

Principle

Serum or plasma is mixed with acid tungstate to protein precipitant filtrate the protein. After centrifugation a portion of the supernatant is mixed with alkaline picrate which there forms a yellow red complex with creatinine. The absorbance of which is measured at 505 nm or using a green filter.

Reagents

1. Picric acid, 0.04 M.
2. Acid tungstate.
3. Sodium hydroxide, 0.7 M.
4. Stock creatinine, 66mg %
5. Working creatinine, 0.066 mg/dl.

Procedure

0.5 ml of plasma is added to 4 ml of tungstate shake and centrifuged 0.3 ml of supernatant taken in a test tube. In blank 3.0 ml of distilled water and for standard 2.5 ml distilled water and 0.5ml of working standard are used 1.0 ml of picric acid is added to fill the tubes and then 1.0 ml of 0.7 M sodium hydroxide is added and mixed well, Kept at room temperature for 15 C at 505nm.

Calculation

$$\text{Serum creatinine in mg /dL} = \frac{\text{O.D. Sample} \times \text{Standard concentration}}{\text{O.D. Standard.}}$$

Normal values

Serum creatinine = 0.4 – 1.5 mg/dL.

Note

Urine samples are diluted to 1 to 10 diluted urine samples is subjected to the conditions as the test. The final result is multiplied by 10 to get the values in mgs creatinine / 100 ml urine.

Storage and stability

The shelf life of the reagents as supplied when stored at 15 to 25^0 C is indicated on the labels the working reagent is stable for 24 hours at 20 to 25 ^{0} C and for one week at 2 to 8 ^{0} C

Estimation of Erythrocyte Sedimentation Rate (ESR)

Westergren's Method

This method consists of an ESR tube, which has a marking of 200 mm. It resembles 1 ml pipette and its internal diameter is 1 mm.

Procedure

0.4 ml of 3.8% sodium citrate solution was taken and then 1.6 ml of whole blood was added and mixed well. From this

mixture, the blood was taken into Westergren's tube up to "O" mark of the ESR pipette and then it was fixed onto the ESR stand. Reading was taken after 1 hour with interval of 20′, 40', 60'.

Normal Value

Male: 9 mm/hr

Female: 20 mm/hr

Rheumatoid Arthritis (RA) Test

Method

1. Qualitative test: [Slide test]

The entire reagent was brought to the room temperature before using for the test.

1. Undiluted serum sample was used in this method. One drop of undiluted serum was placed over the circulated area of a provided, cleaned slide.
2. One drop of latex RA reagent was added to the above drop and the mixture was thoroughly mixed with the disposable applicator sticks and the content was spread to the edge of the circle.
3. The slide was gently rocked to and fro for 2 minutes. Then the slide was macroscopically observed for agglutination under direct light source. The results were recorded and interpreted.

Interpretation

Interpretations of results of qualitative slide test

S.No.	Observation	Conclusion
1.	Agglutination	Positive
2.	Smooth suspension without any noticeable change	Negative

Quantitative Method

1. Serum sample was serially diluted in this test. As it was carried out in slide instead of tubes, it is called semi quantitative test.
2. Series of dilution of the test serum in normal saline was prepared in order to get the dilution factors such as 1:2, 1:4, 1:8, 1:16, 1:32 and 1:64.
3. One drop of each of those dilutions was tested with one drop of latex RA reagent.
4. Agglutination was observed as in the case of qualitative test. The highest dilution at which the agglutination is observed was taken as RA titer of the test serum. The results were recorded and interpreted.
5. The reciprocal of the dilution factor (for example, if the dilution factor is 1: 8, then 8 is the reciprocal) is multiplied with the sensitivity factor to get the concentration of RA.

Anti-Streptolysin (ASO) Test

This test is used to detect prior infection by group A *Streptococcus*, the bacteria responsible for diseases such as bacterial endocarditis, glomerulonephritis, rheumatic fever seen with joint pain, Scarlet fever and strep throat. The ASO antibody may be found in the blood weeks or months after the strep infection has gone away.

Method

1. Qualitative test: [Slide test]

The entire reagent was brought to the room temperature before using for the test.

1. Undiluted serum sample was used in this method. One drop of undiluted serum was placed over the circulated area of a provided, cleaned slide.
2. One drop of latex ASO reagent was added to the above drop and the mixture was thoroughly mixed with the disposable applicator sticks and the content was spread to the edge of the circle.
3. The slide was gently rocked to and fro for 2 minutes. Then the slide was macroscopically observed for agglutination under direct light source. The results were recorded and interpreted.

Interpretation

Interpretations of results of qualitative slide test:

S.No.	Observation	Conclusion
1.	Agglutination	Positive
2.	Smooth suspension without any noticeable change	Negative

Quantitative Method

1. Serum sample was serially diluted in this test. As it was carried out in slide instead of tubes, it is called semi quantitative test.
2. Series of dilution of the test serum in normal saline was prepared in order to get the dilution factors such as 1:2, 1:4, 1:8, 1:16, 1:32 and 1:64.
3. One drop of each of those dilutions was tested with one drop of latex ASO reagent.
4. Agglutination was observed as in the case of qualitative test. The highest dilution at which the agglutination is observed was taken as ASO titer of the test serum. The results were recorded and interpreted.
1. 5.The reciprocal of the dilution factor (for example, if the dilution factor is 1: 8, then 8 is the reciprocal and the result can be given in 800 units) is multiplied with the sensitivity factor to get the concentration of ASO. Anti-streptolysin O more than 200 is considered positive.

C - reactive protein (CRP) Test

Method

1. Qualitative test: [Slide test]

The entire reagent was brought to the room temperature before using for the test.

1. Undiluted serum sample was used in this method. One drop of undiluted serum was placed over the circulated area of a provided, cleaned slide.
2. One drop of latex CRP reagent was added to the above drop and the mixture was thoroughly mixed with the disposable applicator sticks and the content was spread to the edge of the circle.

3. The slide was gently rocked to and fro for 2 minutes. Then the slide was macroscopically observed for agglutination under direct light source. The results were recorded and interpreted

Interpretation

Interpretations of results of qualitative slide test:

S.No.	Observation	Conclusion
1.	Coarse agglutination	Strongly positive
2.	Fine agglutination	Weakly positive
3.	Smooth suspension without any noticeable change	Negative

Quantitative Method

1. Serum sample was serially diluted in this test. As it was carried out in slide instead of tubes, it is called semi quantitative test.
2. Series of dilution of the test serum in normal saline was prepared in order to get the dilution factors such as 1:2, 1:4, 1:8, 1:16, 1:32 and 1:64.
3. One drop of each of those dilutions was tested with one drop of latex CRP reagent.
4. Agglutination was observed as in the case of qualitative test. The highest dilution at which the agglutination is observed was taken as CRP titer of the test serum. The results were recorded and interpreted.
5. The reciprocal of the dilution factor (for example, if the dilution factor is 1: 8, then 8 is the reciprocal) is multiplied with the sensitivity factor to get the concentration of CRP.

RESULTS

Effect of herbal medicinal plants

Pirandai, Champagne, Avaram, were the herbals selected for gout treatment. Ten patients were selected for each herbal and treatment was given.

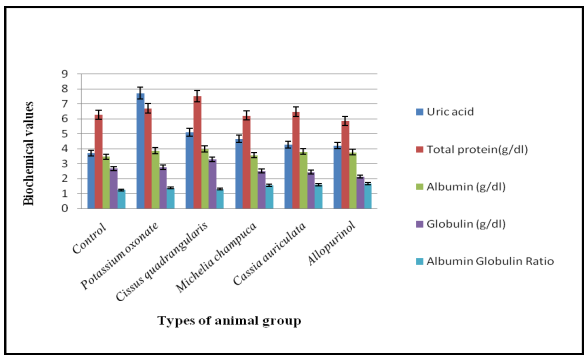


Figure 1 Effect on Serum biochemical parameters in rat

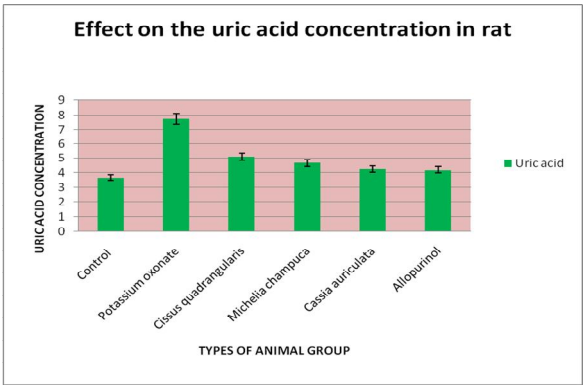
Effectiveness of Cissus quadrangularisis

In using Pirandai, the average level of Serum Uric acid, Albumin, Creatinine and Urine Uric acid were 9.08 mg/ dL, 5.72 g/dL 1.61 mg/ dL and 1133.1 mg/dL respectively. After herbal treatment the values reduced to 5.10 mg/dL, 3.98 g/dL, 0.57 mg/dL and 157.3 mg per 24 hours respectively.

STATISTICAL ANALYSIS

Using the level of Serum Uric acid, Albumin, Creatinine, and Urine Uric acid, the average (mean) was calculated. From the mean value the standard deviation (S.D) are derived the Control tests were followed above method.

Figure 2 Effect on Uric acid concentration in rat



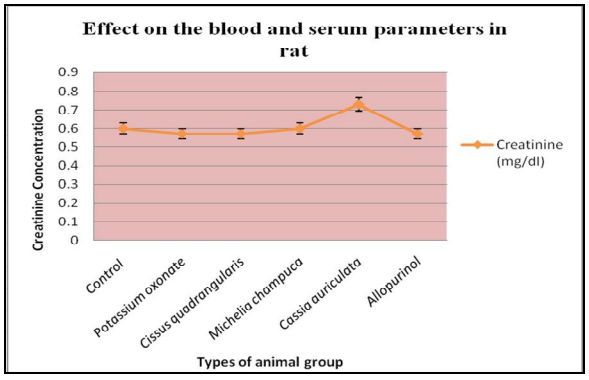


Figure 3 Effect on the blood and serum parameters in rat

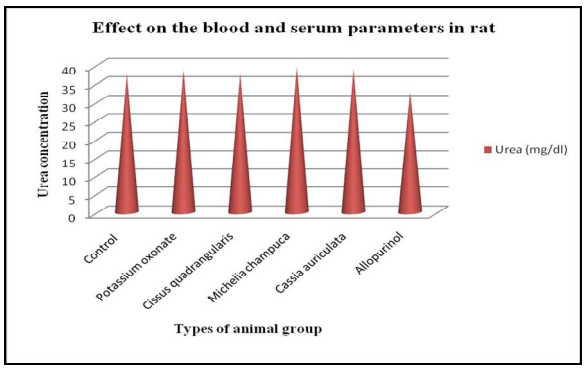


Figure 4 Effect on the blood and serum parameters in rat

DISCUSSION

Gout is a systemic disease caused by the buildup of uric acid in the joints, causing inflammation, swelling and pain. A diet of foods, which puts more uric acid into the blood stream than the kidneys, can filter. This condition is called Hyperuricemia. Uric acid is the final metabolic products of purine metabolism for human and apes. Most other mammals have the ability to catabolism purines one step further to allontoin a much more water-soluble end product.

Experimental rat was taken to induce anti-gout using immobilization method. Potassium oxonate induced rat were treated herbal *Cissus quadrangularis*, *Michelia champaca,*

Table 1 Effectiveness of selected herbals in Potassium oxonate induced Gout in Wistar rats

S.No	Biochemical parameters	Control	Potassium oxonate	*Cissus quadrangularis* Treated	*Michelia champuca* Treated	*Cassia auriculata* Treated	Allopurinol Treated
1	Uric acid (mg/dl)	3.70±0.20	7.73±0.20	5.10±0.10	4.67±0.18	4.27±0.21	4.20±0.26
2	Creatinine (mg/dl)	0.60±0.02	0.57±0.04	0.57±0.06	0.60±0.02	0.73±0.02	0.57±0.05
3	Urea (mg/dl)	37.37±1.98	38.47±2.74	37.50±1.80	39.43±2.61	38.67±1.93	32.70±1.95
4	Total protein(g/dl)	6.27±0.50	6.70±0.20	7.53±0.84	6.22±0.33	6.46±0.43	5.85±0.30
5	Albumin (g/dl)	3.47±0.35	3.87±0.35	3.98±0.50	3.57±0.32	3.80±0.26	3.76±0.25
6	Globulin (g/dl)	2.67±0.15	2.76±0.24	3.28±0.26	2.50±0.40	2.43±0.35	2.13±0.06
7	Albumin Globulin Ratio	1.23±0.11	1.38±0.08	1.30±0.23	1.55±0.11	1.60±0.09	1.67±0.15

Table 2 Effectiveness of selected herbals in Potassium oxonate induced Gout in Wistar rats with Rheumatoid arthritis parameters

S.No	Immunological parameters	Control	Potassim oxonate	*Cissus quadrangularis* Treated	*Michelia champuca* Treated	*Cassia auriculata* Treated	Allopurinol Treated
1	RA	Negative	Negative	Negative	Negative	Negative	Negative
2	ASO	Negative	Negative	Negative	Negative	Negative	Negative
3	CRP	Negative	Negative	Negative	Negative	Negative	Negative

*Cassia auriculata***.** Serum sample collected were analyzed for any changes in biochemical parameter.

RA was done and it was found to be negative compared to control. Similar results were also found for the animals induced with potassium oxonate and treated with *Cissus quadrangularis, Michelia champuca, and Cassia auriculataand with* Allopurinol.

ASO was done and it was found to be negative compared to control. Similar results were also found for the animals induced with potassium oxonate and treated with *Cissus quadrangularis, Michelia champuca, Cassia auriculata* and with Allopurinol.

CRP was done and it was found to be negative compared to control. Similar results were also found for the animals induced with potassium oxonate and treated with *Cissus quadrangularis, Michelia champuca, Cassia auriculata* and with Allopurinol.

Uric acid level was increased than the control.
There is no significant changes biochemical parameter (Creatinine, Urea, Total protein, Albumin, Globulin, and Albumin Globulin Ratio).

Uric acid is a strong scavenger of reactive oxygen species, which are known to contribute to the development of atherosclerosis, while the incidence of atherosclerotic diseases is rather high in patients with gout. Among the established risk factors for atherosclerosis, oxidized LDL is believed to play a major role in its development and progression. Allopurinol and its active metabolite, oxypurinol, have been suggested to possess an antioxidant ability to scavenge the hydroxyl radical. Therefore, allopurinol may be beneficial in the prevention of LDL oxidation (Kaul and Kulkarni, 1978)
Rheumatoid arthritis (RA) is a systemic disease whose morbidity exceeds its mortality (Adzu, 2002).

CONCLUSION

In this present study the anti-gout agents tested, *Cissus quadrangularis* was found to be the better one. Next to that *Michelia champuca L* and the *Cassia auriculata L*, were found to be effective in gout reduction. Allopurinol the common allopathic medicine was found to be more effective than others in reducing gout markers. The animals were tested with gout inducing drug and found that it has not induced Rheumatoid arthritis. Hence, while testing the parameters, there were negative results for RA and the herbals can be declared as anti-gout than anti rheumatoid arthritis. In order to work with RA immunological autoimmune induction is needed. The results have shown that the test plants can be strongly suggested for gout treatment. Anyway the toxicity study is strictly needed to advocate these drugs for gout relief.

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