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Immunostimulant potency of *Cassia alata* petals in *Garra rufa*

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Abstract

Cassia alata, a well-known medicinal and ornamental plant have several biological activities such as antimicrobial, purgative, anti-inflammatory, analgesic, hypoglycemic and antitumor activities. Considering the growing demands of herbal medicine and the need for natural sources for immune stimulants, the immuno stimulant potency of common ethno-medicinally important plant *Cassia alata* was investigated. Petals of *Cassia alata* was used in this study and feed was prepared from it. Phytochemical screenings were also performed using petroleum ether extract of the petals. Freshwater fish *Garra rufa* was used as a specimen model in the study. After feeding period, the blood samples were collected from the fish and haematological parameters were analysed. A significant P value of 0.0093 in the body weight of *Garra rufa* suggests that Cassia alata is a good immunostimulant. The finding suggests that the feed of *Cassia alata* is a good immunostimulant.

Key words

Cassia alata, Garra rufa, Haematological parameters, Immunostimulant.

Introduction

Cassia alata Linn belongs to the class Fabaceae. It is identified as an important medicinal and ornamental plant. *Cassia alata* is a native of tropical America and it is now widespread in warm countries. It has diverse medicinal values like antimicrobial , anti-inflammatory (Paul *et.al.,* 2013) , antifungal (Fuzellier *et al.,* 1982) , analgesic (Palanichamy *et al.,* 1990), purgative (Chatterjee *et al.,* 2013) and antitumor (Biresh *et al.,* 2014). It is commonly called as candlestick plant. It is rich in important phytochemical constituents such as polysaccharides, glycosides, alkaloids, phenols, flavonoids and cardiac glycosides.

Garra rufa is also called as doctor fish, nibble fish, kangal fish and bone fish. They are native of northern and central Middle East. *Garra rufa* are known to remove dead skin and produce diathanol (Wildgoose 2012). Diathanol is a naturally occurring chemical associated with regeneration of skin. The fish are teethless and they nibble the dead or unhealthy skin. This allows the process of regeneration of cells to occur. Though they are hardy and capable of tolerating cool water, *Garra rufa* prefer tropical conditions (Uribe *et al.,* 2011). Ichthyotherapy is a method of treating skin diseases using two different types of therapeutic fish, *Cyprinion macrostomus* and *Garra rufa*. Ichthyotheraphy was applied for the first time in therapeutic purpose only in Kangal Spa in the Central Anatolia region of Turkey (Somchit *et al.,* 2003).

Garra rufa can tolerate a wide range of water temperature between 18-30 ^{0}C. They are omnivores and require a balanced diet. Mortality rate of *Garra rufa* due to *Aeromonas sobria* bacteria is prevalent among ichthyotheraphy treatments.

Among the variety of health issues in fishes, infections caused by bacteria are wide spread and highly pathogenic to warm water fish. The severity of infection caused by bacterial sp in *Garra rufa* has been reported previously (Neil *et al.,* 2013). Antibiotics used against these infections have been identified to increase the risk of developing antibiotic resistant strains, although vaccines have been developed but they are not available commercially (Kum *et al.,* 2012). Medicinal plants are used as immunomodulating agents in traditional medicines (Mukherjee *et al.,* 2014) and their use as immune modulators may show a strong antibacterial effect in preventing bacterial infections. Medicinal plants are used not only to treat diseases but also as growth promoters, stress resistance boosters, preventatives of infections and immuno stimulants (Ali *et al.,* 2008) . Thus, in the light of above the present study aimed at evaluating the effect of *Cassia alata* petals as an immune stimulant in fish and to increase their resistance against microbial infection.

Materials and Methods

Fish rearing and conditioning : The experimental fish *Garra rufa* were purchased from Jeevan Aqua Palace, Chennai. Fish of uniform length (10 cm) and weight (25±5g) were segregated from stock and acclimatized for 10 days under the following laboratory conditions: temperature 30±2°C, pH 7.5- 7.8 and a photoperiod of 12:12-h L/D.

Flower collection and processing : *Cassia alata* was collected from local places of Kanchipuram, Tamil Nadu and was scientifically authenticated. These flowers were shade dried and powdered coarsely.

Sample preparation : The powdered petals of about 500g were extracted with 1L of petroleum ether using soxhlet's apparatus for 72 hrs at 50°C. After extraction, the solvent was removed with the help of a rotator evaporator. The extract obtained was used for phytochemical screening.

Phytochemical screening of C*assia alata* petal extract : The freshly prepared petal extract of C*assia alata* were qualitatively tested for the presence of chemical constituents. They were identified by characteristic color changes and precipitation reactions using standard procedures (Balandrin *et al.,* 1985 and Kiritikar *et al.,* 1975).

Feed preparation : The feed was prepared by mixing the petal powder along with the commercial feed; with the addition of required quantity of water. The pellets were prepared by hand pelletizer having 1.8mm diameter in size. Finally, the pellets were air dried, packed in airtight polythene bags and labelled.

Growth performance : The growth performance was assessed in terms of percentage weight gain.

Weight gain % = [(Final weight - Initial weight) / initial weight] X 100

Experimental design : Fish were divided into four groups of seven fish each. Group 1 served as control and received normal diet throughout the experimental period. Group 2 fish received 5g of commercial feed and 5 g of *Cassia alata* powder. Group 3 fish received 5 g of commercial feed and 10 g of *Cassia alata* powder. Group 4 fish received 5 g of commercial feed and 15 g of *Cassia alata* powder, respectively. Experimental feeding was done for 45 days.

Blood collection : After feeding period, blood samples were collected from each group. Blood from caudal fin was collected and stored in 1.5 ml heparinized eppendorf tubes. Haematological values were measured following the standard protocol. WBC was counted by Neubaur's chamber (Homatowska *et al*., 2002).

Results and Discussion

Table 1 shows the presence of various potential phytochemicals of medicinal property in *Cassia alata* petals. The bioactive compounds glycosides, phenols, terpenoids, anthraquinones, flavonoids, alkaloids, steroids, quinones could enhance the curative process of health in fish (Nguyen *et al.,* 2008) .Variety of plant derived materials such as alkaloids, flavonoids and other phytochemicals have been reported to modulate the immune system (both innate and adaptive) (Kumar *et al.,*2012). Polyphenols can boost T cell

Table 1 : Phytochemical screening of *Cassia alata*

Phytochemicals	Result
Glycosides	+
Cardiac Glycosides	+
Phenols	+
Terpenoids	-
Anthraquinones	+
Flavonoids	+
Alkaloids	+
Steroids	-
Quinones	+

+/- Presence or absence of phytochemicals

Table 2 : Net gain in weight of the experimental groups

Parameters	Group 1	Group 2	Group 3	Group 4	P value
Initial weight (g)	24.33±0.577	24.66±0.577	26 ± 2.64	26 ± 1	0.0045
Final weight (g)	26 ± 1	28 ± 1.73	30.33 ± 1.52	27 ± 1	0.0045
Weight gain (%)	5.49 ± 2.45	14.88 ± 6.19	17.15 ± 7.88	3.84 ± 0.15	0.0093

Table 3: Haematological parameters in control and experimental fish

Cells	Group 1	Group 2	Group 3	Group 4	P value
Lymphocytes	48.66±0.577	50.66±0.577	51.66±0.577	46± 1	0.0000
Neutrophils	42.66±0.577	38.33±0.577	35± 1	39.33±0.577	0.0000
Eosinophils	5.33± 0.577	3.33± 0.577	3.66 ±0.577	4.6± 0.577	0.0100
Monocytes	3.66± 0.577	3.7± 1	3.9 ±1	3.4 ±1	0.0000
Basophil	3.66± 0.577	2.33 ±0.577	1.33± 0.577	2.33± 0.577	0.0078
Total WBC count	11,200±264.57	17,150±132.28	23,372±118.09	16,000± 100	0.0000

*P <0.05 - Statistically significant

immunity by increasing the number of T cells and its sensitivity towards stimulants (John *et al.,* 2011).

During the feeding period the treated fish showed active movements when compared to the control. There was a remarkable increase in the weight of fish fed with petal extract than fish fed with common feed (Table 2). The percentage of weight gain was found to be significant (p value less than 0.05) in the group of fish treated with plant extracts (Wagner *et al.,* 1990).

The primary function of fish lymphocytes seems to act as the cells of specific immune system via antibody production (Jan Raa., 2000). Hematological values are represented in Table 3. The results of the present study showed an increase in the lymphocyte count in the blood samples collected from the treated fish (Group 2 and Group 3) as compared to the control (Group 1). In the present study, *Cassia alata* increased the immune system in *Garra rufa* at both specific and non-specific levels. The herbal extracted as an immuno-modulator and boosted the immune system in *Garra rufa.* A significant increase in the weight gain and change in protein content of fish was noted. This confirmed that the immuno-stimulant herbal incorporated diet increases the humoral elements in serum (Lichenstein *et al.,* 1994).

The results of the present study is in agreement with the findings of Sahu *et al.,* (2007), where WBC counts increased in *Rohita* fed with *Magnifera indica.* There was an increase in WBC count on feeding carp with chitin (Bhattacharjee *et al.,* 2011). The present study proved a gradual increase in the monocytes count in the treated fish groups (Table 3) when compared with control. This enhances the probability of the groups to be more resistant to infections. Immunostimulatory feed enhanced the extracellular respiratory burst activity as compared to control group. Similar results were observed in rainbow (Dugenci *et al.,* 2003) on treatment with various medicinal plant extract. A similar study by Jeremy *et al.* (2001) reported an increase in all the immunomodulatory parameters of various medicinal plant extracts like ginger, viscum album. The probable mechanism shows an enhancement in phagocytosis and extracellular burst activity of blood leukocytes (Table 3). Increase in the level of neutrophils in herbal feed fed fish can be attributed to the specific immune response (Lemberkovics *et al.,* 1998). Most of the phytoconstituents of *Cassia alata* are known to influence the biological system. The secondary metabolites are responsible for the immune stimulating activity in the doctor fish *Garra rufa. Cassia alata* may possess diversified properties with pharmacological benefits and may be contributed in folk medicine. Also the herb possesses a strong immunopotency and is a growth promoter(Mahady, 2002).

The results obtained in the present study indicate that *Cassia alata* is a potent immunostimulant which can stimulate both specific and non-specific immune system. The immunomodulatory activity of the petal extract of *Cassia alata* on *Garra rufa* showed an increase in blood cell profile which is a sign of enhancement of specific and non-specific immune system. The immunomodulatory effect could be attributed for the presence of flavonoids, alkaloids, terpenoids and phenolic compounds in the extract. Therefore *Cassia alata* petals holds a promise for being used as a strong

immunostimulating agent for human health. It has been reported that *Cassia alata* is rich in many phytochemical constituents that are responsible for the immune stimulating property (Table 1). The results proved that there was a significant increase in weight of the fish fed with the extracted product than the fish fed with commercial feed (Table 2). The results of the present study showed an increase in the lymphocytes count in the blood samples collected from the treated fish (Group 2 and Group 3) as compared to control (Group 1) (Table 3).

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