Evaluation of anti-inflammatory activity of polyherbal formulation Vipro™ in Lipopolysaccharide (LPS) induced inflammation in Wistar albino rats

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ABSTRACT

This study evaluated the anti-inflammatory activity of Vipro™ in Wistar albino rats. Healthy young adult Wistar rats were selected and divided into four groups. Each group consisted of 3 male Wistar rats. All the animals were acclimatized for five days. Rats were divided into 4 groups. Group I served as control, in groups II, III & IV, inflammation was induced by single intraperitoneal injection of LPS. Group II served as inflammation control and groups III & IV received Vipro™ and Dexamethasone respectively. A limit dose of 1000 mg/kg body weight of Vipro™ mixed with distilled water was administered to test group through oral route for seven consecutive days. The standard drug Dexamethasone was administered at a dose of 1.5 mg/kg body weight orally on day 7, one hour before LPS injection. All the animals were observed daily for clinical signs. Body weights were taken at the time of receipt, days 1, 3 and 7. On day 7, after 1 hour of dosing, LPS was injected intraperitoneally to all groups of rats except control group. After four hours of LPS injection, Broncho Alveolar Fluid (BALF) was collected, centrifuged and supernatant was collected for TNF-α estimation using ELISA (Enzyme Linked ImmunoSorbent Assay). No mortality and clinical signs observed in any of the animal from day 0 till the end of observation period (day 7). There was no significant change in the body weights in all groups. The level of TNF-α was found to be 200 pg/ml in Vipro™ group. These levels were lower when compared to LPS group (420 pg/ml) and Dexamethasone group (300 pg/ml). Based on the results, it is concluded that Vipro™ Polyherbal Formulation has anti-inflammatory activity when tested for inhibition of TNF-α using Wistar rats at 1000 mg/kg for 7 consecutive days.

KEYWORDS: Vipro™, Anti-inflammatory, Polyherbal formulation, Lipopolysaccharide (LPS)


OBJECTIVE

The objective of the study was to assess the anti-inflammatory activity of Vipro™ Polyherbal formulation in Wistar albino rats after repeated (7 days) administration with the following parameters:

i) Clinical signs including morbidity and mortality

ii) Assessment of TNF-α levels

MATERIALS AND METHODS

This study was initiated after obtaining approval from the Institutional Animal Ethics Committee. Male Wistar albino rats, 8-10 weeks old, weighing 150 to 200 grams were selected and used in the experiment.

Acclimatization

Animals were acclimatized 5 days to laboratory conditions after veterinary examination.

Environmental Conditions

- Temperature: 19.9°C to 24.9°C
- Relative Humidity: 46% to 69%
- Air changes/hour: Minimum 10 to 15
- Photo period: 12 hours dark and 12 hours light

Feed & Water

Conventional laboratory rodent diet were provided *ad-libitum* to the animals. Purified filtered water was provided *ad libitum*.

Bedding

Sterilized corn cob was used as bedding material and changed frequently.

Housing

Animals were group housed in polycarbonate cages in a clean disinfected experimental room during experimental period.

Groups

The rats were divided into 4 groups with 3 rats in each group. The interventions given in all the groups are shown below:

- **Group I** – Normal control. No intervention was given.
- **Group II** – Inflammation control. LPS (5 mg/kg) was injected intraperitoneally on day 7
- **Group III** – Vipro™ (1000 mg/kg) in distilled water, was given orally for 7 days and LPS was injected intraperitoneally on day 7.
- **Group 4** – Standard treatment, Dexamethasone (1.5 mg/kg) was given orally on day 7, 1 hour prior to LPS injection

Dose Selection

1000 mg/kg body weight dose was selected based on the Acute Oral Toxicity Study.

Preparation of Doses

Prior to administration, test item was mixed with distilled water. The maximum dose volume for administration did not exceed 1mL/100g of animal body weight.

Route of Administration and Justification

Oral route was selected because this was one possible and intended route of human exposure and was also recommended by the OECD guideline (OECD 423).

Based on body weight, formulated test item in vehicle was administered as per the below formula:

\[
\text{Dose Volume (in ml)} = \frac{\text{Body Weight of animal (g)} \times \text{Fixed dose volume (ml/kg)}}{}
\]
Individual animals were observed for clinical signs including mortality and morbidity once daily for a period of 7 days. Body weights of animals were recorded at the time of receipt, prior to dosing (day 1) and days 3&7. All animals were euthanized at the end of observation period using CO₂ and Broncho Alveolar Fluid (BALF) was collected from the lungs. After dissection, the lungs and trachea were removed and washed with distilled water. Lungs were lavaged with 1ml of saline five times at controlled room temperature through the cannulated trachea. The collected BALF was centrifuged at 2500g for 10 minutes and the obtained supernatant was stored at -80°C for TNF-α estimation. The estimation of TNF-α was done using commercially available ELISA kit.

RESULTS

Clinical Signs
No Abnormal clinical signs including mortality and morbidity were observed in any of the animal from day 0 till the end of observation period (Table 1).

Body Weight
There was no significant change in the body weights in all groups (Table 2).

TNF-α Estimation
The levels of TNF-α was found to be 200pg/ml in Vipro™ group. These levels are higher when compared to control but lower than in LPS and Dexamethasone groups (Table 3).

CONCLUSION
Based on the results, it is concluded that Vipro™Polyherbal Formulation showed significant anti-inflammatory activity when tested for inhibition of TNF-α using Wistar rats at 1000mg/kg body weight for 7 consecutive days.

CONFLICT OF INTEREST: The authors declare that there is no conflict of interest.

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REFERENCES


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