International Standard Serial Number (ISSN): 2249-6807

International Journal of Institutional Pharmacy and Life Sciences 6(3): May-June 2016

INTERNATIONAL JOURNAL OF INSTITUTIONAL PHARMACY AND LIFE SCIENCES

Pharmaceutical Sciences

Research Article……!!!

Received: 15-03-2016; Revised: 18-05-2016; Accepted: 19-05-2016

EVALUATION OF ANTI-ASTHMATIC ACTIVITY OF AQUEOUS EXTRACT OF *CAMELLIA SINENSIS*

M.Sangeetha[a]*, K.Vasuki[a], R.Priyatharshini[a], J.Gopi Sudheer Kumar[a], S.Umamaheswari[b], S.Kavimani[a]

[a]Department of Pharmacology, College of Pharmacy, Mother Theresa Post Graduate and Research Institute of Health Sciences, Indira Nagar, Gorimedu, Puducherry – 605 006, INDIA

[b]Department of Pharmacology, Faculty of Pharmacy, Sri Ramachandra University, Porur, Chennai – 600 116, Tamilanadu, INDIA.

Keywords:

Anti-asthmatic activity, *Camellia sinensis*, Green tea, flavanoids

For Correspondence:

M.Sangeetha

Department of Pharmacology, College of Pharmacy, Mother Theresa Post Graduate and Research Institute of Health Sciences, Indira Nagar, Gorimedu, Puducherry – 605 006, INDIA

E-mail:

mohansangeetha500@gmail.com

ABSTRACT

The ancient civilizations of the Chinese, Indians and North Africans were providing written evidence for the use of natural resources to treat several human disorders as a source of drug. Management of asthma with present drugs was not effective and poor control and produce side effects due to frequent use. Catechin is a strong antioxidant obtained from green tea and has been proven several pharmacological activities. Hence, the present study was conducted to evaluate and compare the anti-asthmatic activity of aqueous extract of two (Tetley and Lipton) commercial green tea (*Camellia sinensis*) products against the tracheal smooth muscle contraction *in vitro* model. The percentage inhibition of tracheal smooth muscle contractions by histamine in the different concentrations(10 µg/ml, 20 µg/ml, 40µg/ml) of two test aqueous extract were revealed as dose dependent effect and the primary phytochemical screening revealed the presence of flavanoids, saponins, tannins and other constituent. Hence the present study concluded that the commercial green tea product possessed a significant ($P<0.05$) antiasthmatic activity against the histamine reduced contraction *in-vitro*. Lipton was found to be more active than Tetley.

1. INTRODUCTION

For thousands of years Natural products, including plants, animals and minerals have been served as a major source of drugs to treat the human diseases and about half of the pharmaceuticals in use today are derived from natural products[1]. Recently, the number of occurrences and severity of asthma have been rising sharply, especially within certain populations. Overall, inhaled corticosteroids remain the most effective form of asthma treatment. Despite the overall effectiveness some portions of the population do not respond well to inhale corticosteroids. This has led to the investigation and development of new asthma treatments, especially for people who have severe asthma symptom[2]. Asthma is a chronic (long-term) lung disease that narrowing of the airways of the lungs caused by inflammation in the air passages, resulting from both genetic and environmental influences. It is a lifelong disease that can limit a person's quality of life and even lead to death, if not treated properly. Asthma affects people of all ages, but it most often starts during childhood. Today, more than 25 million people and about 7 million of these people are children suffer from asthma, with the prevalence increasing 12 percent in the last decade, according to the Centers for Disease Control and Prevention. Each day, 40,000 Americans miss school or work due to asthma, costing the U.S. economy an estimated $56 billion each year in direct and indirect costs[3].

Plant derived constituents have received considerable attention in recent year due to their presumed safety and potential nutritional and therapeutic effects[4]. Purest and unadulterated form of green tea from *Camellia sinensis* influences the human health from generations and day-by-day research throughout the world make the people aware of health benefits associated with this green tea. *Camellia sinensis* is a large evergreen shrub indigenous to Eastern Asia, have been used to prepare beverages from more than 4000 years. Green tea is a type of cured tea that is non fermented and produced by drying and steaming the fresh leaves and non developed buds of *Camellia sinensis* from the family of Theaceae[5] and is the second most consumed beverage in the world[6]. Tea has been used medicinally from centuries in the Traditional Chinese Medicine (TCM). In recent times, there has been renewed interest in green tea, for the prevention of several disease risks and other important health benefits due to the presence of several phytoconstituents such as Polyphenols (Catechins and Flavonoides), Alkaloids (Caffeine, Theobromine, Theophylline and etc.), Volatile Oils, Polysaccharides, Amino Acids, Lipids,

Vitamins (E.G., Vitamin C), Inorganic Elements (e.g., Aluminium, Fluorine and Manganese), etc. in leaves of *Camellia sinenssi*[7,8]. Previous researchers have reported the effects of several pharmacological and toxicological properties of green tea on animals and humans[9,10] including antioxidant and anti-inflammatory activities[11,12,13,14]. Anti-carcinogenic[15], Anti-atheriosclerotic and Anti-bacterial effects[16], Cardiovascular diseases[17], Antiarthritic[18], Antibacterial[19], Antiangiogenic[20], Antiviral[21], Neuroprotective[22] and Cholesterol-lowering effects[23]. However, the polyphenols are primarily responsible for the beneficial healthful properties and the flavonoides have antioxidant, anti-inflammatory, antiallergic and anti microbial effects. Green tea contains six primary catechin compounds namely Catechin, Gallocatechin, Epicatechin, Epigallocatechin, Epicatechin Gallate and Epigallocatechin Gallate (EGCG), the later being the most active component[24].Hence, the present research work has been found worthwhile to evaluate and compare the possible anti-asthmatic activity of two commercial green tea products of *Camellia sinensis* by *in-vitro* method, as their anti asthmatic effects have not been studied in this model so far.

2. MATERIALS AND METHODS

2.1. Plant material

Two commercial Packaged green tea products such as Tetley and Lipton from *Camellia sinensis* were selected and procured in the month of August, 2013 from the local markets in and around of the Mother Theresa Post Graduate and Research Institute of Health Sciences, Indira Nagar, Gorimedu, Puducherry - 605 006, India, and kept into an air-tight container for use in the study. Chemical such as Histamine, Sodium chloride, Sodium bicarbonate, Magnesium sulfate, Potassium dihydrogen phosphate, Potassium chloride, Calcium chloride, Dextrose were procured from Subra Scientific Pvt. Ltd, No. 54, Balathandayutham street, Ellaipillaichavady, Puducherry – 605 005.

2.2. Sample Preparation

Aqueous extracts of *Camellia sinensis* was prepared by mixing 20 gm of dry powder of the product with 200 ml of sterile distilled water in a round bottom flask (no.72) by boiling under reflux for 30minutes with occasional shaking. Before placing, the flask was washed properly and then dried. Extracts were filtered through a muslin cloth for coarse residue and finally filtered through Whatman No.1 filter paper with 150µm diameter, evaporated to concentrate. Extracts kept in vacuum desiccator until used[34,35] for qualitative, quantitative analysis of phytochemicals.

2.3. Phytochemical Screening

Preliminary qualitative phytochemical screening of AQGT-T and AQGT-L were evaluated for the presence of Phytoconstituents such as carbohydrates, protein, alkaloids, amino acids, tannins, flavonoids, glycosides, saponins and inulin[25].

2.4. Statistical Analysis

The results of the study were expressed as mean ± SEM and analyzed statistically using one-way ANOVA, followed by Dunnet's Multiple Comparison Test by Graph-pad Prism software to find out the level of significance. $P < 0.05$ was considered statistically significant.

2.5. Preparation of Kerb's Solution

Physiological salt solution was prepared by accurate weight of NaCl-6.9 gm; $NaHCO_3$-2.1 gm; $MgSO_4$-1.28 gm; KH_2PO_4-0.16 gm; KCl-0.35 gm; $CaCl_2$-0.28 gm and dextrose-2 gm were dissolved in distilled water and make up to 1L and the Krebs solution was maintained at 37^0C and gassed with 95% O_2 and 5% CO_2. Tissue was suspended under isotonic tens.

3. *In vitro* Anti-asthmatic Activity

Histamine contracts the tracheal-bronchial muscle of guinea pig, goat, horse, dog and man[4]. Goat tracheal chain is much more sensitive and easier to handle[26]. This method is a modification to the tracheal chain model where the knitting/connecting of the tracheal rings is not performed. In this method, fresh isolated goat tracheal chain was obtained from slaughter house and cut into zigzag fashion thereby exposing large portion of the tissue using the method described by *Kulkarni., 2007*[27]. Trachea was suspended in an organ bath of 20 mL containing Krebs-Henseleit solution maintained at 37 ± 0.5^0C a stream of O_2 was bubbled through the organ tube and connected to the isotonic frontal writing lever on kymograph paper. Tissue was allowed to equilibrate for 45 min. during which, the bathing solution was changed at every 15 min. under to the load of 400 mg[28]. Dose Response Curve (DRC) of different concentration of histamine (100 µg/ml) in absence and presence of different doses (10 µg/ml, 20 µg/ml and 40 µg/ml) of AQGT-T and AQGT-L of *Camellia sinensis* were recorded and the percentage of inhibition was measured in different doses of tests[29,30].

4. RESULTS AND DISCUSSION

4.1. Preliminary Phytochemical Screening

Preliminary qualitative phytochemical screening of AQGT-T and AQGT-L were showed the presence of carbohydrates, protein, alkaloids, amino acids, tannins, flavonoids, glycosides, saponins and inulin.

4.2. Anti-asthmatic activity

Asthma is a very commonly occurring condition that is most difficult to control in chronic stage. Bronchial asthma is a chronic inflammatory disease, characterized by both bronchoconstriction and airway inflammation which leads to bronchial hyper responsiveness to various stimuli, in which many cell types play a role, more important being mast cells, eosinophils and T-lymphocytes. Histamine is a biologically active component secreted from the mast cells and also derived from human epidermis, gastric mucosa and CNS neurons. High concentration of histamine present in lung, skin, GI mucosa, bone marrow and CSF.

Histamine is one of the major inflammatory mediators in the immediate phase of asthma, causing airway hyper responsiveness and bronchial airway inflammation. Besides the triple response caused by it, histamine has spasmogenic response on intestinal smooth muscle by acting on H_1 – histamine receptor that causes the contraction of intestinal smooth muscle[31]. Histamine produces bronchoconstriction by interacting with H_1 receptor in bronchial smooth muscle. In asthmatic condition very low concentration of histamine causes bronchoconstriction.

In the present study, the percentage of smooth muscle contractions were increased with increase the concentrations of histamine (100 µg/ml), the maximum response (100%) was observed at the dose of 160 µg in absence of the test extracts. In the presence of different doses of test extracts were reduced the percentage of maximum response as 62%, 51%, 42% (Figure-1) of AQGT-T and 71%, 56%, 37% (Figure-3) of AQGT-L in the different doses (10µg/ml, 20 µg/ml, 40 µg/ml) of both extracts. Hence the results revealed that the dose response curve of the histamine shifted to right side in the presence of AQGT-T and AQGT-L respectively in figure-2 and figure-4.

Figure 1: *In-Vitro* Anti Asthmatic Activity of aqueous extract of Green Tea (Tetley)

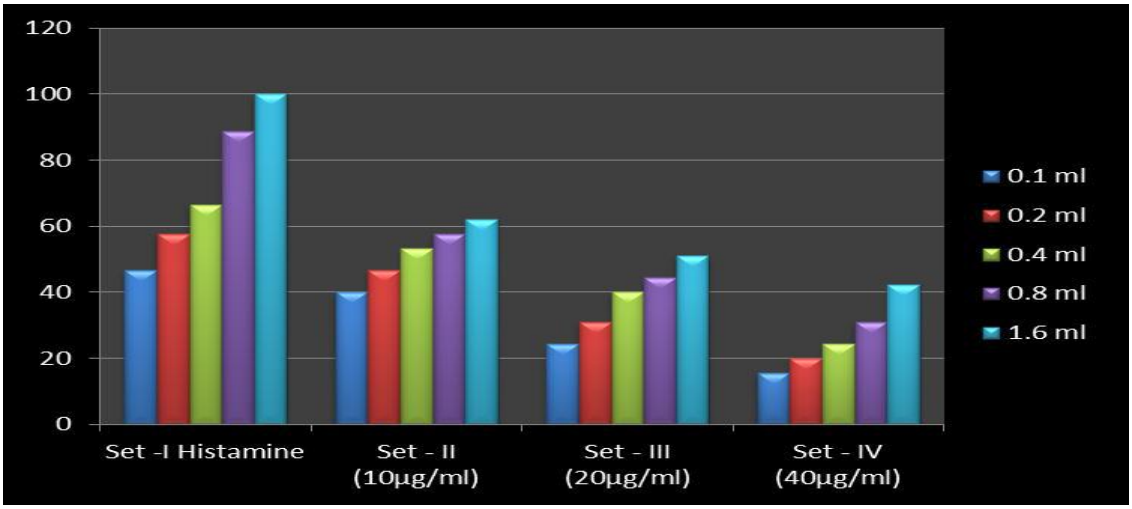
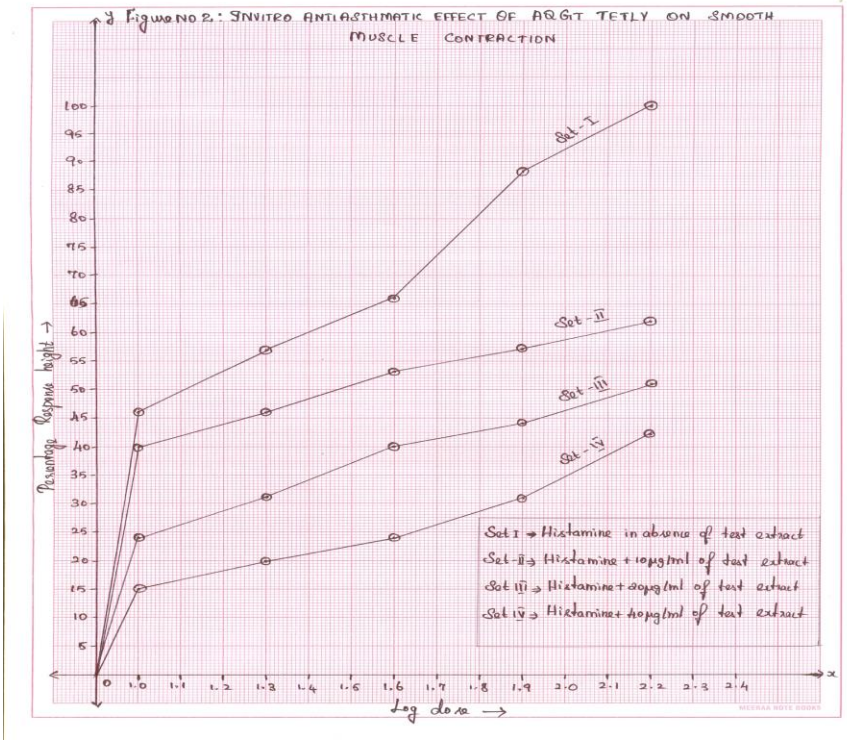


Figure 2: *In-Vitro* Anti Asthmatic Activity of aqueous extract of Green Tea (Tetley) on smooth muscle contraction



The aqueous extracts of green tea products contain more flavonoids which includes Catechins, Epicatechins, Epicatechin-3-gallate, Epigallocatechin and Epigallocatechin-3-gallate (EGCG)[32]. These are well known to possess several notable biological properties such as Anti-Inflammatory and Antioxidant [24], Antimicrobial and Thrombolytic[33]and etc. In the present study, the results

concluded the response of histamine reduced by blocking the histamine-receptor interaction with different doses (10µg/ml, 20µg/ml and 40µg/ml respectively set-II, III & IV, Figure-1 & 3) of both test extracts and the maximum percentage of inhibition was observed as 42% and 37% respectively with Tetley and Lipton at the concentration of 40 µg/ml. Moreover, the aqueous extract of Lipton was showed more anti asthmatic effect than Tetley.

Figure 3: *In-Vitro* Anti Asthmatic Activity of aqueous extract of Green Tea (Lipton)

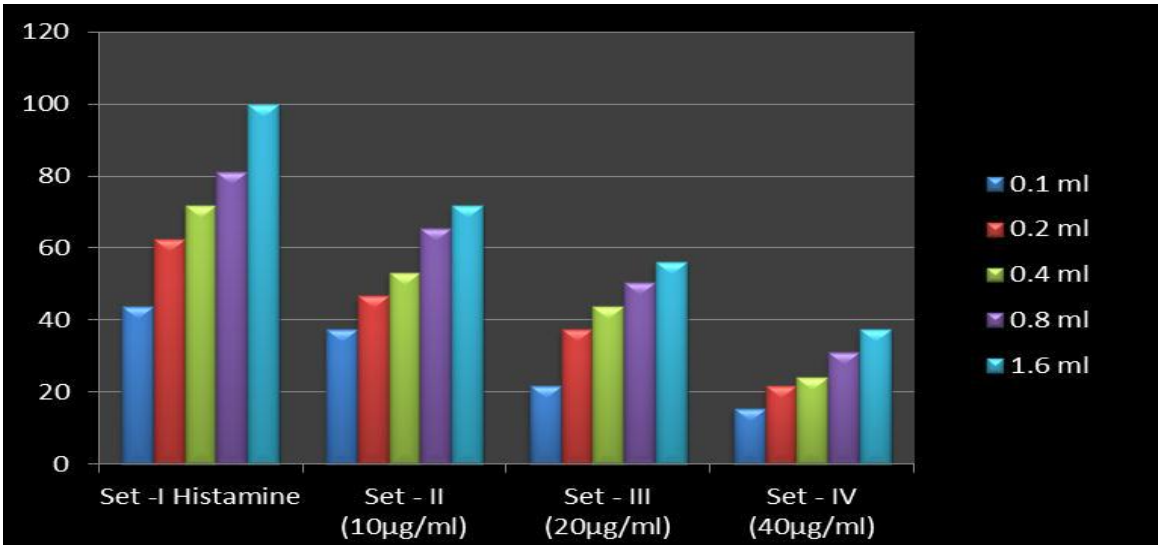
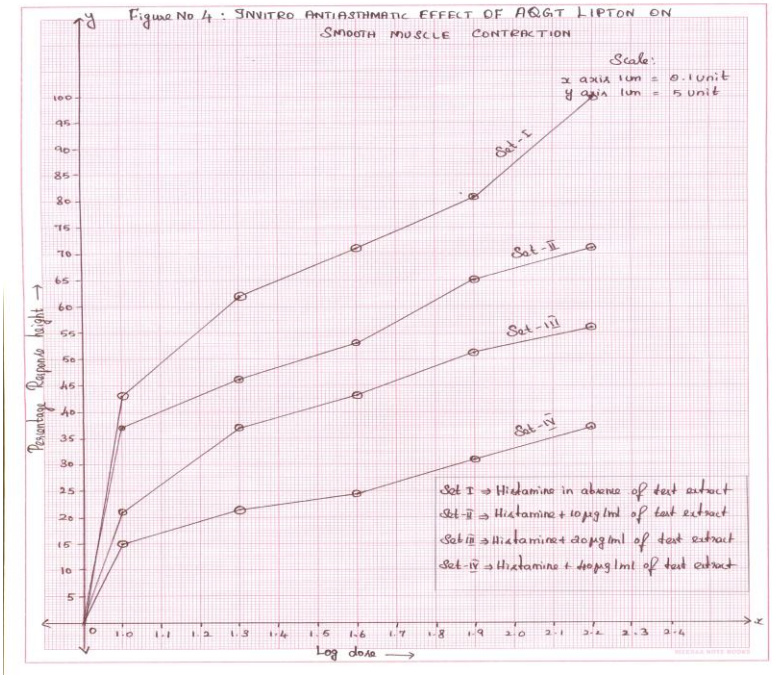


Figure 4: *In-Vitro* Anti Asthmatic Activity of aqueous extract of Green Tea (Lipton) on smooth muscle contraction



The anti-histamines especially second generation antihistamine such as Azelastine, Cetrizine, Ketotifen are approved to relieve the asthmatic symptoms. Hence, in the present study the anti-asthmatic activity of *Camellia sinensis* evaluated by blocking H_1 receptors in bronchial smooth muscle and significantly ($p < 0.05$) inhibited the contractile effect of histamine thus produces bronchodilator effect in dose dependent manner with different doses (10µg/ml, 20µg/ml and 40µg/ml respectively set-II, III & IV) of aqueous extract of *Camellia sinensis* commercial products (Tetley and Lipton). By comparing these two aqueous extracts, Lipton had more potent anti-asthmatic effect than Tetley by anti-histaminic effect on tracheal smooth muscle.

CONCLUSION

In conclusion, the present study confirmed that the aqueous extract of *Camellia sinensis* exhibits significant dose dependent anti-asthmatic activity in various doses. Both products showed significant H_1 receptor antagonistic activity when compared to both products, AQGT-L found have a better anti-asthmatic activity. It was suggested that the anti-asthmatic effect of the commercially available green tea products of *Camellia sinensis* could be further evaluated in other experimental animal methods.

ACKNOWLEDGEMENT

We acknowledge the Department of Pharmacology, College of Pharmacy, Mother Theresa Post Graduate and Research Institute of Health Sciences, Indira Nagar, Gorimedu, Puducherry – 605 006.

REFERENCES

1. M. Lahlou, "Screening of Natural Products for Drug Discovery," *Expert Opinion on Drug Discovery*, Vol. 2, No. 5, 2007, PP: 697-705.
2. Patwardhan B, A. D. B. Vaidya and M. Chorghade, "Ayurveda and Natural Products Drug Discovery," *Current Science*, 2004, 86(6): 789-799.
3. Asthma management handbook, National Asthma Council Australia, 2006:8-10.
4. Pandi Selvi A., S. Rajkumar and G. Sandhya,"Anti asthmatic activity of leaves of Cordia subcordata Lam. (Boraginaceae)",Asian Journal of Pharmaceutical Science & Technology.,2011: 1(1): PP: 1 – 3.
5. Duke JA, Bogenschuz-Godwin MJ, duCellier J, Duke PK. Boca Raton:"Handbook of medicinal plants" CRC Press; 2002.
6. Rietveld A, Wiseman S. "Antioxidant effects of tea: evidence from human clinical trials." J Nutr. 2003; 133: 3275–3284.

7. DerMarderosian A. Missouri:"The reviews of natural products" *Facts and Comparisons.2001.*
8. Sabu M Chacko, Priya T Thambi, Ramadasan Kuttan, and Ikuo Nishigaki "Beneficial effects of green tea: A literature review" Chin Med. 2010; 5: 13.
9. McKay DL, Blumberg JB. The role of tea in human health: An update. J Am Coll Nutr. 2002; 21: 1–13.
10. Cabrera C, Artacho R, Gime'nez R. Beneficial effects of green tea - a review. J Am Coll Nutr. 2006; 25: 79–99.
11. Osada K, Takahashi M, Hoshina S, Nakamura M, Nakamura S, Sugano M "Tea catechins inhibit cholesterol oxidation accompanying oxidation of low density lipoprotein in vitro." Comp Biochem Physiol C Toxicol Pharmacol. 2001 Feb; 128(2): 153-64.
12. Aneja R, Odoms K, Denenberg AG, Wong HR. Theaflavin, a black tea extract, is a novel anti-inflammatory compound. Crit Care Med. 2004; 32: 2097–103.
13. Tipoe GL, Leung TM, Hung MW, Fung ML. Green tea polyphenols as an anti-oxidant and anti-inflammatory agent for cardiovascular protection. Cardiovasc Hematol Disord Drug Targets. 2007; 7: 135–44.
14. Cavet ME, Harrington KL, Vollmer TR, Ward KW, Zhang JZ. Anti-inflammatory and anti-oxidative effects of the green tea polyphenol epigallocatechin gallate in human corneal epithelial cells. Mol Vis. 2011; 17: 533–42.
15. Kavanagh KT, Hafer LJ, Kim DW, Mann KK, Sherr DH, Rogers AE, Sonenshein GE "Green tea extracts decrease carcinogen-induced mammary tumor burden in rats and rate of breast cancer cell proliferation in culture." J Cell Biochem. 2001; 82(3): 387-98.
16. Koo MW, Cho CH "Pharmacological effects of green tea on the gastrointestinal system." Eur J Pharmacol. 2004 Oct 1; 500(1-3): 177-85.
17. Sueoka N, Suganuma M, Sueoka E, Okabe S, Matsuyama S, Imai K, Nakachi K, Fujiki H "A review: A new function of green tea: prevention of lifestyle-related diseases." Ann N Y Acad Sci. 2001 Apr; 928: 274-80.
18. Haqqi TM, Anthony DD, Gupta S, Ahmad N, Lee MS, Kumar GK, Mukhtar H"Prevention of collagen-induced arthritis in mice by a polyphenolic fraction from green tea." Proc Natl Acad Sci U S A. 1999 Apr 13; 96(8): 4524-9.
19. Sudano Roccaro A, Blanco AR, Giuliano F, Rusciano D, Enea V "Epigallocatechin-gallate enhances the activity of tetracycline in staphylococci by inhibiting its efflux from bacterial cells."Antimicrob Agents Chemother. 2004 Jun; 48(6): 1968-73.

20. Sartippour MR, Shao ZM, Heber D, Beatty P, Zhang L, Liu C, Ellis L, Liu W, Go VL, Brooks MN "Green tea inhibits vascular endothelial growth factor (VEGF) induction in human breast cancer cells." J Nutr. 2002 Aug; 132(8): 2307-11.
21. Weber JM, Ruzindana-Umunyana A, Imbeault L, Sircar S "Inhibition of adenovirus infection and adenain by green tea catechins." Antiviral Res. 2003 Apr; 58(2): 167-73.
22. Weinreb O, Mandel S, Amit T, Youdim MB "Neurological mechanisms of green tea polyphenols in Alzheimer's and Parkinson's diseases." J Nutr Biochem. 2004 Sep; 15(9): 506-16.
23. Raederstorff DG, Schlachter MF, Elste V, Weber P "Effect of EGCG on lipid absorption and plasma lipid levels in rats." J Nutr Biochem. 2003 Jun; 14(6): 326-32.
24. Sharangi A.B. "Medicinal and therapeutic potentialities of tea (Camellia sinensis L.) – A review"., *Food Research International*.2009(42): 529–535.
25. Kokate CK,Purohit A.P,Gokhale S.B Pharmacogonsy., 2009;15(2): A1-A6.
26. Vadnere GP., Somani RS and Singhai AK "Studies on Antiasthmatic activity of aqueous extract of *Clerodendron Phlomidis" Pharmacologyonline;* 2007; 1: 487-94.
27. Kulkarni Sk (2007). Hand Book of Experimental Pharmacology, 92-95.
28. Savita D. Patil., Sameer V. Ahale and Sanjay J. Surana "Evaluation Of Antiasthmatic And Antianaphylactic Activity Of *Balanites Aegyptiaca* (Delile), (Balanitaceae)" *Asian Journal Of Pharmaceutical And Clinical Research*; 2011; 4(1): 52 – 55.
29. Castillo JC and De-Beer EJ "The Tracheal Chain-l - A Preparation for the Study of Antispasmodics with Particular Reference to Bronchodilator Drugs" *J. Pharmacol. Exp. Ther.*, 90; 1947: 104 – 109.
30. Chaudhari KN, Lahiri SC "Role of Goat Trachea for an Isolated Tracheal Chain Preparation" *Indian J. Pharmacology;* 6(3); 1974: 149 – 151.
31. Kulakarni SK "Hand book of Experimental Pharmacology" 3rd Ed. New Delhi. Vallabha Prakashan, 2003: 92 – 94.
32. Zhu HB, Li BM, Liu C, Chen RY. "Chemical constituents of *Camellia sinensis* var. assamica." Zhongguo Zhong Yao Za Zhi. 2013 May; 38(9): 1386-9.
33. Monir Hossain et al.,□ In Vitro Studies On Antibacterial And Thrombolytic Activities Of Black Tea Or *Camellia Sinensis*□., Int.J.Inv.Pharm.Sci.,1(4) 2013; 292—299.
34. Sherwani SK, Nazim K, Khan TM, Ahmed M, Malik MW, Noor AA, Khan MU, Ali QM and Alam SI. Phytochemical and antibacterial screening of crude extract of Sargassum tenerrimum J. Agardh against potential human pathogens. FUUAST J. BIOL.,2012; 2(2): 65-68 18.
35. Sherwani SK, Gilani AS, Masroor S, Kazmi SU. (2013). In vitro anthelminthic activity of crude leaf extract of Bougainvillea spectabilis. FUUAST J. Biol (Accepted).