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Potencies of *Aurum metallicum* and *Colchicum* Ameliorate Adjuvant Arthritis in Albino Rats

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Abstract

One objective of this study is to see whether *Aurum met* 30 and *Colchicum* 30 could ameliorate adjuvant-induced arthritis in albino rats. Another objective is to assess the relative efficacy of conventional *Aurum met* 30 and the same drug produced from the nano particles of gold and called here as *Aurum met* 30 (nano). Freund's complete adjuvant (FCA) was injected into the plantar region of the left hind paw of 4 batches of rats at 0.025 ml/rat on day 0. From day 1 until day 18, each of the four injected batches was treated orally with a homeopathic potency, one dose daily, in the following way : *Ethanol 30, Aurum met* 30, *Aurum met* 30 (nano) and *Colchicum* 30. One batch served as the uninjected untreated control. *Aurum met* 30 (nano) was prepared in the laboratory from ~8 nm gold particles by the standard procedure of initial trituration with lactose followed by successive dilution and succussion with 90% ethanol. Paw volume of each rat was measured before injection on day 0 and again after injection on day 18. The subjective symptom of arthritic pain felt by rats was measured in terms of their movement score in the open field and also on an inclined wire grid. The data were analyzed by one-way analysis of variance (ANOVA) followed by multiple comparison *t*-test. *Aurum met* 30, *Aurum met* 30 (nano) and *Colchicum* 30 significantly reduced paw oedema and enhanced movement as compared to the *Ethanol* 30-treated control. *Colchicum* 30 produced the maximum therapeutic effect. *Aurum met* 30 (nano) was significantly more effective than *Aurum met* 30. Smaller particle size of gold appears to be the main factor for the higher efficacy of *Aurum met* 30 (nano).

Key words : Nano particles, Paw oedema, Movement score, Homeopathy, Adjuvant arthritis.

Rheumatoid arthritis is a chronic crippling disease affecting the synovial membrane of many joints. The main subjective symptom is pain, which is related to prostaglandin-mediated sensitization of the primary afferent nociceptive nerves (1). The objective symptoms include swelling of soft tissue around joints, fibrillation of cartilages, subchondral sclerosis, peri-articular bone erosion and formation of osteophytes (2, 3). The disease usually affects people of advanced ages. Nonsteroidal anti-inflammatory drugs are mainly used in symptomatic treatment and these act by inhibiting prostaglandin G/H synthase enzymes. These drugs have adverse side reactions (4). Pharmacological management of arthritis is a big problem and for this, new drugs and the efficacy of current drugs are tested in animal models. Arthritis can be induced in rats by Freund's complete adjuvant (FCA) (5—7). More and more patients seek homeopathic treatment for arthritis, and in Israel the percentage of such patients is 44 (8). Homeopathic materia medica mentions many remedies including the two used here, presenting symptoms of arthritis, but very few of them have been tested on animal models to prove their efficacy by standard scientific methods.

In the present study we have tested two homeopathic remedies, namely *Aurum met* and *Colchicum* on rat adjuvant arthritis, and assessed their efficacy in terms of both subjective and objective symptoms. According to homeopathic pharmacy, substances, which are not soluble in water, are triturated initially with lactose powder to liberate their dormant medicinal properties. *Aurum met* is such a substance and we have applied modern technique to obtain nano particles from gold. In this study we have used traditional *Aurum met* 30 and the same prepared from the

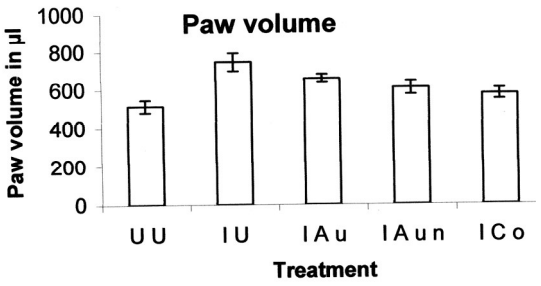

Figure 1. Left hind paw volume in µl of rats of batch I uninjected untreated (UU), batch II injected and treated with *Eathanol* 30 (IU), batch III injected and treated with *Aurum met* 30 (IAu), batch IV injected and treated with *Aurum met* 30 (nano) (IAun) and batch V injected and treated with *Colchicum* 30 (ICo). All the batches differ from each other significantly (P<0.000, F-value 6.744, one way ANOVA, n=10). Each column represents mean±SE. Left hind paw was injected with FCA at 0.025 ml/rat.

nano particles of gold. The purpose is to see whether the nano potency is more effective than the usual one.

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Methods

Animals

Fifty female albino rats of Charles Foster strain weighing 75—125 g were divided randomly into five batches, each comprising 10 individuals. They were kept in cages in groups of 5 with food (germinated Bengal gram) and water *ad lib.* All the animals were kept in the animal house at a room temperature of 26±2 C with natural light and dark cycle during May-June, 2007. Experiments were started 15 days after shipment. The animals were handled with extreme care following international norms, and the protocols were approved by the Animal Ethics Committee of the University.

Drugs

Freund's complete adjuvant (FCA) was purchased from GENEI, Bangalore. *Aurum met* 6 and *Colchicum* 6 were purchased from Seth Dey and Co, Kolkata. The potency 30 of each drug was prepared in the laboratory in 90% ethanol following the standard procedure of successive dilution and succussion. The control consisted of *Ethanol* 30 prepared in the same way. The concentration of the diluent medium was fixed at 90% for all the potencies used.

Besides the usual *Aurum met* 30, we have prepared the same drug from the nano particles of gold. An aliquot of 50 ml aqueous solution of $HAuCl_4$ (0.25 M) was continuously stirred and heated to boiling point when 2 ml of trisodium citrate (1%) was introduced for 8 nm gold colloid. The boiling solution turned faintly blue in about 20 sec. After ~ 7 sec of boiling the blue color changed to deep red. The solution was allowed to boil for 15 more min to obtain stable gold nano particles in a solution phase. The solution was allowed to cool down to room temperature (9). Because nano particles are susceptible to oxidation they were capped with citrate ions using sodium citrate. The size of the gold nano particles used in this experiment was ~ 8 nm. The gold nano particles were triturated with lactose upto the centesimal potency 3 and then converted into the liquid potency following the standard procedure of successive dilution followed by succussion (10). This potency would be called as *Aurum met* 30 (nano).

Treatment

Arthritis was induced by injecting 0.025 ml of FCA in the plantar region of the hind paw of the left leg in batches II, III, IV and V on day 0. Batch I remained as uninjected untreated control and batch II as injected control. From day I until day 18, each rat of each batch received an oral dose daily of a particular potency such as *Ethanol* 30 for batch II, *Aurum met* 30 for batch III, *Aurum met* 30 (nano) for batch IV and *Colchicum* 30 for batch V. Each potency was mixed with sterile distilled water 1 : 100 and administered orally by a micropipette at 100 µl/rat.

Paw Volume

The volume of the left hind paw of each rat was measured before injection on day 0 by dipping the

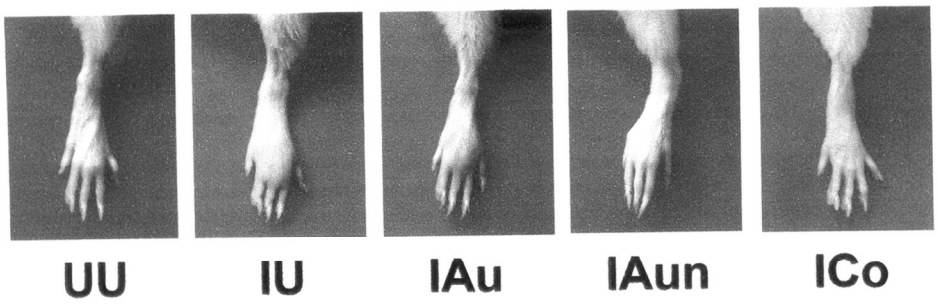

Figure 2. Photograph of left hind paws of rats of batch I uninjected untreated (UU), batch II injected and treated with *Eathanol* 30 (IU), batch III injected and treated with *Aurum met* 30 (IAu), batch IV injected and treated with *Aurum met* 30 (nano) (IAun) and batch V injected and treated with *Colchicum* 30 (ICo).

clean paw in water in a hard glass tube upto the ankle-joint. The tube had a narrow side tube 6 mm below the tip. It was filled with water upto the outlet. Water displaced by the immersed paw was accurately measured by a microsyringe. The paw volume in µl was similarly measured once again on day 18. FCA arthritis in rats peaks within 14—16 days (11).

To measure the subjective symptom of pain in the hind paw we conducted two tests on the movement of rats. A rat with a painful paw would be slow in movement.

Open Field Activity

Rats were releazed individually on a checkered sheet, 45 × 45 cm, inside an aluminium tray having walls 21 cm high. Each square of the checkerboard measures 2 by 2 cm. The number of squares traversed by a rat in 60 sec was recorded as the locomotor activity score for that rat. The sheet was wiped with ethanol-soaked cotton ball after each test with a rat. The experiment was conducted on day 18.

Movement On An Inclined Wire Grid

Rats were released individually on the upper end of inclined wire grid (31 × 15.5 cm), set at a 70 incline. The walls of the grid were 12 cm high. The time a rat took to come down to the bottom of the grid was recorded as the movement score for that rat. The cut-off time was 90 sec. The test has been used to measure the catalepsy of a mouse (12, 13).

All the data were analyzed by one way analysis of variance followed by multiple comparison *t*-test.

Results

Paw Volume

Paw volumes did not show any significant difference before FCA injection between the five batches of rats tested and the mean volume was almost same as in batch I (Fig. 1). Paws were swollen and redish in all the test batches of rat following FCA injection right from day 1. Paw volumes increased significantly on day 18 in all the FCA-injected batches, and the volumes also differed from each other significantly among the batches ($P<0.000$, ANOVA one way, F-value 6.744, Fig. 1). Among the treatment batches, the paw volume was lowest with *Colchicum* 30 followed by *Aurum met* 30 (nano) (Fig. 1). A photograph of paws, taken on day 18, selected at random from each batch is presented in Figure 2. *Aurum met* 30, *Aurum met* 30 (nano) and *Colchicum* 30 different each significantly from the injected untreated control by P-values less than 0.05, 0.005 and 0.005, respectively (t-test). *Colchicum* 30 was more effective significantly ($P<0.05$, t-test) than *Aurum met* 30.

Open Field Activity

The locomotor activity scores showed signifi-

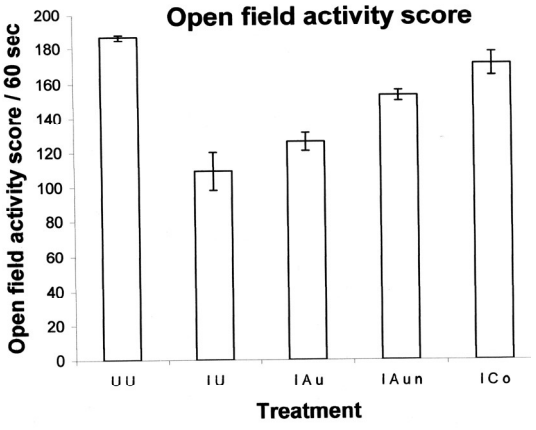

Figure 3. Open field activity score in 60 sec in rats of batch I uninjected untreated (UU), batch II injected and treated with *Eathanol* 30 (IU), batch III injected and treated with *Aurum met* 30 (IAu), batch IV injected and treated with *Aurum met* 30 (nano) (IAun) and batch V injected and treated with *Colchicum* 30 (ICo). All the batches differ from each other significantly (P<0.000, F-value 24.834, one way ANOVA, n = 10). Each column represents mean ± SE. Left hind paw was injected with FCA at 0.025 ml/rat.

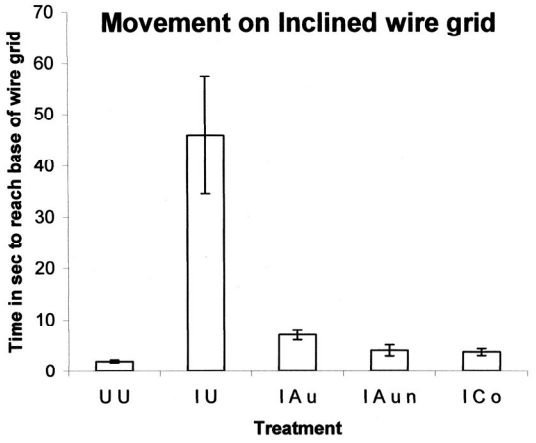

Figure 4. Movement score in sec of rats of batch I uninjected untreated (UU), batch II injected and treated with *Eathanol* 30 (IU), batch III injected and treated with *Aurum met* 30 (IAu), batch IV injected and treated with *Aurum met* 30 (nano) (IAun) and batch V injected and treated with *Colchicum* 30 (ICo). All the batches differ from each other significantly (P<0.000, F- value 13.209, one way ANOVA, n=10). Each column represents mean ± SE. Left hind paw was injected with FCA at 0.025 ml/rat.

cant difference between batches (P<0.000, ANOVA one way, F-value 24.834, Fig. 3). Among the treatment batches, *Colchicum* 30 showed the highest score and the next highest was with *Aurum met* 30 (nano) (Fig. 3). *Aurum met* 30, *Aurum met* 30 (nano) and *Colchicum* 30 differed each significantly from the injected untreated control by P-values less than 0.05, 0.0005 and 0.0005, respectively (t-test). *Aurum met* 30 (nano) was more effective significantly (P-<0.005, t-test) than *Aurum met* 30. *Colchicum* 30 was more effective than *Aurum met* 30 (P < 0.0005, t-test) and *Aurum met* 30 (nano) (P<0.025, t-test).

Movement on Wire Grid

Movement scores on the inclined wire grid showed significant difference between the five test batches (P<0.000, ANOVA one way, F-value 13.209, Fig. 4). Among the treatment batches *Colchicum* 30 showed the lowest score closely followed by *Aurum met* 30 (nano) (Fig. 4). Here the lowest score signifies fastest movement of rats on the grid. *Aurum met* 30, *Aurum met* 30 (nano) and *Colchicum* 30 differed significantly from the injected untreated control by P-values less than 0.005, 0.0005 and 0.0005, respectively (t-test).

Discussion

The results indicate that *Colchicum* 30 produced the maximum therapeutic effect with respect to both the objective symptom of paw oedema and the subjective symptom of pain in the injected paw. Since the treated rats showed faster movement in the open field and inclined wire grid, it is assumed that they felt lesser pain. Further, *Aurum met* 30 (nano) produced significantly better effect than *Aurum met* 30. This shows that nano particles make more effective potencies than their traditional counterparts. This is because the traditional method of trituration or grinding has never brought down the particle size to the nano level. This shows that particle size of a drug plays an important role in inducing the efficacy of a potency. Hahnemann recommended that all medicines should be prepared by trituration up to the third attenuation from which higher potencies could be prepared by successive dilution with aqueous ethanol

followed by succussion (10, 14). The idea was to break the particles of a drug as small as possible. The gold nano particles carried negative charge and were decapped during trituration and potentization with aqueous ethanol due to place exchange reaction. So, the gold nano particles directly influenced the diluent medium during dynamization.

Gold therapy is employed for rheumatoid arthritis patients who show progressive deterioration and do not respond to nonsteroid anti-inflammatory agents. Gold produces serious adverse reactions in skin, mucous membranes, kidneys and blood (4, 15). Auranofin, a synthetic gold compound, inhibits antibody production in adjuvant arthritis in rats (15). The potencies 30 of *Aurum met* do not contain any gold particles, and for this, there is no possibility of adverse side reactions.

Colchicine is an alkaloid derived from the plant *Colchicum autumnale.* The plant extract has been used for joint pain for about 2000 years (16). Colchicine can relieve pain of acute gout, but it has many side reactions.It arrests mitotic activity, inhibits secretion of insulin from pancreatic β-cells, lowers body temperature, depresses the respiratory center, constricts blood vessels and induces hypertension, alters neuromuscular function, causes gastro-intestinal disorder, bone-marrow depression, ascending paralysis of the CNS, proteinuria, haematuria (4, 16). Since *Colchicum* 30 does not contain any Colchicine molecules, the question of side effects does not arise. In our study *Colchicum* 30 produces the maximum therapeutic effect. Earlier we reported that potentized *Causticum* and *Rhustox* ameliorated rat adjuvant arthritis (17).

Homeopathic potencies are thought to be steriospecific water structures preserved by the non-polar tail of ethanol molecules. They initiate their therapeutic effect on the integral membrane proteins in the oral mucosa where the potency first makes contact with the body of the patient (10).

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