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Short Communication

Immunomodulatory activities of different crude fractions of *Nepeta juncea*

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In this study, we are reporting the immunomodulatory behavior of different crude fractions of *Nepeta juncia*. In case of immunomodulatory study, the ethyl acetate and chloroform friction demonstrated significant modulatory effect for oxidative burst of polymorphoneutrophils, while the aqueous fraction demonstrated moderate inhibition. Hence, both the $CHCl_3$ and ethyl acetate fractions of *N. juncia* are suggested further for the isolation and identification of active constituents may be responsible for the observed effects.

Key words: *Nepeta juncia*, Lamiaceae, immunmodulatory activities.

INTRODUCTION

Genus *Nepeta* belong from Family Lamiaceae is multi region genus and consist of about 250 species (Li and Hedge, 1977). Different *Nepeta* species are employed as traditional medicine in different part of the world. In some area, they are in practice to treat dysentery, kidney and liver ailments and teeth problems (Baser et al., 2000); employed also as diuretic, vulnerary, diaphoretic, antispasmodic, antiasthmatic, stimulant, febrifuge and sedative agents (Baser et al., 2000; Dabiri and Sefidkon, 2003; Rapisarda et al., 2001; Zargari, 1990). Genus *Nepeta* are also accounted to hold biological behavior especially anti-inflammatory effects and reduction of serum lipids (Agarwal et al., 1978; Prokopenko and Spiridonov, 1985). *Nepeta juncea* Benth wildly grow up in mountainous NA and north western mountains of Pakistan.

Essential oils have been also reported by various investigators (Kobaisy et al., 2006; Tripathi et al., 2004, 2008). Essential oils of species *N. juncea* have revealed antimicrobial potential (Kobaisy et al., 2006; Tripathi et al., 2004, 2008). Hussain et al. (2009) reported that the water fraction showed highest inhibition in platelet aggregation as well as in brine shrimp lethality bioassay similarly, chloroform fraction showed significant antiglycation activity. The aim of the present study was to monitor the active fractions of *N. juncea* and assay for immunomodulatory activities.

MATERIALS AND METHODS

Plant material

The whole parts of the plant *N. juncia were* collected from the Kurram Agency KPK, Pakistan in June 2005 and were identified by plant taxonomist: at the Department of Botany, University of Peshawar NWFP Pakistan. Herbarium specimens were deposited in Department of Botany, University of Peshawar, KPK Pakistan.

Extraction and fractionation

The whole plant of *N. juncea* was dried in dark, cut and ground to coarse powder. The powdered plant (4 kg) was initially extracted with CH_3OH (7 days × 3) at room temperature. The combined CH_3OH extract was evaporated under reduced pressure leaving behind a greenish, syrup residue (150 g). The methanol extract was partitioned in various fractions through separating funnel. It was partitioned into n-hexane (30 g), $CHCl_3$ (50 g), ethyl acetate (20 g), n-butanol (10 g) and water fractions (18 g), successively.

Immunomodulatory activity

Luminol-enhanced chemiluminescence assay was performed as described by Helfand et al. (1982). Briefly, whole blood (diluted 1:200) neutrophils (1 × 107) and PMNs (1 × 106) were suspended in Hank's balance salt solution with calcium and magnesium (HBSS) and incubated with 50 uL of test compounds concentrations (1.6 to 50 ug/ml) for 30 min. To each well, 50 ul (20 mg/ml) zymosan (Sigma Chemical Co. USA), followed by the addition of

Table 1. Immunomodulatory activity of *Nepeta juncia* various fractions and the control drug, Ibuprofen.

Plant samples	With whole blood (µg/ml)	With PMNs (µg/ml)
Ethyl acetate	111.0 ± 2.6	55.5 ± 4.8
Chloroform	37.8 ± 5.9	19.8 ± 0.3
Aqueous	191 ± 1.5	195 ± 2.3
Ibuprofen	11.2 ± 1.9	2.88

PMNs = Polymorphoneutrophils.

50 uL (7 × 10s M) luminol (G-9382 Sigma Chemical Co.) and then HBSS were added to adjust the final volume to 0.2 ml. HBSS was used as a control. Chemiluminescence's peaks were recorded with a Luminometer (Luminoskan RS Lab Finland).

Statistical analysis

The data expressed are median inhibitory concentrations (IC) with ± standard error of mean.

RESULTS AND DISCUSSION

The oxidative burst of polymorphoneutrophils (PMNs) and their ability to inhibit reactive oxygen species (ROS) were analyzed for the various fractions of *N. juncia* including ethyl acetate, aqueous and chloroform fractions. Phagocytic cells on activation induce release of reactive oxygen free radicals (oxidative burst), which is then quantified by a luminol enhanced chemiluminescence assay. A measurement of chemiluminescence is an efficient and highly sensitive to investigate the different kinds of reactive oxygen species (HO, O-2 and HO). Luminol dependent chemiluminescence is a convenient method for detection of super oxide radicals anion in a biological system. Various concentrations of the crude extract of *N. juncia* were incubated with PMNs for 30 min. After the addition of serum treated zymosan and luminol phagocytic cells were scanned at 37°C for their chemiluminescence's activity. Ibuprofen was used as positive control.

Ethyl acetate, aqueous and chloroform fractions from *N. juncia* were screened over a wide range of concentration (6.25 to 200 µg/ml) for their possible modulatory effect on the oxidative burst in whole blood and PMNs, using a luminol based chemiluminescence assay (Hadjimitova, 2002). The result of different assays employed in this study showed that ethylacetate fraction has a potential suppressive effect and clear inhibitory activity for oxidative burst of PMNs at a concentration of 15.8 µg /ml as compared to chloroform and aqueous fraction, while $CHCl_3$ has a significant potential suppressive effect in whole blood at a concentration of 31.8 µg /ml in this assay as shown in the Table 1. This exhibited a clear suppressive effect on phagocytosis response upon activation with serum opsonized zymosan in a dose dependent manner. Proposed implications of the immunomodulatory potential are inhibitors for ROS inflammation control or other immunomodulatory uses.

Conclusion

In conclusion, the results of the present study indicate that fractionated samples of *N. juncia* possess significant immunomodulatory activities. In order to further exploit the *in vivo* immunomodulatory activities of this indigenous medicinal plant and to come up with a potent, safe and economically affordable formulation, further investigations are to be required.

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