Anti-hyperuricemic potential of *Rhododendron tomentosum* Harmaja syn. *Ledum palustre* L. 30c and 1M in potassium oxonate induced rat model

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Hyperuricemia is a common metabolic disorder and several homeopathic ultra-high dilutions are being used in the treatment of hyperuricemia and its related diseases. Conventional treatment for the hyperuricemia is allopurinol but it gives many side effects like allergic reactions, gastrohepatic ailment, hepatic and renal complaints. It is the need of the hour to introduce an alternative system of medicine with minimal side effects. The study aimed to find hypouricemic effects of *Ledum palustre* 30c and 1M in potassium oxonate induced hyperuricemia rat model. The study comprised of 11 groups of rats (E = 33). All the groups except normal control were treated with potassium oxonate. Normal control group received distilled water, hyperurecemic control group succussed alcohol mixed in distilled water. Allopurinol, *Ledum palustre* 30c and 1M were administered for one day, 3 day and 7 days (single dose/day) in different study groups (3×3 = 9 groups). Blood samples were collected by rat tail vein bleeding. Serum uric acid and serum creatinine levels were checked by using standard kits. Student's *t*-test for independent means was used for statistical analysis of difference between the groups. *p* ≤ 0.05 (two tailed value) was considered significant. Oral administration of *Ledum palustre* 30c and 1M decreased serum uric acid levels of hyperurecemic rats in time dependent manner. 3 day and 7 day administration of *Ledum palustre* 30c and 1M reduced serum uric acid level more significantly as compared to one day administration. However, allopurinol normalized serum uric acid levels in all study groups. The present study indicated marked hypouricemic effects of *Ledum palustre* 30c and 1M in hyperuricemia induced by potassium oxonate in rats. However, clear conclusion of hypouricemic potential of *Ledum palustre* required replication of experiment.

**Keywords:** Anti-hyperuricemia, *Rhododendron tomentosum* Harmaja syn. *Ledum palustre* L., Potassium oxonate, Animal mode, Silkworm

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Hyperuricemia is a common metabolic disorder worldwide with an increased level of serum urate/ uric acid (UA) up to 6.8 mg/dL. It may be caused by under excretion or over production of uric acid or combination of both. It is also known as rich man disease. Several medical conditions are also responsible for this condition, i.e., obesity, hepatitis, kidney failure, glomerulonephritis and nephritic syndrome. It is a precursor of gout, a rheumatic inflammatory disease characterized by the deposition of uric acid in the form of monosodium urate (MSU) crystals in joints. MSU crystals stimulated a powerful inflammatory reaction resulted in acute pain in joints. There are various marketed drugs used for the treatment of hyperuricemia. Currently used hypouricemic medicines allopurinol has some side effects such as gastrointestinal problems, which limit their use in patients. Therefore, there is need for new hypouricemic agents. It becomes need of the hour and era to introduce an alternative system of medicine with minimal side effects. Homeopathic system of medicine is an alternative system of medicine with least side effects and this system is based on presence of nano bubbles ultra-high dilutions.

*Ledum palustre* also known as marsh tea is an aromatic medicinal plants belongs to Ericaceae family. It is an ever green shrub and well known since 18th century. The flowers are white in color ad native to native to America, Manchester, Norway and Canada. Traditionally it is a folk medicine and use in the sore throat, inflammation, fever and in rheumatism.
Ledum palustre is used for the treatment of acute and chronic gout in homeopathy. The old literature of homeopathy showed that patients of Ledum palustre have gouty nodosities in joints like knee, wrist, fingers, toes, etc. Ledum palustre is indicated in ascending rheumatism associated with swelling, shooting, throbbing pains that are aggravated by heat, touch, motion, and ameliorated by cold. Fifteen days qualitative study on acute gout gives an evidence of use of Ledum palustre in treatment of hyperuricemia. Ledum palustre 30c was indicated in male patients that have bad effects of alcohol, hot to touch and pale > bathing with cold water. Mean reduction in serum uric acid was 0.42 mmol/L. A single blind randomized trial reported marked effectiveness of Ledum palustre tincture against hyperuricemia and gouty arthritis. In old literature of homeopathy, Ledum palustre is ranked as first aid remedy in emergency situations due to its remarkable anti-inflammatory property. Ledum palustre is included in various homeopathic formulations used for reducing pain and inflammation. These findings suggested that Ledum palustre might prevent or treat disorders related to hyperuricemia and gout.

Potassium oxonate blocks the effect of hepatic uricase and causes hyperuricemia in rodents. The oxonate-treated rats can serve as a useful animal model to evaluate drugs/medicines that affect serum UA and to evaluate therapeutic agents in disorders associated with uric acid.

The primary objective of study was to find hypouricemic effects of Ledum palustre 30c and 1M on serum UA in potassium oxonate induced hyperuricemia in Wister albino rats. The secondary objective of study was to appreciate distinguishing effects of homeopathic medicine administration on diseased rats for three different time durations.

Materials and methods

Plant material

Rhododendron tomentosum Harmaja syn. Ledum paustre L.

2.1 Ethical statement

The research proposal went through a process of ethical review by Pharmacy Research Ethics committee (PREC), The Islamia University Bahawalpur prior to the study commencement. The potential for application of the 3R’s was analyzed before finalizing research proposal. Every opportunity was taken during the course of the study to implement each of them.

2.2 Replacement

Prior to in vivo study, Ledum palustre 6x, 30c, 200c and 1M undergone in vitro testing for estimation of its anti-hyperuricemic effects. For this purpose, xanthine oxidase inhibition assay and oxidative stress inhibition assay in MSU-treated RAW264.7 macrophages were performed. Only Ledum palustre 6x has shown slight inhibition (< 50 %) of xanthine oxidase while no potency showed any inhibition of reactive oxygen species in MSU-treated RAW264.7 macrophages.

The secondary action of the vital force results in restoration of health by smallest doses of highly diluted homeopathic medicines. So, homeopathic ultrahigh dilutions could show their efficacy in animal models.

After in vitro trials, in vivo models of antihyperuricemic agents have been searched. The most common and widely used animal model for assessing hypouricemic effects of medicines/drugs is mouse or rat animal model. Another animal model, i.e., silkworms to screen and evaluate gouty therapeutic drugs has been described in literature. The hypouricemic effect of allopurinol in silkworm was shown by the transparency of epidermis of silkworm and reduction of uric acid in hemolymph and fat bodies of silkworms. This model seems to be more suitable as it involved invertebrate instead of mammalian animals. This model was studied in detail for possible option. Literature review of silkworm showed that silkworm has a common parasite, Botrytis (Beauveria bassiana), that consumed the fatty parts of the silkworm by leaving the transparent skin. This study describes the effects of extract from silkworm larvae with Botrytis on lowering serum uric acid levels.

The use of silkworms to screen and evaluate gouty therapeutic drugs is the new method described and silkworms used in the study were not ruled out for Botrytis parasite infection before conduction of study. Although it can be assumed that researchers use healthy colonies but the reported hypouricemic effects of silkworm larvae with botrytis created confusion that either allopurinol reduced uric acid levels or the silkworm is affected with Botrytis (a common parasite of silkworm) that might be responsible for lowering uric acid levels and turned epidermis transparent. Silkworm as an animal model for hyperuricemia was searched on PubMed and only this methodology paper was found. No other drug, medicine, or plant extract for their antihyperuricemic effects were evaluated by following this paper or used silkworm as an animal model.
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model. (Although this methodology paper is cited in various other papers). So, further validation of silkworm as an animal model for hyperuricemia is warranted before its final application as an animal for testing anti-hyperuricemic effects. After consideration of other possible in vitro and in vivo (Invertebrate) models, rat model was selected for assessing the anti-hyperuricemic effects of homeopathic ultra-high dilutions.

2.3 Reduction
For reduction of animal’s number in study, sample size was calculated through "resource equation" method. Any sample size, which keeps E between 10 and 20 should be considered as an adequate. If E is less than 10 then adding more animals will increase the chance of getting more significant result, but if it is more than 20 then adding more animals will not increase the chance of getting significant results. The E can be measured by following formula:

\[ E = \text{Total number of animals} - \text{Total number of groups} \]

The required sample size per group in this experiment is 3 with E value '22'. Final sample size should be adjusted for expected attrition%. 20 % attrition was expected in this study and sample size was selected as 4 animals per group. In this experiment, total no of groups were 11 and total no of animals were 44 and calculated E value was 33 that was more than 20.

In this way, confident statistical analysis and avoidance of wastage of animals have been attained in this study. Moreover, animals were returned to laboratory instead of euthanized. Blood samples were collected by rat tail vein bleeding by the method of Lee. In laboratory, animals will be treated humanely according to PREC rules and would be reutilized. Urate is a risk factor of diabetes. Animals of this study were reutilized for another project related to find an impact of high uric acid levels on diabetes and its organopathic complications.

2.4 Refinement
That animal husbandry and care was in accordance with EU Directive 2010/63/EU for animal experiments. All individuals involved with the care and use of animals were trained and skilled. PREC follows OECD animal welfare guidelines. Blood samples were collected by rat tail vein bleeding by the method of Lee. In current study, efforts were made to minimize harm to animals. However, animals suffer injection stress and pain during rat tail vein bleeding. Moreover, same animals were used for another project that requires hyperuricemic rats. So, for current study, only harm to animals is some pain and distress that was due to injection and blood collection. Moreover, during injection and blood collection ethics were followed carefully. The benefits of study relate to evaluation of therapeutic potential of a medicine from poorly studied alternative system of medicine, Homeopathy. Gout is a metabolic disorder related to hyperuricemia that can cause disability in a person. Conventional system of medicine has unsatisfactory treatment options while homeopathy can prove beneficial to the treatment of gout and its related disorders. If homeopathic medicines have any effect on experimental hyperuricemic models, the effects can be observed in human beings also.

2.5 Experimental animals
Wistar albino rats (150-250 g) were purchased from animal house of the Faculty of Pharmacy and Alternative Medicine, The Islamia University of Bahawalpur, Pakistan. Rats were housed in polycarbonate cages of size 47 × 34 × 18 in² with a maximum of 4 animals per cage. The standard conditions of humidity (50 – 55 %) and temperature (25±1 °C) along with 12/12 h light dark cycle were maintained throughout the study period. Rats were acclimatized to laboratory environment for one week prior to study and fed standard diet and water ad libitum. Only well trained and skilled individuals were involved with the care and use of animals. That animal husbandry and care was in accordance with EU Directive 2010/63/EU for animal experiments. Rat tail vein blood was collected by the method of Lee. The whole manuscript complied to the ARRIVE guidelines. The experimental protocol was approved by animal ethics committee (PREC - Pharmacy Research Ethics Committee), The Islamia University Bahawalpur, Ref No. 41-2016/PREC dated 23-08-2016.

2.6 Drugs and chemicals
Potassium oxonate (Sigma - Aldrich, Germany), Allopurinol (Mega pharmaceutical, Pakistan), succussed alcohol, Ledum palustre 30c and 1M were obtained from Masood homoeopathic pharmaceuticals, Pakistan.

3. Experimental setup
The animals were divided into 11 different groups and each group consisted of 4 animals. Sample size was calculated through "resource equation" method
and was sufficient for statistical analysis. Animals were randomly allocated to different groups by a lab attendant unknown to the nature of experiment. All the animals were weighed and blood samples were collected from rat tail vein bleeding for checking baseline serum uric acid levels. Animals were also monitored for adverse events like immobility, inability to eat and drink, change in body weight of animals or death of any animal during the whole course of study. The animals were treated and assessed in the order given in Fig. 1. Experimenters were not blinded to experimental groups during medicine administration.

3.1 Hyperuricemia induction
Potassium oxonate was used to induce the hyperuricemia in rats, as described previously. Potassium oxonate at a dose of 250 mg/kg was injected intra peritoneal, one hour before final medicine administration to increase serum UA levels.

3.2 Medicine administration
Animals were withdrawn from the food but not from water, one hour before medicine administration daily. All the medicines including allopurinol, homeopathic medicine, and succussed alcohol were given orally once daily at 10 am - 11 am. As shown in Fig. 1, nine groups were orally administered with allopurinol 10 mg/kg, Ledum palustre 1M, Ledum palustre 30c for 1, 3 and 7 days, respectively. Two groups, normal control and hyperuricemic control were given distilled water and succussed alcohol 70 % (vehicle of used homeopathic medicines as per company mentioned) respectively. Homeopathic ultrahigh dilutions and 70 % succussed alcohol were mixed (2 drops) in 1 cc distilled water for oral administration.

3.3 Uric acid assay
One hour after medicine administration on day 1st, 3rd and 7th, blood samples were collected from rats by tail vein bleeding. The blood was allowed to clot for one hour at room temperature. Blood was centrifuged at 3000 rpm for 5 min to get serum. Serum was preserved at -20 °C until analyzed. Serum uric acid and creatinine levels were analyzed by using Microlab 300. Uric acid liquicolor kit and Creatinine liquicolor kit (human diagnostic worldwide) were used respectively. Primary outcome of study was to find any effect of Ledum palustre on reducing serum uric acid and creatinine levels of hyperuricemic rats.

Statistical analysis
Results obtained by this activity were analyzed by SPSS version 20.0 software (I.B.M.SPSS. statistics.v20_32bit_oxava.com). Data was expressed as mean ± standard error of mean (S.E.M). Student's t-test for independent means was applied for calculating statistical significance of the differences between the groups. p ≤ 0.05 (Two tailed values) was considered significant. Statistician was blinded to experimental groups. (Only codes for different groups were mentioned).

Results
The baseline characteristics of different study groups were mentioned in Table 1. Intra peritoneal injection of potassium oxonate caused hyperuricemia in rats, as indicated by drastic increase in serum UA levels.
levels. One day administration of *Ledum palustre* 30c and *Ledum palustre* 1M effectively reduced serum UA levels in rats compared to the hyperuricemic control group, though still higher than the normal control level. The hypouricemic action of homeopathic medicines was slower as compared to allopurinol that required only one day for normalizing serum UA levels.

Three and seven day treatment of *Ledum palustre* 30c and *Ledum palustre* 1M reduced the elevated serum UA level to the normal value. Moreover, three day and seven day treatment of *Ledum palustre* 30c and 1M was found to be as effective as Allopurinol (10 mg/kg) which was taken as standard control of the study as shown in Fig. 1. There were no adverse event (immobility, loss of weight, an inability to eat or drink or death of animals) observed during course of study.

Similar to above results, serum creatinine levels were also reduced in time dependent manner (Fig. 2). In hyperuricemic control group, serum creatinine levels were markedly high as compared to other groups. Serum creatinine levels of 3 day and 7 day study groups treated with medicines were almost similar to normal control group. 4 animals in each group were analyzed for serum uric acid and creatinine levels.

**Discussion**

The present study evaluated the effects of *Ledum palustre* 30c and *Ledum palustre* 1M in hyperuricemia in potassium oxonate induced hyperuricemic model of rats. A significant increase in serum UA levels in all the groups compared to normal control group indicating that the hyperuricemia rat model was effectively established. This finding matched to the previous studies reporting hyperuricemia induced by potassium oxonate intra peritoneal in rats and mice. Hyperuricemia triggered when there are disturbances in uric acid regulating pathways. Potassium oxonate causes hyperuricemia by inhibition of uricase or by blocking of an electrogenic urate transporter activity in renal proximal tubule that controls serum UA levels.

Anti-hyperuricemic drugs reduced hyperuricemia by inhibiting xanthine oxidase and action on renal or extra-renal urate transporters. In the present study, orally administered allopurinol (10mg/kg) significantly decreased potassium oxonate induced hyperuricemia in rats. It has action on a urate transporter and Cl/urate transporter and inhibits xanthine oxidase. Results matched to the several studies presented the reduction of hyperuricemia with allopurinol at same dosage.

The experimental model used in this study to evaluate antihyperuricemic action has explored what is allopathically indicated as pharmacological action on one symptom (e.g. urate reduction). While allopurinol has the action on a urate transporter and Cl/urate transporter, homoeopathic medicines has supposed action on the regulation of pathological changes as the phenomenon by itself is seen as an expression of dynamic vital force of the organism. In many species, major quantity of extracellular urate is transported into hepatocytes. Uricase in hepatic peroxisomes degraded uric acid into allantoin that is excreted by kidneys. Liver uric acid is partially transformed into allantoin and rest is exported to the blood. In rodents, serum uric acid level is also regulated by kidneys. Some pharmacological studies have suggested that uric acid produced in liver is transported to proximal tubules. Hyperuricemia is produced when basolateral uptake of uric acid from peritubular plasma is defected. An electrogenic urate transporter (UAT) is present in renal proximal tubule that regulates blood uric acid levels and has some similarities to the enzyme uricase. Many studies confirmed that oxonate blocks channel activity of UAT in mammalian. Moreover, urate transporter 1 in rodent kidney transported urate similar to human urate transporter 1, the gene responsible for hereditary renal hypouricemia. So, it can be speculated that potassium oxonate induced hyperuricemia in rats was due to either blocking of UAT activity or inhibition of...
uricase activity. It can be speculated that dynamic vital force regulates the pathological changes produced by oxonate. However, action of homeopathic medicines through regulatory effect of vital force is merely a theoretical concept. If highly diluted homeopathic medicines act through an influence on dynamic vital force of the organism, at least theoretically, this action could be very sensitive to smallest changes in experimental conditions.

One day study depicts the anti-hyperuricemic potential of allopurinol, *Ledum palustre* 30c and 1M in potassium oxonate induced rat model that received medicines after one hour of intra peritoneal injection of potassium oxonate. The rats of 3 day and 7 day study groups received homeopathic medicines as well as allopurinol for consecutive 2 or 6 days respectively and then received single potassium oxonate intra peritoneal injection before final dose of medicines. Potassium oxonate is a hyperuricemia inducing agent, but when rats previously treated with allopurinol, *Ledum palustre* 30c and 1M received potassium oxonate, serum uric acid levels remained normal in all the groups. It is previously reported that allopurinol has hypouricemic effects in normal and hyperuricemic animals. So, allopurinol might exert its hypouricemic effects on normal rats and after increase in serum uric acid level by potassium oxonate administration, hypouricemic effects reversed to normal serum uric acid levels. Moreover, final allopurinol dose administration after potassium oxonate injection might show more reduction in serum uric acid level.

Hahnemann, founder of homeopathy, described in aphorism 43 that when two similar diseases meet together in an organism, it resulted in cure.

During one day study, *Ledum palustre* administration reduced serum uric acid level. However, single dose of homeopathic medicine administration can’t turn increased serum UA to normal levels. A homeopathic medicine gently annihilates similar acute disease with slight homeopathic aggravation (aphorism 154, 155). The initial elevated serum UA levels may be due to homeopathic aggravation.

During 3 day and 7 day study, *Ledum palustre* administration normalized serum uric acid level just like allopurinol. Homeopathic medicines could act as prophylactic when administered to healthy individuals having risk of disease. *Arsenicum album* and *Veratrum album* are the two well-known homeopathic medicines used as a prophylactic of cholera. It might be possible that homeopathic medicines administered in 3 day or 7 day study groups produce prophylactic effect in rats that inhibit the action of potassium oxonate in those rats. Moreover, a homeopathic drug proving (HDP) is the application of a substance in nontoxic dilutions to healthy individuals. The tested material causes mental, physical or psychological symptoms that can be reversed. *Ledum palustre* is a chilly remedy and patients of this remedy have violent thirst for cold water. It might be possible that the rats of *Ledum palustre* groups develop violent thirst and drink more water than other groups resulting in reduced uric acid levels. In other words, *Ledum palustre* might have no or little hypouricemic potential and drinking more water causes excretion of more uric acid.

The specific effects of homeopathic medicines are of non-molecular origin, yet provide powerful clinically effective biological activities. The potencies selected in current study were medium (30c) and higher (1M) potencies of *Ledum palustre*. Both potencies showed similar hypouricemic potential in rats. It has been assumed that highly diluted substances transfer biological activity to cells by electromagnetic fields. A magnetic resonance imaging study on various serial dilutions showed that vigorous shaking or succession continuously alter the hydroxyl groups in the solvent of solution as dilutions become higher. Nano bubbles (NBs) are present in homeopathic dilutions. These NBs have superstructures of increasing size with increasing dilutions and create superstructures related to specific solute. However, the exact mechanism of action of these ultrahigh dilutions is not known.

The results follow from blinded and controlled trial and thus are valid. However, to transfer an experimental result into a scientific fact requires several further replications by independent groups.

**Conclusion**

The current study indicated antihyperuricemic potential of *Ledum palustre* 30c and 1M in potassium oxonate induced hyperuricemia model of rats in time dependent manner. In one day study the level of uric acid reduced but not normalized but in 3 day and 7 days study Homeopathic medicines not only reduced but normalized the level of uric acid. The homeopathic medicines work due to presence of ultrahigh dilutions. It is concluded that homeopathic medicine *Ledum palustre* 30c and 1M significantly reduced the uric acid level and it can be a good alternative to allopurinol and other therapeutic agents used as Hypouricemic agents however further researches on large samples are required.
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Competing interests
None of the authors have any competing interests.

References