Acta Scientific Pharmaceutical Sciences (ISSN: 2581-5423)

Volume 5 Issue 5 May 2021

Research Article

Investigation of Protective Effects of Graviola (*Annona muricata*) Plant against Disinfectants Used in Different PHs in Covid-19 Process

Sidika Genc1, Yesim Yeni[1], Ahmet Hacımüftüoğlu[1] and Ali Taghizadehghalehjoughi[1,2]*

[1]*Department of Medical Pharmacology, Faculty of Medicine, Ataturk University, Turkey*

[2]*Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, Ataturk University, Turkey*

***Corresponding Author:** Ali Taghizadehghalehjoughi, Department of Medical Pharmacology, Faculty of Medicine, Ataturk University, Turkey.

Received: March 01, 2021

Published: April 14, 2021

Abstract

Coronavirus disease 2019 (COVID-19) caused by acute respiratory syndrome coronavirus-2 (SARS-CoV-2) is a major threat to global health. The rapid spread of the epidemic around the world has caused it to become a global pandemic. Disinfection is one of the best practice measures used to eradicate viral/bacterial pathogens in homes and community settings and reduces the spread of infection. However, it has been shown in studies that the long-term use of degenerate agents with different pH values triggers the development of itching, rash, urticaria and even allergic rhinitis. Graviola (*Annona muricata*) and its fruits, leaves, stems and roots are known to be rich in flavonoids and isoquinoline alkaloids. It has been used for thousands of years to treat many diseases from arthritis to liver problems. It is also used to treat a wide variety of human diseases such as inflammation, rheumatism, diabetes, hypertension, insomnia, parasitic infections and cancer.

The aim of this study is to prevent toxicity that develops against different pH changes with the use of Graviola plant. For this reason, Human Fibroblast cell line was grown under the conditions specified by the manufacturer and inoculated into 96 well plates and culture medium with different pH (5 - 6 - 7 - 8) was prepared and different doses of Graviola (20-40-80-160 µgr/ml) were prepared. It is planned to eliminate the toxicity caused by using it for 24 hours. At the end of the study, cell viability was determined by performing MTT analysis and LDH and GR.

According to the results of our study, cell viability increased from %71 to 132 compared to positive control at pH 5. The vitality that decreased to %79 at 6 pH increased to 132 as a result of the application of the highest concentration of Graviola (160 µgr/ml). While it increased from % 71 to %74 at pH 7, the damage received by fibroblast cells at pH 8 was determined to be high (viability rate %50) and by removing this toxicity, it was observed that the vitality was increased up to %75. Considering the results obtained, Graviola is recommended to be used at doses of 80 and 160 µgr/ml, as it has a protective effect. The LDH and GR results show correlation with MTT.

Keywords: Graviola; Covid 19; pH; MTT; LDH

Abbreviations

SARS-CoV-2: Coronavirus-2; COVID-19: Coronavirus Disease 2019; MTT: 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide; LDH: Lactate Dehydrogenase; GR: Glutathione Reductase; WHO: World Health Organization; GST: Glutatyon-transferaz; CAT: Chloramphenicol Acetyltransferase; SOD: Superoxide Dismutase

Introduction

The coronavirus disease 2019 outbreak (COVID-19), which emerged in Wuhan, China in December 2019, was declared by the World Health Organization (WHO 2020b) as an emergency case of international importance and threatening public health. WHO has announced the official name of the virus as "COVID-19 virus" previously known as "2019-nCoV" or "Wuhan Coronavirus" [1]. In the first three months of the epidemic, this epidemic spread rapidly across the country and the world and became a global epidemic. The COVID-19 pandemic has shaken the entire scientific community, and researchers around the world have turned to work to devise various intervention strategies to treat and prevent the disease. There are two main routes of transmission of SARS-CoV-2: infected respiratory droplets and transmission by contact [2]. Current evidence suggests that droplets containing SARS-CoV-2 can survive for hours on surfaces made of various materials [3] in temperature ranges from 4°C to -70°C (4°C to -70°C) [4]. For this reason, the use of disinfectants has gained importance and the use of degenerates with different chemical formulations has become widespread.

Disinfection is one of the best practice measures used to eradicate viral/bacterial pathogens in homes and community settings and reduces the spread of infection. Therefore, as COVID-19 spread around the world, the use of disinfectants has increased at the same rate. However, its use is thought to cause global secondary disasters in human health and ecosystems. These chemicals, when used regularly, increase the risk of chronic obstructive pulmonary disease (COPD), asthma and eye irritation in healthcare workers and individuals [5-7]. Chemical residues left on a surface can be airborne and inhaled, and often cause reactions in asthmatic, allergic or sensitive individuals. These residues have been shown to cause cancer, reproductive disorders, respiratory disorders (including occupational asthma), skin reactions with symptoms such as eye and skin itching, rash, urticaria, central nervous system (CNS) deterioration, and oxidative damage [8].

Herbal treatments are traditionally used in many parts of the world. Annona muricata (also known as Graviola) is a tropical fruiting tree of the *Annonaceae* family found in the rainforests of Africa, South America and Southeast Asia [9,10]. Its leaves, pericarp, fruits, seeds and roots are widely used in traditional medicine. Different parts of A. muricata have been used in the treatment of diabetes [11,12], cough, cancer [13,14], arthritis [15], hypertension [16], skin diseases [17] and malaria [18]. In addition, there are studies showing that it is used as antimicrobial [19], antidiabetic [12], anti-inflammatory [20], antiprotozoan [21], antioxidant [22], insecticide, larvicide [23]and anticancer [24].

Aim of the Study

The aim of this study is to prevent the toxicity of the fibroblast cell line with the use of Graviola against different pH changes caused by increased disinfectant use with Covid 19.

Material and Method

Chemicals and reagents

It was obtained from Graviola Solgar (U.K). All chemicals derived from Dulbecco Modified Eagles Medium (DMEM), Fetal bovine serum (FBS), phosphate buffer solution (PBS), antibiotic antimitotic solution (Penicillin/Streptomycin/Amphotericin B) (100 ×), L glutamine and trypsin-EDTA has been. HCl and NaOH were obtained from Sigma Aldrich (St. Louis, MO, USA).

Cell culture

For the study, a fibroblast cell line, Ataturk University (Erzurum, Turkey) were obtained from medical pharmacology department. Briefly, the cell suspension was centrifuged at 1200 rpm for 5 minutes. Cells were resuspended in fresh medium DMEM, %10 FBS and %1 antibiotic (penicillin, streptomycin and amphotericin B) and cells were collected in a 25 cm^2 flask. (Corning, USA) and stored in incubator (%5 CO_2; 37°C). When %80 of the flask was covered with the cell, it was removed with Trypsin-Ethylene di amine tetra acetic acid (EDTA) (%0.25 trypsin-%0.02 EDTA) and planted in 96 well plates to be centrifuged.

PH toxicity

DMEM medium was prepared as full medium and added to the cells by adjusting various pH values (5, 6, 7 and 8) with the use of HCl and NaOH by pH meter.

Graviola application

After the cells reached %85 confluence, graviola of different concentration (20, 40, 80 and 160 µg/ml) was added to the corresponding wells and the plates were left in the incubator for 24 hours.

MTT test

At the end of the experiment (after 24 hours of treatment) 10 µL of MTT solution was added to each well plate (1 mM final concentration). Plates were then incubated for 4 hours at 37°C in a CO_2 incubator. After 4 hours, 100 µL of DMSO solution was added to each well to dissolve the formazan crystals. The density of the

formazan crystals was read by the Multiskan™ GO Microplate Spectrophotometer reader at a wavelength of 570 nm [25-27].

LDH (Lactate Dehydrogenase) assay

LDH assay test was performed using a commercially available test kit from Cayman Chemical Co. Ltd, (Ann Arbor, MI, USA). Briefly, the cell culture medium was centrifuged at 400g for 5 minutes at the room temperature 100 µl of the supernatant was added to 100 µl of the reaction solution (LDH Assay Buffer, LDH Substrate Mix) and incubated with gentle shaking on an orbital shaker for 30 min at room temperature. Finally, the absorbance was read at 490 nm wavelength [28,29].

GR (Glutathione Reductase) assay

In the activity measurement of the GR enzyme, the principle that the reacted NADPH gives maximum absorbance at 450 nm was used. The GR enzyme causes a decrease in NADPH in the reaction it catalyzes. Enzyme activity was determined by following this reduction spectrophotometrically at 450 nm [30].

Statistical analysis

Statistical analysis was performed using one-way analysis of variance (ANOVA) with Tukey's HSD for posthoc comparisons using SPSS 22.0 software. $P < 0.05$ was accepted as the statistical threshold for each analysis.

Results

MTT analysis

MTT analysis results are shown in figure 1. Viability percentages of all groups were calculated by comparing with the positive control (pH application only) values and the control values were set as %100 viability. The lowest effect was seen in the Graviola (20 µg/ml) group among the treatment groups compared to the PH control groups ($P > 0.05$). In addition, it was observed that the survival rate in the Graviola (20-40-80 and 160 µg/ml) groups increased depending on the concentration (Figure 1). According to the results of our study, cell viability increased from %71 to 131 compared to positive control at pH 5. The vitality that decreased to %79 at 6 pH increased to 132 as a result of the application of the highest concentration of Graviola (160 µg/ml). While it increased from %71 to %74 at pH 7, the damage received by fibroblast cells at pH 8 was determined to be high (viability rate %50) and it was observed that this toxicity was removed and the vitality was increased up to %83 ($P < 0.05$).

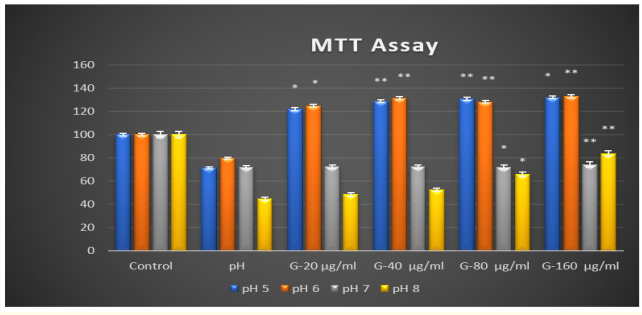

Figure 1: Different PH concentrations and Graviola treatments effects on fibroblast cells viability ratio *P < 0.05, **P < 0.01.

LDH assay

Damage to the cell membranes is reflected as elevated LDH levels in the cell medium after the cells were exposed to Graviola (20- 40- 80 and 160 µg/ml) for 24 h. The LDH activity of the control group was defined as %100, and the other groups were rated accordingly. Our results show that pH 8 was most toxic as indicated by the greatest amount of LDH activity in the media from the fibroblast cells in comparison to the other pH group. Figure 2 shows that combinations of Graviola treatment group at all pH parameters reduce cytotoxicity in fibroblast cells in a time- and dose-dependent manner. Also, the high concentration Graviola groups (80 and 160 µg/ml) differed statistically from the PH control group ($P < 0.05$). A linear correlation was observed between LDH activity and cell viability.

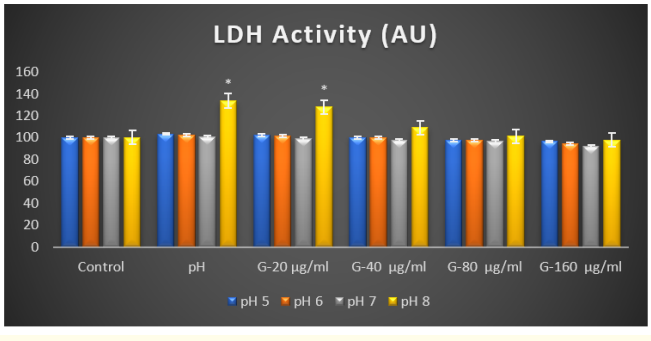

Figure 2: LDH activity (AU) was measured and the result proportioned to control group.
*P < 0.05.

Glutathione reductase assay

Glutathione reductase levels in the cell medium after the cells were exposed to Graviola (20-40-80 and 160 µg/ml) was evaluated for 24h. The GR activity of the control group was defined as %100, and the other groups were rated accordingly. Figure 3 shows that combinations of Graviola treatment group at all pH parameters increased GR level in fibroblast cells. Also, the high concentration Graviola groups (80 and 160 µg/ml) differed statistically from the PH control group ($P < 0.05$ and $P < 0.001$).

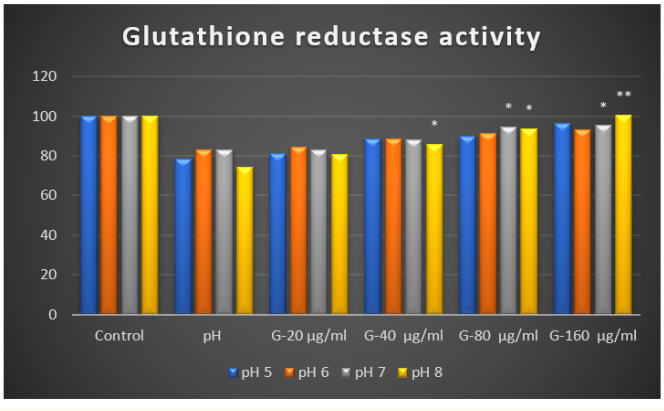

Figure 3: Glutathione reductase activity was measured and the result proportioned to control group.
*P < 0.05, **P < 0.01.

Discussion

Various studies have shown that A. muricata has chemical components of different alkaloids and essential oils [31,32]. However, species belonging to the *Annonaceae* family, including A. muricata, have various acetogenin compounds that act as the main bioactive compounds [33]. Studies have shown that Annona species have wound healing capacity. An *in vivo* study on the wound-healing activity of Graviola trunk bark revealed a significant reduction in the wound area after topical treatment with ethanol extract of the root bark [34]. In another study, Annona squamosa leaves showed a promising wound-healing effect against streptozotocin-induced diabetic rats [35]. Given the wound healing activity of graviola extract, the wound healing process is thought to be accompanied by skin ischemia, which promotes the formation of reactive oxygen species by activated leukocytes in the tissue region.

Prolonged exposure to disinfectant agents may cause skin damage or hypersensitivity. Such exposures can cause immediate or delayed skin reactions. Highly concentrated solutions have caused severe chemical skin burns. The long-term use of these agents is thought to result in greater release of oxygen-derived free radicals and increased tissue oxidative damage [36].

Under normal conditions, body homeostasis balances the level of free radicals by utilizing the human body's endogenous antioxidant capacity. However, when this level exceeds the normal balancing capacity of antioxidants, highly activated radicals will cause different structural changes and cell damage [37,38]. The cells' antioxidant defense systems contain a variety of enzymatic and non-enzymatic scavengers. Enzymatic antioxidants of cells such as GR, glutathione-transferase (GST), chloramphenicol acetyltransferase (CAT), Superoxide Dismutase (SOD) play a critical role in weakening oxidative stress caused by reactive oxygen species [34]. The first defense mechanism against reactive oxygen species is provided by the GR, which reduces oxidative stress through the reduction of NADPH. The antioxidant activity of Graviola leaves has also been reported in previous *in vitro* and *in vivo* studies [39,40].

Among the different Annona species, Graviola leaves were found to have the highest antioxidant activity assessed by FRAP (Ferric reducing antioxidant property), DRSA (DPPH radical scavenging activity) and HRSA (hydroxyl scavenging activity) techniques [37]. An *in vivo* study also showed that ethyl acetate extract of Graviola leaves caused an increase in CAT, glutathione and SOD activity on stomach cells. In our *in vitro* study, it was observed that Graviola concentrations increased fibroblast proliferation in a dose-dependent manner, and also increased antioxidant levels by eliminating oxidative damage. After treatment with Graviola for 24 hours against toxicity applied at different PH, it was observed that the high concentration of Graviola extract (160 µg/ml) at pH 5 and 6 had a statistically significant positive effect on enhancing cellular viability and alleviating oxidative stress.

Conclusion

Today, the rapidly spreading coronavirus is a great danger threatening humanity. Despite the rapid development of vaccine and drug studies, disinfectants continue to be a part of our lives. If the use of disinfectants destroys viral agents, it also damages our skin cells. According to our results, skin cells suffer great damage during PH change. According to our study, Graviola has a strong protective effect on PH between 6 – 8 damage induction. Therefore, we recommend the use of Graviola after hand disinfections.

Bibliography

1. WHO. "Rolling updates on coronavirus disease (COVID-19)" (2020b).

2. Wu SM., *et al*. "Comprehensive transcriptome profiling of Taiwanese colorectal cancer implicates an ethnic basis for pathogenesis". *Scientific Report* 10.1 (2020): 4526.

3. van Leerdam ME., *et al*. "Endoscopic management of Lynch syndrome and of familial risk of colorectal cancer: European Society of Gastrointestinal Endoscopy (ESGE) Guideline". *Endoscopy* 51.11 (2019): 1082-1093.

4. Chin YH., *et al*. "Evolving perspectives on stool testing for colorectal cancer: a qualitative systematic review". *European Journal of Cancer Prevention* 29.5 (2020): 416-423.

5. Casey ML., *et al*. "Health problems and disinfectant product exposure among staff at a large multispecialty hospital". *American Journal of Infection Control* 45.10 (2017): 1133-1138.

6. Weinmann T., *et al*. "Association Between Occupational Exposure to Disinfectants and Asthma in Young Adults Working in Cleaning or Health Services: Results From a Cross-Sectional Analysis in Germany". *Journal of Occupational and Environmental Medicine* 61.9 (2019): 754-759.

7. Dumas O., *et al*. "Association of Occupational Exposure to Disinfectants With Incidence of Chronic Obstructive Pulmonary Disease Among US Female Nurses". *JAMA Netw Open* 2.10 (2019): e1913563.

8. Choi JY., *et al*. "Clinical Implication of Concordant or Discordant Genomic Profiling between Primary and Matched Metastatic Tissues in Patients with Colorectal Cancer". *Cancer Research Treatment* 52.3 (2020): 764-778.

9. Moghadamtousi SZ., *et al*. "Annona muricata (Annonaceae): A Review of Its Traditional Uses, Isolated Acetogenins and Biological Activities". *International Journal of Molecular Sciences* 16.7 (2015): 15625-15658.

10. Coria-Téllez AV., *et al.* "Annona muricata: a comprehensive review on its traditional medicinal uses, phytochemicals, pharmacological activities, mechanisms of action and toxicity". *Arabian Journal of Chemistry* (2016): 30.

11. Karou SD., *et al*. "Ethnobotanical study of medicinal plants used in the management of diabetes mellitus and hypertension in the Central Region of Togo". *Pharmaceutical Biology* 49.12 (2011): 1286-1297.

12. Florence NT., *et al.* "Antidiabetic and antioxidant effects of Annona muricata (Annonaceae), aqueous extract on streptozotocin-induced diabetic rats". *Journal of Ethnopharmacology* 151.2 (2014): 784-790.

13. Liu N., *et al*. "Functional proteomic analysis revels that the ethanol extract of Annona muricata L. induces liver cancer cell apoptosis through endoplasmic reticulum stress pathway". *Journal of Ethnopharmacology* 189 (2016): 210-217.

14. Syed Najmuddin SU., *et al*. "Anti-cancer effect of Annona Muricata Linn Leaves Crude Extract (AMCE) on breast cancer cell line". *BMC Complementary and Alternative Medicine* 16.1 (2016): 311.

15. Prabhakaran K., *et al.* "Polyketide Natural Products, Acetogenins from Graviola (Annona muricata L), its Biochemical, Cytotoxic Activity and Various Analyses Through Computational and Bio-Programming Methods". *Current Pharmaceutical Design* 22.34 (2016): 5204-5210.

16. Hajdu Z and J Hohmann. "An ethnopharmacological survey of the traditional medicine utilized in the community of Porvenir, Bajo Paragua Indian Reservation, Bolivia". *Journal of Ethnopharmacology* 139.3 (2012): 838-857.

17. Longuefosse JL and E Nossin. "Medical ethnobotany survey in Martinique". *Journal of Ethnopharmacology* 53.3 (1996): 117-142.

18. Asase A., *et al.* "Uses of multiple plants prescriptions for treatment of malaria by some communities in southern Ghana". *Journal of Ethnopharmacology* 144.2 (2012): 448-452.

19. Pai BM., *et al*. "Anti-microbial Efficacy of Soursop Leaf Extract (Annona muricata) on Oral Pathogens: An In-vitro Study". *Journal of Clinical and Diagnostic Research* 10.11 (2016): ZC01-ZC04.

20. Oliveira AP., *et al*. "Exploratory Studies on the in Vitro Anti-inflammatory Potential of Two Herbal Teas (Annona muricata L. and Jasminum grandiflorum L.), and Relation with Their Phenolic Composition". *Chemistry and Biodiversity* 14.6 (2017).

21. Boyom FF., *et al*. "Potent antiplasmodial extracts from Cameroonian Annonaceae". *Journal of Ethnopharmacology* 134.3 (2011): 717-724.

22. Santhosh SB., *et al*. "Spectral and HRTEM analyses of Annona muricata leaf extract mediated silver nanoparticles and its Larvicidal efficacy against three mosquito vectors Anopheles stephensi, Culex quinquefasciatus, and Aedes aegypti". *Journal of Photochemistry and Photobiology B: Biology* 153 (2015): 184-190.

23. Santhosh SB., *et al.* "Annona muricata leaf extract-mediated silver nanoparticles synthesis and its larvicidal potential against dengue, malaria and filariasis vector". *Parasitology Research* 114.8 (2015): 3087-3096.

24. Paul J., *et al*. "Anti cancer activity on Graviola, an exciting medicinal plant extract vs various cancer cell lines and a detailed computational study on its potent anti-cancerous leads". *Current Topics in Medicinal Chemistry* 13.14 (2013): 1666-1673.

25. Genc S., *et al*. "Does Picnogenol and Melatonin Combination Decrease Tumor Cells Viability in Comparison to Platin Base Anti-Cancer Drug?" *Acta Physiologica* 227 (2019): 71-71.

26. Yeni Y., *et al*. "Antitumor Effect of Combination Momordica and Melatonin on Prostate Cancer Pc-3 Cell Line". *Acta Physiologica* 227 (2019): 71-71.

27. Taghizadehghalehjoughi A., *et al*. "Na+ channel blocker enhances metformin effects on neuroblastoma cell line". *Medicine Science International Medical Journal* (2019).

28. Taghizadehghalehjoughi A., *et al*. "Melatonin receptors increase Momordica's anticancer effects against PC-3 and HT-29". *Journal of Contemporary Medicine* 11.2 (2021): 1-8.

29. Taghizadehghalehjoughi A., *et al.* "Combination of Pycnogenol and Melatonin Reduce PC-3 and HT29 Cell Migration: Comparison to the Actions of Cisplatin". *Acta Scientific Pharmaceutical Sciences* 4.12 (2020): 176-188.

30. Carlberg I and B Mannervik. "Purification and characterization of glutathione reductase from calf liver. An improved procedure for affinity chromatography on 2',5'-ADP-Sepharose 4B". *Analytical Biochemistry* 116.2 (1981): 531-536.

31. Cosme Kossouoh MM., *et al.* "Essential Oil Chemical Composition of Annona muricata L. Leaves from Benin". *Journal of Essential Oil Research* 19.4 (2007): 307-309.

32. Moghadamtousi SZ., *et al*. "Annona muricata leaves accelerate wound healing in rats via involvement of Hsp70 and antioxidant defence". *International Journal of Surgery* 18 (2015): 110-117.

33. Rupprecht JK., *et al.* "Annonaceous acetogenins: a review". *Journal of Natural Prodution* 53.2 (1990): 237-278.

34. Paarakh PM., *et al*. "Wound healing activity of Annona muricata extract". *Journal of Pharmacy Research*, 2.3 (2009): 404-406.

35. Ponrasu T and L Sugun. "Efficacy of Annona squamosa on wound healing in streptozotocin-induced diabetic rats". *International Wound Journal* 9.6 (2012): 613-623.

36. Slaughter RJ., *et al.* "The clinical toxicology of sodium hypochlorite". *Clinical Toxicology (Phila)* 57.5 (2019): 303-311.

37. Soneja AM., *et al.* "Role of nitric oxide, nitroxidative and oxidative stress in wound healing". *Pharmacology Report* 57 (2005): 108-119.

38. Valko M., *et al*. "Free radicals and antioxidants in normal physiological functions and human disease". *The International Journal of Biochemistry and Cell Biology* 39.1 (2007): 44-84.

39. Caxton-M., *et al.* "Morphological changes and hypoglycemic effects of Annona muricata linn. (annonaceae) leaf aqueous extract on pancreatic β-cells of streptozotocin-treated diabetic rats". *African Journal of Biomedical Research* 9.3 (2006).

40. Baskar R V., *et al.* "Kumar, In vitro antioxidant studies in leaves of Annona species". *Indian Journal of Experimental Biology* 45.5 (2007): 480-485.