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COMPARATVE URIC ACID LOWERING STUDIES OF ALLOPURINOL WITH AN INDIGENOUS MEDICINAL PLANT IN RABBITS

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Abstract: The aim of this research was to carry out a comparative study of lowering of uric acid by the use of dried powder of *Colchicum luteum* and allopathic drug (allopurinol) in rabbits, to determine whether herbal drugs can be used by patients instead of allopathic drugs. The herbal medicine, dried corm powder of *Colchicum luteum* 2.5 mg/kg/day and dried powder of allopurinol 2 mg/kg/day an allopathic medicine, was used in the study. The results of these medicines were observed in animal model, using 12 adult rabbits, which were divided into three groups A, B and C, respectively, where group C was taken as control. The SPSS version 17 was used for statistical analysis and analysis of variance (ANOVA) was used for comparing the data in different groups and the level of significance was 5%. It was resulted that dried corm of *Colchicum luteum* significantly reduced the uric acid in adult rabbits as reduced by allopathic medicine – allopurinol. In the light of present research we concluded that the herbal medicines can be used in lieu of allopathic drugs. Thus, the risk of side effects that are associated with the prolonged use of allopathic drugs can be minimized.

Keywords: herbal medicine, *Colchicum luteum,* allopurinol, reduction in uric acid

Hyperuricemia, a common biochemical abnormality can be termed as the increased concentration of serum uric acid beyond the limit of solubility (approximately 6.8 mg/dL) (1). Hyperuricemia is the abnormally raised uric acid concentration in the blood (2), usually defined as more than 6.8 mg/dL (3). Serum uric acid levels greater than 6 mg/dL in females and 7 mg/dL in males deal with hyperuricemia (4). Hyperuricemia can also be termed as uric acid concentration greater than 7.0 mg/dL in the blood (5), which results in diseases like gout and arthritis, i.e., rheumatoid arthritis. For a long time, hyperuricemia and gout were used interchangeably, but uric acid is now confirmed as a nuisance factor for many other abnormalities in the body metabolism. It is well established that hyperuricemia is the actual cause of gout (6). Deposits of crystals of monosodium urate in tissues lead to arthritis and gout i.e., gouty arthritis characterized by swelling of one or more joints in the lower extremities, erythema and recurring attacks of pain (7). Elevated serum uric acid level in combination with joint symptoms help in diagnosing gouty arthritis (8). Hyperuricemia may lead to an autosomal dominant renal disease i.e., familial juvenile hyperuricemic nephropathy (FJHN). The diseases caused by hyperuricemia include gout or arthritis, multiple complications and metabolic syndromes like insulin resistance and dyslipidemia and it may also be a cause of severe cardiovascular diseases and chronic kidney diseases and renal stones, if not managed properly (9).

Uric acid is the final product of protein metabolism in human beings (10). Allantoin is the end product in lower animals, which is much more soluble than uric acid found in humans. Deficiency of hepatic enzyme, uricase and lower fractional excretion of uric acid are the main causes of higher levels of uric acid in the blood in human beings (11). Hyperuricemia may result from decreased excretion (90%) of uric acid, overproduction (10%) of uric acid, or as a result of combined mechanism (12).

Food and Drug Administration approved allopurinol in 1966 for treatment of hyperuricemia and gout (13). Allopurinol helps in the management of hyperuricemia and thus helps in the treatment of

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gout and arthritis by inhibiting the biosynthesis of purines and pyrimidines in man (14).

Colchicum luteum is being used generally in the Unani system of medicines as a main ingredient in many herbal formulations for the treatment of several diseases since a long time. *Colchicum luteum* belongs to the family Liliaceae (15). The genus colchicum includs almost 42 species mostly endemic to the Middle East and South Africa to Western Europe and Asia (16). Corms of the *Colchicum luteum* are widely used in the treatment of gout, arthritis and several diseases of the spleen and liver. The corms are also used for the purification of blood (17). The seeds and corms of the plant are used by practitioners for the treatment of arthritis, gout, rheumatic fever and several complications of spleen and liver (18).

The active principle in colchicum is the alkaloid "colchicine", which provides dramatic relief from acute attacks of gouty arthritis. This antirheumatic effect is highly specific for gout and colchicum has little effect on non-gouty arthritis and no analgesic property.

MATERIALS AND METHODS

All the drugs used were of pharmaceutical/analytical grade with known assay. Fructose (BDH Laboratory Supplies, London), allopurinol USP30/BP2008, Batch No. 20111102, (Shanghai Chemicals & Pharmaceuticals Co. Limited, China) provided by Pharmedic Laboratories, Lahore, dried corms of *C. luteum* (R&D Department, Qarshi Laboratories, Lahore) and uric acid determination kit (Crescent Diagnostic, Lahore). Analytical balance (Mettler Toledo AB54-S), centrifuge (BHG), incubator/oven (Memmert Schwabach, Germany), spectrophotometer (UV-1700, Shimadzu), refrigerator/freezer (Dawlance), BD syringes, capsule shells and wooden restraining boxes were use in the study.

Animals

Twelve adult healthy albino rabbits with weights ranging from 1.3 kg to 1.6 kg were bought from the city local market and they were housed in clean metal hutches in the animal house of University College of Pharmacy, University of the Punjab, Lahore, Pakistan. The animals were exposed to their usual feeding pattern and green fodder and water were provided to all of the rabbits. The temperature of the animal house was maintained at 22.5 ± 2^{o}C. Before conducting the study, the rabbits were acclimatized in the animal house for a week.

Study protocols

All the twelve rabbits were administered with oral fructose syrup for ten days to induce hyperuricemia. A daily dose of fructose syrup, i.e., 1 gram per kg per day was given to all of the rabbits (19). The weighed amount of fructose was dissolved in distilled water at 37^{o}C. Syringes were used to dispense the appropriate preparation and amounts into the mouth of the animals. After attaining a specific

Table 1. Comparison of uric acid at different follow-ups in group "A" (allopurinol).

No.	Follow up duration	Mean (mg/dL)
0	Base line (day 0)	8.50 ± 1.41
1	1st Follow up (day 01)	6.96 ± 0.42
2	2nd Follow up (day 04)	6.95 ± 1.87
3	3rd Follow up (day 07)	3.99 ± 0.26
4	4th Follow up (day 10)	5.85 ± 0.72
5	5th Follow up (day 13)	4.75 ± 0.68
6	6th Follow up (day 16)	4.16 ± 0.57
7	7th Follow up (day 19)	3.78 ± 0.39
8	8th Follow up (day 22)	3.53 ± 0.40
9	9th Follow up (day 25)	3.16 ± 0.29
10	10th Follow up (day 28)	2.94 ± 0.29
11	11th Follow up (day 31)	2.85 ± 0.28
12	12th Follow up (day 34)	2.63 ± 0.24

level of animal's uric acid, they were divided into three groups with four rabbits in each group. Each group was labeled and received the treatment as follows:

GROUP A: In this group, all rabbits were administered with allopurinol 2 mg/kg/day for six weeks.

GROUP B: In this group, all rabbits were administered with fine powder of *C. luteum* filled in capsules with dose of 2.5 mg/kg/day and fructose syrup (1 g/kg/day) for six weeks.

GROUP C: This group was kept as control group and all rabbits of this group received fructose syrup 1 g/kg/day for six weeks.

Serum uric acid was measured as an indicator of hyperuricemia.

Collection of blood samples

Blood samples of all the rabbits were collected at the start of the experiment to measure the normal values of serum uric acid. Then, blood samples were collected after an intervals of two days till the end of study.

Before the start of sampling, the rabbit was held in the wooden restraining box in such a way that its head protrudes outwards. The rabbit's ear was shaved with the help of the sterilized blade, to make vein more prominent. Blood of each rabbit was collected into the blood collecting tubes after puncturing the vein of the right ear with 21-gauge syringes after an interval of two days. After collecting the blood samples, tubes were marked for identification with specific codes and kept in container containing ice cubes in order to coagulate the blood samples for almost an hour. The coagulated blood samples were then centrifuged at 4000 rpm for about 15 min at room temperature to separate the serum. The clear supernatant serum was then collected in previously marked Eppendroff tubes and stored at –23°C to –18°C in a freezer before analysis.

In order to make multi blood sample collection possible from the one rabbit, the blood sampling started from the apex to base of the ear, so that vein would not be blocked.

Analysis of serum uric acid

Analysis of uric acid was performed by using the enzymatic colorimetric test, URICASE/PAP method.

Quantitative analysis of uric acid helps in diagnosing hyperuricemia, gout, renal dysfunction, diabetes and other several conditions. Uricase catalyzes the reaction and uric acid is oxidized to allantoin and H_2O_2 reacts with 4-aminoantipyrine and 3,5-dichloro-2-hydroxybenzenesulfonate in the presence of peroxidase, which resulted in the formation of a quinoneimine dye, the concentration of which is in direct proportion to the concentration of uric acid at 546 nm (20).

Statistical analysis

Statistical analysis was performed using SPSS version 17. The quantitative data were given as the mean ± SD along with their SEM and range (lowest thorough highest value). The appropriate line charts

Table 2. Comparison of uric acid at different follow-ups in group "B" (*Colchicum luteum*).

No.	Follow up duration	Mean (mg/dL)
0	Base line (day 0)	7.87 ± 1.79
1	1st Follow up (day 01)	7.77 ± 0.41
2	2nd Follow up (day 04)	7.37 ± 0.85
3	3rd Follow up (day 07)	4.20 ± 0.75
4	4th Follow up (day 10)	6.34 ± 0.50
5	5th Follow up (day 13)	5.27 ± 0.29
6	6th Follow up (day 16)	4.40 ± 0.14
7	7th Follow up (day 19)	4.05 ± 0.13
8	8th Follow up (day 22)	3.81 ± 0.10
9	9th Follow up (day 25)	3.57 ± 0.24
10	10th Follow up (day 28)	3.36 ± 0.17
11	11th Follow up (day 31)	3.28 ± 0.18
12	12th Follow up (day 34)	3.11 ± 0.13

along their description were incorporated for visual presentation and visual comparison of data. For statistical analysis, analysis of variance (ANOVA) was used for comparing the data in different groups. Friedman test (alternative to repeated measurement ANOVA), was used for the comparison of different follow ups in three treatment groups. The level of significance was established at (less or equal) the 5% probability level.

RESULTS AND DISCUSSION

In this study, hyperuricemia was induced in twelve rabbits for the comparative study of indigenous medicinal plant (*Colchicum luteum*) and allopathic drug (allopurinol) for lowering uric acid level. At each follow up study, it was observed that there was a prominent fall in uric acid level, which gave significant results till the end of studies. Allopurinol is an isomer of hypoxanthine and inhibits the activity of xanthene oxidase, the main enzyme responsible for oxidation of hypoxanthine and xanthene, which give rise to uric acid synthesis (21). Increased concentration of xanthene and hypoxanthine are converted to closely related ribotides i.e., adenosine and guanosine monophosphates. The raised level of ribotides can cause inhibition of amidophosphoribosyl transferase i.e., the rate limiting and the first step in the synthesis of purines in the body, by feedback mechanism (22).

In the present study, allopurinol dramatically reduced uric acid in Group A of rabbits from 8.50 mg/dL to 2.63 mg/dL in twelve follow ups. Allopurinol helps in lowering serum concentration of uric acid by decreasing the purine biosynthesis. This reduction in the level of uric acid was very significant as shown in Table 1.

In group A (treated with allopurinol), the mean uric acid at base line was 8.50 mg/dL, which decreased at 12th follow up to 2.63 mg/dL, respectively. The mean uric acid was statistically significantly decreased over a period of 12 follows up with a p-value < 0.05 as compared to Group C (control group).

On the other hand, in Group B of rabbits the herbal medicine *Colchicum luteum* showed similar significant effects as it gradually reduced the uric acid from 7.87 mg/dL to 3.11 mg/dL in 12th follow ups as shown in Table 2.

The mean uric acid was statistically significantly decreased over a period of 12 follows up with p-value < 0.05 as compared to Group C (control group).

The main phytochemical principles in *Colchicum luteum* possess alkaloids i.e., colchicine, lumicolchicine, N-desacetyl-N-formylcolchicine, 2-desmethylcolchicine and luteidine (23). Among these alkaloids, colchicine is believed to be one of the most important anti-inflammatory compounds procured from *Colchicum luteum* and its anti-inflammatory activity is characterized by inhibiting microtubules in the proinflamatory cells comprising of macrophages (24). The mean uric acid in group C (control group i.e., hyperuricemic) at base line 7.74 mg/dL was raised to 10.58 mg/dL at the end of study (p-value > 0.05).

Table 3. Comparison of uric acid at different follow-ups in group "C" (control).

No.	Follow up duration	Mean (mg/dL)
0	Base line (day 0)	7.74 ± 0.66
1	1st Follow up (day 01)	8.18 ± 0.2
2	2nd Follow up (day 04)	8.23 ± 0.25
3	3rd Follow up (day 07)	8.48 ± 0.91
4	4th Follow up (day 10)	8.81 ± 5.08
5	5th Follow up (day 13)	9.86 ± 0.85
6	6th Follow up (day 16)	10.78 ± 0.54
7	7th Follow up (day 19)	10.53 ± 1.34
8	8th Follow up (day 22)	10.38 ± 0.71
9	9th Follow up (day 25)	9.99 ± 0.38
10	10th Follow up (day 28)	10.14 ± 0.84
11	11th Follow up (day 31)	10.0 ± 0.59
12	12th Follow up (day 34)	10.58 ± 0.00

Group A (treated with allopurinol) gave the best results and lowered uric acid level to the lowest level as compared to the Group B (treated with *Colchicum luteum*). However, *Colchicum luteum* can be used as a good alternative in case of patients suffering from severe liver damage or renal failure or those patients who are found hypersensitive to allopurinol.

CONCLUSION

The *Colchicum luteum* reduced the uric acid at significant level as does allopurinol, which is a synthetic allopathic drug. *Colchicum luteum* is not only used in gout but it can also be used as analgesic and in rheumatoid arthritis. One of the major problems with this herbal formulation is that the active ingredients are not well defined. It is important to know the active component and their molecular interaction, which will help to analyze therapeutic efficacy of the product and also to standardize the product. Efforts are now being made to investigate the mechanism of action of some of these plants using model systems. Care must be taken while using *Colchicum luteum*. It must be free from toxic adulterations. Due to the poisonous nature of the herb, it must be used only under strict professional supervision. Dose of the herbal drug must not exceed beyond limits, as it may cause severe gastric disturbances. If this herb is used according to the prescribed instructions, then it can be a better and competent alternative of allopathic medication in the management of hyperuricemia and for the treatment of diseases associated with it. Newer approaches utilizing collaborative research and modern technology in combination with established traditional health principles will yield rich dividends in the near future in improving health, especially among people who do not have access to the use of more costly western systems of medicine.

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