Abstract

Aim and Objective
Hecogenin is a steroidal sapogenin obtained from the leaves of species such as Agave genus, including A. cantala, A. sisalana, A. avellanidens, A. cerulata, A. cocui, A. goldmaniana, and A. aurea. That plays an important role in the treatment of variety of inflammatory diseases. The management of inflammatory disorders is very difficult because of the development of drug resistance after their chronic and prolonged treatment. These treatments are ineffective and associated with variety of side effects on long term use. Therefore, currently research has been thrown on searching effective anti-inflammatory treatments of herbal origin associated with minimal side effects. We have investigated the anti-inflammatory effects of Hecogenin and combination of Hecogenin with low dose Fluticasone on various inflammatory models such as Croton oil induced ear edema, Cotton pellet induced granuloma, Tri-Nitro-Benzene-Sulphonic acid induced colitis, 2, 4-dinitrofluorobenzene induced contact dermatitis, Ovalbumin induced lung edema and Complete Freund Adjuvant induced arthritis.

Material and Methods
The selection of plant steroid was done by comparing all the phytosteroids (Hecogenin, Diosgenin, Solasodine, Glycyrrhizin, Boswellic acid, Guggulsterone, Sarsasapogenin or Withaferin-A) by using croton oil induced ear edema model.

The ear edema was induced by topical, single administration of croton oil (20 µl of 2.5% v/v) on the inner surface of right ear, while the left ear received 20 µL of vehicle acetone only. After 15 minutes the drug treatments were topically applied on the right ear. Ear edema was evaluated 6 h after croton oil application and was expressed as increase in ear weight (mg). The anti-inflammatory effect of Hecogenin was evaluated by measuring the ear weight, % inhibition of edema and histopathological analysis of ear tissues.

The cotton pellets induced granuloma model was used to assess anti-inflammatory activity of Hecogenin in rats. The autoclaved cotton pellets of 10 ± 1.0 mg were aseptically implanted sub-cutaneously in the back region of anesthetized rats. On day 8 cotton pellets were removed surgically & dried in hot air oven at 60°C to a constant weight. Mean weight of the granuloma tissue in each group was recorded and percentage inhibition was calculated by comparing the mean weight of test group with the control group. Blood samples were collected from rats for...
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tumor necrosis factor-α and interleukin-12 cytokines analysis. The adrenal gland and thymus gland were removed from rats and weighed.

The colitis was induced by instillation of 10 mg of tri-nitro-benzene-sulphonic acid dissolved in 0.25 ml of 50 % ethanol (v/v) in rats by trans-rectal route. Hecogenin and combination were orally administered 48, 24 and 1 h by using catheter prior to the induction of colitis and 24 h after colitis induction. The rats were killed after 72 h of colitic induction colon weight, colon weight to length ratio were recorded. The inflammatory response was assessed by macroscopic, microscopic, myeloperoxidase activity, tumor necrosis factor alpha and Interleukin-12 levels and a histological study of rat colon tissue.

The dermatitis was induced by repeated application of 25 μL of 0.15% 2, 4-dinitrofluorobenzene in acetone/olive oil (3:1) to the inner and outer surfaces of the ears, and 100 μL of the same solution applied to shaved back skin once on days 1 and 4. On days 7, 10, and 13, sensitized mice were challenged by applying 0.2% 2, 4-dinitrofluorobenzene to the back and ear skin surfaces. On day 7 to 13 drug treatment was done. Normal control group was treated with the same volumes of vehicle (Acetone). On day 14 the animal were sacrificed and ear thickness, ear weight, dermatitis score, myeloperoxidase activity, tumor necrosis factor-α, interleukin-12 and histological study of ear tissue was performed.

Balb/c mice were injected intra-peritoneal with a mixture of ovalbumin (50 μg) and alum (1 mg) in 0.2 ml of normal saline except for the NC group on 0 and 7 days. At 14 and 21 days, the mice were challenged with 2.5 % (w/v) ovalbumin aerosol through a nebulizer for 20 min. The normal control mice were exposed to saline aerosol for 20 min on days 14 and 21 (Epstein, 2006). 1 h before each Ovalbumin sensitization and challenge on 14 and 21 day after the initial sensitization 20 μl of drug treatments were administered by intra-nasal route on once daily on 14 to 21 days respectively (Vasconcelos et al, 2008). The animals were sacrificed 48 h after the last challenge on day 22 and total, differential cell count, spleen, thymus weight, body weight, blood glucose level, Myeloperoxidase, Tumor Necrosis Factor-α, Interleukin-12 levels and histopathological analysis of ear tissue was performed.

Male Wistar rats were immunized by intradermal injection of 0.1 ml of complete Freund adjuvant into the left hind paw on day 1. The dosing of Hecogenin and combination was started from day 12 once daily, topically. Anti-arthritic activity was evaluated by measuring paw volume, arthritic score, joint diameter, body weight, spleen and thymus weight, serum
biochemical parameters, haematological parameters, myeloperoxidase, serum cytokines levels, X-rays of hind paw joints, histological analysis of joint tissue and COX-2 expressions study was done.

In acute toxicity study, the Swiss albino mice were orally administered with single dose of HG 2000 mg/kg body weight and were continuously observed for behavioral and autonomic profile for 2 h and for any signs of toxicity or mortality up to 48 h. In safety pharmacological study, the Swiss albino mice were orally administered with HG (50 µg/mice) once daily for 14 days and control groups mice were received with acetone, orally. The mice were observed for any indications of toxicity effect within the first six hours after the treatment period, and daily further for a period of 14 days. Surviving animals were weighed and visual observations for mortality, behavioral pattern, changes in physical appearance, injury and signs of illness were conducted daily during the period.

**Results**

In selection of phytosteroid, from the comparison of various plant steroids it was found that Hecogenin at 50 µg/mice showed maximum inhibition of ear edema in mice.

The topical application of Hecogenin and combination to mice ear showed significant decrease in the ear weight and showed maximum % inhibition of edema weight as compared to croton oil treated mice. The myeloperoxidase levels and pro-inflammatory cytokines levels (TNF-α and IL-12) was also significantly lowered in HG and combination group as compared to croton oil treated mice. Further histopathological analysis of ear tissue showed significant decrease in the dermal thickness and epidermal hyperplasia of mice treated with Hecogenin and their combination as compared with croton oil treated mice.

The treatment of rats with Hecogenin and combination significantly inhibited the formation of granulomatous tissue in cotton pellet induced granuloma model along with significant decrease in the level of serum pro-inflammatory cytokines. The adrenal and thymus gland indices were found to be significantly increased in the rats treated with Hecogenin and combination as compared to Fluticasone treated rats.

Treatment of rats with Tri-Nitro-Benzene-Sulphonic acid was characterized by increased colonic wall thickness, edema, inflammatory cell infiltration, increased myeloperoxidase activity, tumor necrosis factor-α and interleukin-12 levels. Treatment of rats with HG and
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Combination trans-rectally significantly suppressed the severity of colonic injury as evidenced by amelioration of macroscopic damage, colon weight/length ratio, histopathological alterations, leukocyte influx and myeloperoxidase activity and pro-inflammatory cytokines levels such as tumor necrosis factor-α and interleukin-12.

The adrenal, thymus and spleen gland indices in Tri-Nitro-Benzene-Sulphonic acid treated rats were significantly increased in rats treated with Hecogenin and combination as compared to Fluticasone treated rats. Trans-rectal administration of Fluticasone showed significant increase in body weight and blood glucose level in rats. The treatment of rats with Tri-Nitro-Benzene Sulphonic acid, Hecogenin and combination showed significant decrement in the body weight and blood sugar level as compared to Fluticasone.

In Balb/c mice, the topical application of Hecogenin and combination effectively inhibited the enlargement of ear thickness and weight induced by repeated application of mice with 2, 4-dinitrofluorobenzene. Topical application of Hecogenin and combination also inhibited hyperplasia, edema and infiltration of mononuclear cells in ear tissue. In addition, production levels of tumor necrosis factor-α, interleukin-12 and myeloperoxidase also decreased by drug treatment. The treatment of Balb/c mice with Hecogenin and combination topically for 13 days significantly improved the skin thickness as compared to Fluticasone treated mice.

In Ovalbumin induced lung edema model, the results revealed a significant increase in total and differential leucocyte count, myeloperoxidase, cytokines levels (Tumor necrosis factor-α, Interlekins-6, 12 and Thromboxane B₂) in Balb/c mice. However, these parameters were significantly decreased in Hecogenin and combination treated mice. Histopathological analysis of lung tissue by Haematoxylin & Eosin and Toludine blue staining revealed the effectiveness of treatment.

The adrenal, thymus and spleen gland indices of Ovalbumin treated mice were significantly increased in mice treated with HG and combination as compared to Fluticasone treated mice. Intra-nasal administration of Fluticasone showed significant increase in body weight and blood glucose level in mice. The treatment of rats with Ovalbumin, Hecogenin and combination showed significant decrement in the body weight and blood sugar level as compared to Fluticasone.
The treatment of rats with Hecogenin and combination has significantly suppressed the paw swelling, arthritic score and joint diameter in Complete freund adjuvant treated rats as compared to normal control rats. The Hecogenin and combination treatment to rats also increases body weight, decreases thymus and spleen indices, increases myeloperoxidase levels, inhibits serum lysosomal enzymes, pro-inflammatory cytokines (Tumor necrosis factor-α, Interlekins-6, 12 and Thromboxane B₂), haematological parameters (increasing the levels of red blood cells, haemoglobin and by decreasing levels of white blood cells) along with inhibition of joint destruction (histopathological and radiological analysis).

In acute toxicity study, the mice do not show any toxic symptoms and mortality and was found to be safe up to the dose of 2000 mg/kg. The safety pharmacological study of Hecogenin was performed for 14 days. During the period of 14 days mice showed no observable symptoms of neither toxicity nor deaths and gained weight and displayed no significant changes in behavior. The haematological, biochemical parameters and histopathological analysis of various systemic organs also exhibits no significant changes.

**Conclusion**

Thus, from the present study it can be concluded Hecogenin and combination was proved to be a better therapeutic regimen for the treatment of variety of inflammatory conditions such as croton oil ear edema, cotton pellets granuloma, ulcerative colitis, atopic dermatitis, asthma and rheumatoid arthritis by virtue of their serum pro-inflammatory cytokines, myeloperoxidase level inhibition and reduced COX-2 expressions in joint tissue of rats. The present investigations suggest that, Hecogenin is interesting molecules for further research for inflammatory disorders, with an approach through pro-inflammatory inhibitory pathway and combination of Hecogenin with Fluticasone also shown reduced side effects associated with Fluticasone alone.