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Evaluation of Immuno-Stimulant Action of Prickly Pear Fruit Juice *In-Vitro*

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Abstract : Immune system is the main defense mechanism of our body. Strengthening of the immune system is nowadays the major research target of the researchers, as there is a dire need of new safe, economical immune stimulant which is readily available to the common people. Currently, the plant Prickly Pear is reported to possess Immuno-Stimulant activity due to its phytochemicals. The aim of the research was toevaluate immuno-stimulant action of Prickly pear fruit juice *in vitro*. Prickly pear fruits are collected from local market of Tramba (Gujarat-India). The seed free juice of the fruits prepared and immunostimulant action of prickly pear is evaluated using *In-vitro* Phagocytosis test. Confirmation of immuno-stimulant action of prickly pear is done using nitro blue tetrazolium dye test. Anti-oxidant and free radical scavenging potential of Prickly pear perusing DPPH and H_2O_2 test. And Statistical analysis is performed.The results of Phagocytic activity data indicate were significantly different when compared with control and level of significance which was P>0.001. As per DPPH model, indicate that result data were significantly different when compared with control and level of significance which was P>0.001. H_2O_2 model indicate that data were significantly different when compared with control and level of significance which was P>0.001. Thus, Immunostimulant and free radical scavenging activity were confirmed. According to the obtained results, we can conclude that prickly pear possesses strong immune-stimulant potential as well as better free radical scavenging potential.
Keywords : Anti-oxidant, Free radical Scavenging potential, Fruit Juice, Immunostimulant, *Opuntia ficus indica*, Phagocytic action.

Introduction

Immunostimulants, are substances comprising of drugs and nutrients that stimulate the immune system by inducing activation of components of the immune system[1]. During the last two decades, investigations have been carried out for the production in producing a novel category of biologically active substances, the immunostimulants these are the products derived from natural sources or synthetically made with different

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chemical characteristics and varied modes and mechanism of action[2]**.**Currently available immunostimulants like levamisole, Thalidomide, Imuunocynin, Isoprinosine produce adverse effects like allergy, CNS depression, increase uric acid levels in serum, and muscle pain[3]**.**Thus, over past two decades researchers are looking for safe, potent and economic immunostimulant to boost up the immunity. The prickly pear (*Opuntia ficus indica*) is a member of the Cactaceae family, has a unique composition of nutrients, including high levels of vitamin C, B-Family vitamins, magnesium, potassium, calcium, copper, and dietary fiber. In terms of organic compounds, cactus fruit has high levels of flavonoids, polyphenols, and betalains, all of which have a positive impact on health. Advantages of Prickly pear fruit are, Immune system booster, builds strong bones and teeth, digestive health, heart health, cancer Prevention, anti-oxidant potential, weight loss effects, Helps in inflammatory condition[4]**.**

Review of literature suggests that plant with various vitamins like vitamin C, vitamin A, etc can improves body immunity and help in management of immuno-deficiency diseases like AIDS. Prickly pear is one of the most widely used fruit in recent era and containing many required vitamins and anti-oxidants who can act as iron chelator as well as reported to have anti-oxidant potential. Ethnopharmacology also suggests that fruits of prickly pear can be useful as immunostimulant. Considering this principle as well as looking at the dire need to find out a cheaper, easily available and homemade remedy for improvising immunity, we focused on evaluation of immunostimulant action of prickly pear[5]**.**

Figure 1 Prickly pear fruits

The objectives of our study was to prepare seed free prickly pear fruit juice, evaluation ofimmuno-stimulant action of prickly pear fruits using *In-vitro* phagocytic test and establish antioxidant potentialof prickly pear fruits using DPPH and H_2O_2 model.

Experimental

Preparation Of Juice

Fresh fruits of prickly pear which was obtained from the local market of Rajkot (Gujarat). The pulp of the same were mixered (100 g) and filtered to obtain the seeedless juice.

DPPH Assay

The samples were reacted with the stable (0.008 mM) DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) radical in an ethanol solution. The reaction mixture consisted of adding 0.5 mL of sample, 3 mL of absolute ethanol and 0.3 mL of DPPH radical solution 0.5 mM in ethanol.

0.5 ml of test samples were reacted with the stable 0.008mM. When DPPH reacts with an antioxidant compound, which can donate hydrogen, it is reduced. The changes in color (from deep violet to light yellow) were read the absorbance at 517 nm after 100 minutes of reaction using a UV-VIS spectrophotometer. The decrease in absorbance of samples were measured. EDTA (100 µg/ml) is used as standard[6-7]**.**

Hydroxyl Radical Scavenging Activity

The hydroxyl radicals were generated from 40mM H_2O_2 solution. The ability of prickly pear fruit juice to scavenge the radicals determined by decreased pink chromophore generation at 532 nm using double beam UV spectrophotometer. For Comparison of scavenging potential of prickly pear fruit juice, EDTA (100 µg/ml) is used asstandard[8].

Phagocytosis Assay

Polymorphonuclear leucocytes (PMN cells) was collected from volunteers in a sterile heparinized tube. The cell density adjusted by pellet formation to 10^6 cells/ml by suspending in the minimum essential medium (MEM) which contain Phosphate buffer and 0.9 % saline solution. *Candida albicans* cells were incubated at 37^o C for 24 hours, in the presence of the test juice. Cytosmears were prepared after incubation. The smear was fixed with methanol, stained with Giemsa and studied under 100X 'oil immersion objective' to determine the phagocytic activity of PMN cells. Neutrophils (100 nos.) were scanned and the cells with ingested microorganisms were counted. The parameters evaluated were percentage phagocytosis (percentage of PMN cells involved in phagocytosis) and phagocytic index (ratio of number of *Candida albicans* engulfed to the total number of neutrophils)[9].

Results and Discussion

DPPH Assay

In DPPH model, juice of prickly pear shown significant DPPH scavenging potential reduced absorbance i.e. 0.4876 ± 0.0004513which is found significantly lower than that of control group i.e. 1.0183 ± 0.002805, which clearly suggests anti-oxidant and free radical scavenging potential of prickly pear juice. Similar kind of results were found with Standard group.The obtained results are shown in Figure 2. The results are shown in form of mean ± SEM.

Here, *** indicate data were significantly different when compared with control and level of significance which was *P<0.001*

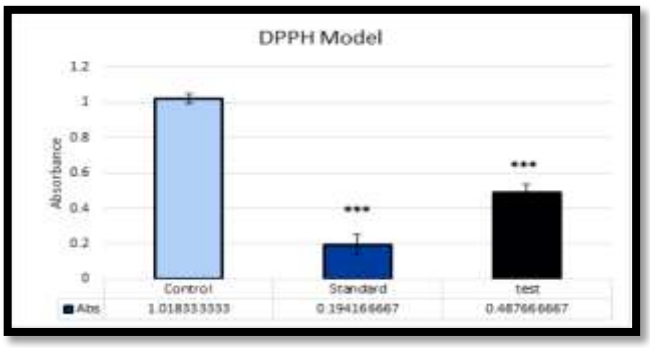

Figure 2Spectrocopic findings ofDPPH assay

Hydroxyl Radical Scavenging Activity

In H_2O_2 model, juice of prickly pear shown significant H_2O_2 radical scavenging potential by reduced absorbance i.e. 0.2565 ± 0.0003908which is found significantly lower than that of control group i.e. 1.0773 ± 0.0004513which clearly suggests anti-oxidant and free radical scavenging potential of prickly pear. Similar kind of results were found with Standard group. The obtained results are shown in Figure 3. The results are shown in form of mean ± SEM.

Here, *** indicate data were significantly different when compared with control and level of significance which was *P<0.001*

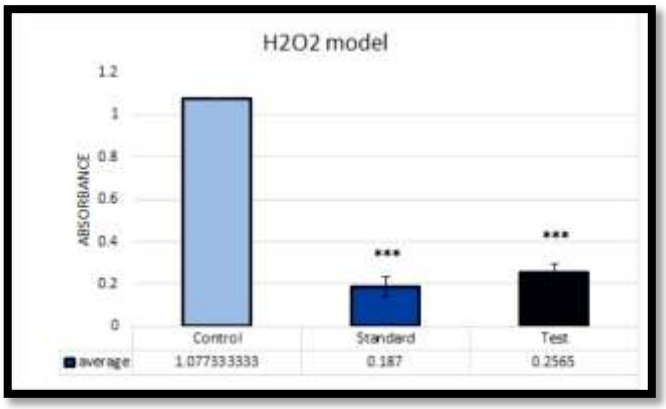

Figure 3Spectrocopic findings ofH_2O_2 assay

Phagocytosis Assay

In phagocytic index model, juice of prickly pear increased mean number of neutrophils in unit area i.e.15.345 ± 0.003908 which was found significantly higher than that of control i.e. 7.050 ± 0.05137.This finding clearly suggests immunostimulant action of prickly pear fruit.Similar findings were observed with Standard group. The obtained results are shown in Figure 4. The results are shown in form of mean ± SEM.

Here, *** indicate data were significantly different when compared with control and level of significance which was *P<0.001*

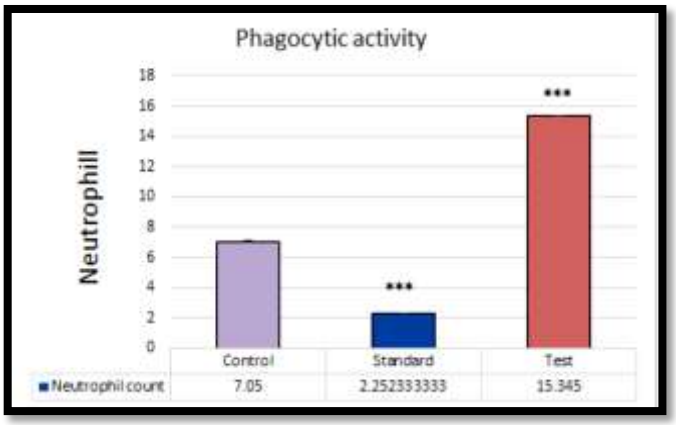

Figure 4Findings fromPhagocytic activity analysis

In nutshell, From interpretation of DPPH and H_2O_2 model, juice of prickly pear shown decreased in mean absorbance and shown significant scavenging action for free radicals generated by these two compounds compared to that of control which clearly suggests anti-oxidant and free radical scavenging potential of prickly pear. In phagocytic index model, juice of prickly pear increased mean number of neutrophil in unit area and shown significant higher absorbance phagocytic index compared to that of control which clearly suggests immunostimulant action of prickly pear.

Conclusion

From the work done and results shown here, we can conclude that prickly pear possesses strong immune-stimulant potential as well as better free radical scavenging potential as same dose. The exact mechanism is not yet clear, so further studies are recommended. Pre-clinical and clinical studies may give the exact idea about the immunostimulant activity and its mechanism of action.

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