



Investigating the Immunomodulatory Effect of *Cassia fistula* on Albino Rats

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Abstract | The present study was aimed to evaluate the immunomodulatory activities of hot aqueous extract of pods & leaves (HAEP & HAEL) of *C. fistula* in albino rats. HAEP/HAEL were given oral dose of 125, 250 & 500 mg/kg to healthy rats divided into one unfed (control group) and six fed groups. The humoral immune response was carried out by testing antibody titer against *Salmonella typhimurium* 'O' antigen by tube agglutination test and cellular immune response was carried out on DNCB (1-Chloro-2, 4-dinitrobenzene) sensitized albino rats. Results showed that agglutination titer in all HAEP treated rats ranged from 153.6±25.6 to 256.0±62.71 and HAEL treated rats exhibited antibody titer from 358.4±62.71 to 409±51.2 in relation to control & other groups. HPLC analysis of HAEP, HAEL for determination of active immunomodulants have confirmed the presence of Quercetin dihydrate within HAEP, HAEL and Kaempferol only within HAEL. Thus the present study concludes that rise in humoral immune response may be due to the stimulation of B- lymphocytes, T-helper Cells & macrophages subsets by pods, leaf extracts containing immunomodulants Quercetin dihydrate and Kaempferol. Hence it is concluded that *C. fistula* plant has immunomodulatory activity, although detailed study regarding digestibility, hepatic toxicity & molecular mechanism of action are needed to be carried out.

Keywords | *Cassia fistula*, 'O' antigen, Immunomodulatory activity, Quercetin dihydrate, Kaempferol

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INTRODUCTION

The greatest threat to human population on Earth comes from microbes (Casadevall and Relman, 2010). These microbes are ubiquitous in our environment and have affected the development, evolution of every human civilization. To combat these ubiquitous killers and to survive in an environment hosted by pathogens a remarkable, versatile, defense system has evolved in animals called as Immune system (Delves et al., 2010). Although Immune system is an autonomous, inbuilt defense system present in all living animals, yet humans have always shielded themselves against these microbes using antibiotic (Tortora et al., 2009).

The commercialization of these antimicrobial agents by pharmaceutical companies and related economic benefits has fascinated scientists to explore new broad spectrum antibiotics (Monnet, 2005). The practice of indiscriminate use of these antibiotics have led to the development of multi drug resistant (MDR) strains of microbes, evidenced from the recent epidemic caused by influenza virus, ebola virus, methicillin resistant *Staphylococcus aureus*, etc. (Davies and Davies, 2010; Monnet, 2005; Vimalraj et al., 2009; Spellberg et al., 2008). The gridlocks associated with modern medicines such as several side effects, ever increasing immunological disorders, development of MDR strains have enforced researchers globally to move back to basic approach, to combat microbes with the use of medic-

inal plants as an immunostimulant (Vimalraj et al., 2009).

One of the most well documented plants in Vedas & Upanishads is *Cassia fistula* (Caesalpinaceae) (Bahorun et al., 2005). Also known as golden shower tree, *C. fistula* is a native of Indian subcontinent & occupies significant position in the indigenous system of medicine of many Asian, African and South American countries (Bahorun et al., 2005; Bhalerao and Kelkar, 2012; Danish et al., 2011). In ancient ayurvedic system *C. fistula* has been used for the treatment of various ailments such as pruritus, leucoderma, diabetes, haematemesis, etc (Danish et al., 2011; Rizvi et al., 2009; Seyyednejad et al., 2014; Yadav and Jain, 1999). Besides *C. fistula*, other species of *cassia* such as *C. alata*, *C. sophora*, *C. javanica*, *C. auriculata* plants have also been studied and are claimed to be of therapeutic importance (Abo et al., 2000; Bhalerao and Kelkar, 2012; Bhuvaneswari and Gobalakrishnan, 2014). Although the plant has a high therapeutic value and reported to possess hepatoprotective, anti-inflammatory, antitussive, antifertility, antimicrobial and antitumor activities within itself, yet little information is available regarding its role in immunomodulation. Thus the present study was taken up to investigate out the folklore use of the plant, determining immunomodulatory property of its aqueous pod & leaf extract with scientific validation.

MATERIALS AND METHODS

COLLECTION OF PLANT MATERIALS, PREPARATION OF HOT AQUEOUS EXTRACT OF PODS & LEAVES

Fresh pods and leaves of *Cassia fistula* were collected separately from the month of May to June from Mathura and its adjoining areas. *Cassia fistula* plant was identified and authenticated at the department of Botany, B.S.A. College, Dr B.R. Ambedkar University. The pod, leaves were dried in shade and coarsely powdered. The dried powder was extracted with triple distilled water using soxhlet apparatus. The extracts obtained were evaporated to dryness at 45°C using hot air oven and weighted.

EFFECT OF HAEP AND HAEL ON HUMORAL IMMUNE SYSTEM

The humoral immune response was measured by determining the amount of antibody raised in different groups of rats fed with 'O' antigen and different concentration of HAEP, HAEL extract.

PREPARATION OF SALMONELLA TYPHIMURIUM 'O' ANTIGEN

Salmonella typhimurium culture was obtained from the department of Microbiology and immunology, Pandit Deen Dayal Upadhyay Pashu Chikitsa Vigyan Vishwavidyalaya Evam Go Anusandhan Sansthan (DUVASU), Mathura.

Advances in Pharmaceutical and Ethnomedicines

Prior to experimentation, the bacterial isolates were characterized on the basis of their shape, microscopic examination, gram staining, ability to ferment sugars, biochemical test such as catalase, oxidase, etc (Cruickshank et al., 1975). After confirmation *S. typhimurium* 'O' antigen was prepared by standard methods (Bhatia et al., 2003). Smooth colonies of *S. typhimurium* grown on Tryptose agar medium were selected and inoculated in nutrient broth. Inoculated broth was incubated for eight hours at 37°C and then centrifuged at 3000 rpm for 20 minutes, subsequently supernatant was discarded and pellet obtained was washed with normal saline and then boiled at 100°C for two hours thirty minutes. This heated culture was then used as 'O' antigen for determination of humoral immune response in albino rats.

IMMUNIZATION OF RATS

Experimental rats were divided in seven groups of albino rats with 5 rats in each group. The Ist group of rats in which only *S. typhimurium* 'O' antigen was inoculated served as the negative control, as no plant extract was provided to the rats of this group. Group IInd, IIIrd & IVth rats were fed orally, with 125 mg/kg, 250 mg/kg & 500 mg/kg of HAEP respectively for 21 days, (Weichert, 1940) while albino rats of group Vth, VIth and VIIth were also fed orally with similar concentration of HAEL respectively for 21 days along with 'O' antigen in all the above groups. One week after last dose of 'O' antigen was provided, blood serum was collected for determining antibody titer in rats against *Salmonella* by serum agglutination test. The presence of active immunomodulants within HAEP and HAEL was determined through HPLC analysis of both the extracts using different standards.

EFFECT OF HAEP AND HAEL ON CELL MEDIATED IMMUNE SYSTEM

Study was conducted to access the effect of HAEP/HAEL on DNCB (1-chloro-2,4-dinitrobenzene) sensitized albino rats (Tiwary and Goel, 1985). In this experiment DNCB acted as allergen. The experiments were conducted over seven groups of albino rats, with five rats in each group. The Group Ist rats were used as a negative control as only DNCB was provided with no plant extract. Group IInd, IIIrd & IVth rats were fed with 125 mg/kg, 250 mg/kg & 500 mg/kg of HAEP and similar concentrations of HAEL were provided to the rats of group Vth, VIth and VIIth for 21 days.

Hair were removed from thigh of rats of all groups and cleaned properly. 100 µl of 0.2% of DNCB (dissolved in acetone) was applied on hairless thigh skin of albino rats in all the groups on 7th, 14th, 17th day, respectively. Challenge dose was applied on 22nd day at the same site. Skin

thickness was measured with the help of Vernier Caliper at 0, 24, 48 and 72 hrs post challenge. Difference in the skin thickness measured at 0, 24, 48 and 72 hrs was calculated. The inoculated site was also examined for erythema, induration and vesicle formulation.

RESULTS AND DISCUSSION

INDUCTION OF HUMORAL IMMUNE RESPONSE

Results obtained clearly illustrated that antibody titer in the serum samples of all treated rats was higher in relation to control. Further, the result establish the fact that HAEL fed rats exhibited much higher antibody titer (358.4 ± 22.71 , 409.6 ± 22.71 , 409.8 ± 21.2), over the serum antibody titer raised in HAEP fed group (153.6 ± 15.6 , 256.0 ± 12.7 , 256.0 ± 12.6) and that of control (115.28 ± 12.7) (Figure 1).

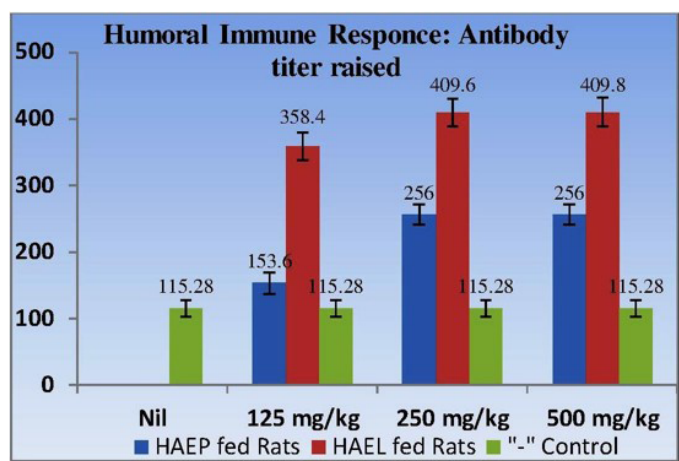


Figure 1: Determining the humoral immune response of albino rats by measuring antibody titer in rats fed with 125, 250 and 500 mg/kg of HAEP and HAEL with respect to control

This study confirms that there is a significant rise in antibody titer, thus humoral immune response is induced in HAEL and HAEP fed rats with significant higher immunomodulatory effect in HAEL fed rats. The determination of active immunomodulants within HAEP and HAEL through HPLC confirms the presence of Quercetin dihydrate among both the extracts and Kaempferol only within HAEL (Figure 2) (Laxmi et al., 2015).

The presence of Quercetin dihydrate in both extracts might be responsible for elevated humoral immune response as Quercetin has been reported to up regulate IFN- γ & Th-2 gene expression and production, which in turn modulate NK cell function and act as immunostimulant (Nair et al., 2002). Quercetin has also been shown to display anti-inflammatory and antibacterial activities (Toit et al., 2009). Further the hyper immune response in case of HAEL

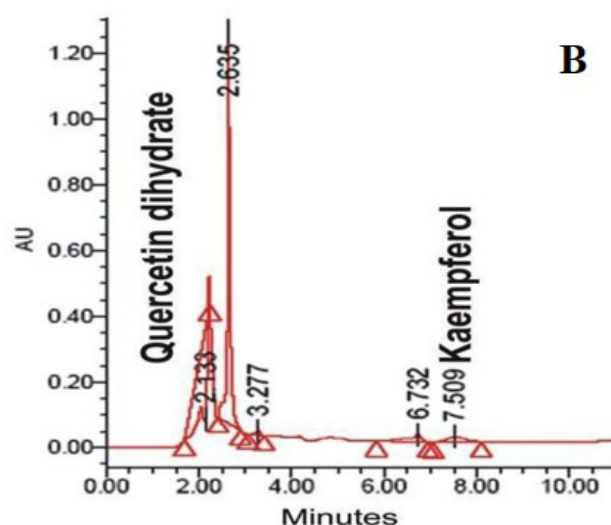
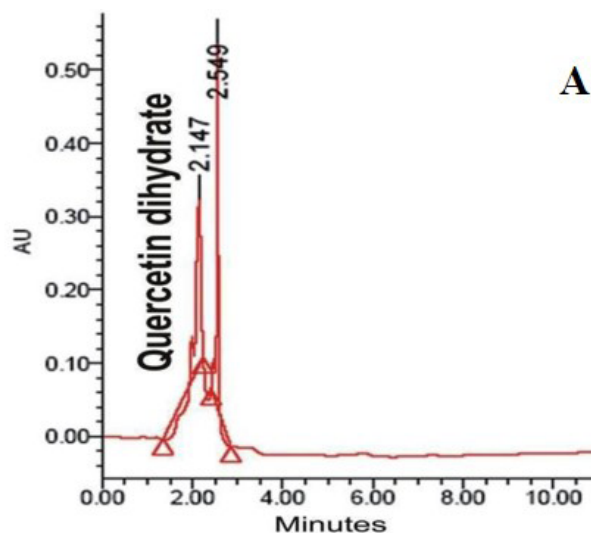


Figure 2: A) HPLC analysis of hot aq. Extract of pods (HAEP) displaying the presence of Quercetin dihydrate; B) HPLC analysis of hot aq. Extract of leaves (HAEL) displaying the presence of Quercetin dihydrate and Kaempferol

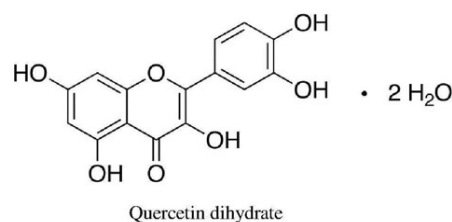
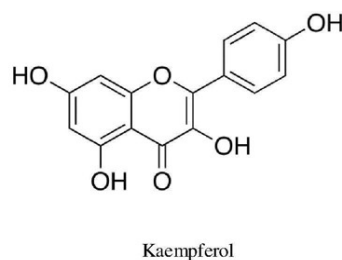


Figure 3: Structure of Quercetin dihydrate and Kaempferol

could be credited to the presence of Kaempferol which has been reported to display a significant increase in phagocytic index and has been shown to exhibit dose dependence enhancement in the production of T-Lymphocytes (CD3 and CD19), Th1 Cytokines (IL-2, IFN- γ and IL-4) (Figure 3) (Swarnalatha and Puratchikody, 2014).

INDUCTION OF CELL MEDIATED IMMUNE RESPONSE

Almost all doses of both extracts exhibited a marked enhancement in skin thickness. Rats fed with 250 mg/kg of body weight HAEP displayed max increase in skin thickness by 27.1%, 21.1% & 14.5% at 24, 48 and 72 hrs,

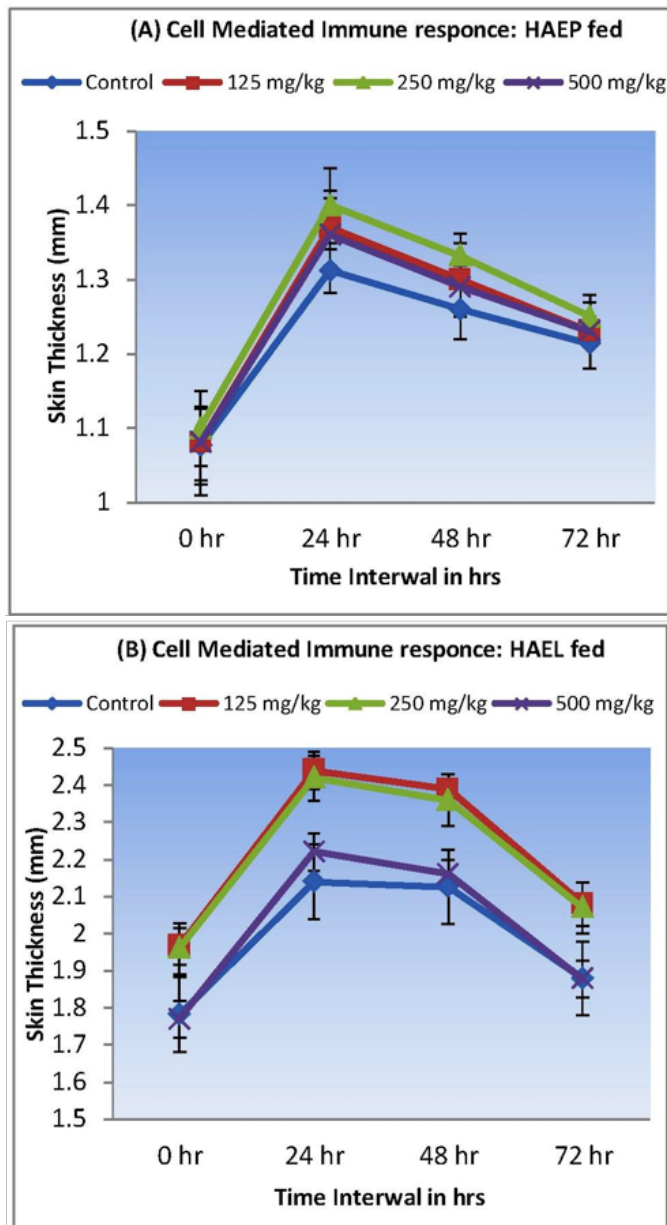


Figure 4: A) Increase in skin thickness of albino rats fed with 125, 250 and 500 mg/kg of HAEP recored at 24, 48 and 72 hrs time intervals with respect to control; B) Increase in skin thickness of albino rats fed with 125, 250 and 500 mg/kg of HAEL recored at 24, 48 and 72 hrs time intervals with respect to control

respectively (Graph-II). Study on rats fed with 125 mg/kg HAEL indicated best increase in skin thickness by 33.15%, 22.67% & 16.33% at 24, 48 and 72 hrs with respect to control (Graph-II). Results also indicated that albino rats fed with 500 mg/kg body weight of HAEP & HAEL induced less increase in their skin thickness with respect to albino rats fed with 125 mg/kg body weight of either extract. The best cell mediated immune response has been achieved in rats fed with 250 mg/kg of HAEP & 125 mg/kg of HAEL (Figure 4).

Comparative analysis with parallel studies suggest patients treated with *C. fistula* pulp showed a significant decrease in serum IgE level producing significant relief in patients suffering from Eczema or Leprosy (Das et al., 2008). Similarly several researchers have demonstrated the immune system modulation activities of *C. auriculata* & *C. tora* (Chakraborty, 2009; Tilwari, 2011).

CONCLUSION

From the results of present study, it was observed that *C. fistula* had stimulatory influence on humoral and cell mediated immunity as evident from increased antibody titer against *Salmonella Typhimurium* 'O' antigen and significant enhancement in skin thickness in DNCB sensitized albino rats. HAEL in comparison to HAEP was more effective in stimulating both immune responses. The humoral immune response obtained in HAEL & HAEP fed rats might be due to the presence of quercetin dihydrate within both extracts while increased immune response within HAEL fed rats might be credited to the presence of Kaempferol (Laxmi et al., 2015; Swarnalatha and Puratchikody, 2014).

The augmented humoral and cell mediated immune responses in the presence of HAEP and HAEL of *C. fistula* reflect the induced proliferation and activation of both B and T lymphocytes. Further studies are although needed regarding digestibility, hepatic toxicity, molecular mechanism of action HAEL & HAEP.

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CONFLICT OF INTEREST

The authors state that they have no conflict of interest to declare.

A. K. Bhatia & Anubhuti Sharma conceptualized the study; work was carried out by Vijay Laxmi with the help of Anjana Goel, while Nitin Wahi is credited for formulating and editing the research paper.

REFERENCES

- Abo KA, Adeyemi AA, Jegede IA (2000). Spectrophotometric estimation of anthraquinone content and antimicrobial potential of extracts of some *Cassia* species used in herbal medicine in Ibadan. *Nigeria Sci. forum.* 3(2): 56-62.
- Bahorun T, Neergheen VS, Aruoma OI (2005). Phytochemical constituents of *Cassia fistula*. *African J. Biotech.* 4(13): 1530-1540.
- Bhalariao SA, Kelkar TS (2012). Traditional medicinal uses, phytochemical profile and pharmacological activities of *Cassia fistula* Linn. *Int. Res. J. Biol. Sci.* 1(5): 79-84.
- Bhatia AK, Sharma SK, Kumar H (2003). Preparation of 'O' antigen laboratory manual for detection of enterotoxigenic *E. coli* (ETEC 22-23).
- Bhuvaneshwari R, Gobalakrishnan R (2014). Antimicrobial potential and structural elucidation of bioactive compounds from flower extract of *Cassia javanica* L. *Indian J. Nat. Prod. Resour.* 5(1): 34-39.
- Casadevall A, Relman DA (2010). Microbial threat lists: obstacles in the quest for biosecurity? *Nat. Rev. Microbiol.* 8(2): 149-154. <http://dx.doi.org/10.1038/nrmicro2299>
- Chakraborty GS (2009). Evaluation of immunomodulatory activity of *Cassia auriculata* Linn. *J. Herb. Med. Toxicol.* 3: 111-113.
- Cruickshank R, Duguid JP, Marmion BP, Swain RHA (1975). *Medical microbiology: The practice of medical microbiology.* Edinburgh, New York: Churchill Livingstone, 12th ed.: 237-400.
- Danish M, Singh P, Mishra G, Srivastava S, Jha KK, Khosa RL (2011). *Cassia fistula* Linn. (Amulthus)- An important medicinal plant: A review of its traditional uses, phytochemistry and pharmacological properties. *J. Nat. Prod. Plant Resour.* 1(1): 101-118.
- Das S, Sarkar PK, Sengupta A, Chattopadhyay AA (2008). Clinical study of Aragvadha (*Cassia fistula* Linn.) on Vicharchika (Eczema). *J. Res. Edu. Indian Med.* 14(2): 27-32.
- Davies J, Davies D (2010). Origins and evolution of antibiotic resistance. *Microbiol. Mol. Biol. Rev.* 74(3): 417-433. <http://dx.doi.org/10.1128/MMBR.00016-10>
- Delves PJ, Martin SJ, Burton DR, Roitt IM (2010). *Roitt's Essential Immunology.* 11th ed. Blackwell Pub, New Delhi, Chapter 1: 1-19.
- Laxmi V, Bhatia AK, Goel A, Wahi N, Sharma A (2015). Phytochemical screening and analysis using HPLC to

determine the Antimicrobial efficacy of *Cassia fistula* extract. *Adv. Biores.* 6(3): 1-7.

- Monnet DL (2005). Antibiotic development and the changing role of the pharmaceutical industry. *Int. J. Risk Safety Med.* 17: 133-145.
- Nair MPN, Kandaswami C, Mahajan S, Chadha KC, Chawda R, Nair H, Kumar N, Nair RE, Schwartz SA (2002). The flavonoid, quercetin, differentially regulates Th-1 (IFN γ) and Th-2 (IL4) cytokine gene expression by normal peripheral blood mononuclear cells. *BBA- Mol. Cell Res.* 1593(1): 29-36.
- Rizvi MM, Irshad M, Hassadi GE, Younis SB (2009). Bioefficacies of *Cassia fistula*: An Indian labrum. *African J. Pharm. Pharmacol.* 3(6): 287-292.
- Seyyednejad SM, Motamedi H, Vafei M, Bakhtiari A (2014). The antibacterial activity of *Cassia fistula* organic extracts. *Jundishapur J. Microbiol.* 7(1): 1-5. <http://dx.doi.org/10.5812/jjm.8921>
- Spellberg B, Guidos R, Gilbert D, Bradley J, Boucher HW, Scheld WM, Bartlett JG, Edwards J (2008). The epidemic of antibiotic-resistant infections: A call to action for the medical community from the Infectious diseases Society of America. *Clin. Infect. Dis.* 46: 155-164. <http://dx.doi.org/10.1086/524891>
- Swarnalatha S, Puratchikody A (2014). Cytokines mediated immunomodulatory properties of Kaempferol-5-O- β -D-glucopyranoside from methanol extract of aerial parts of *Indigofera aspalathoides* Vahl ex DC. *Int. J. Res. Pharm. Sci.* 5(1): 73-78.
- Tilwari A, Shukla NP, Uma DP (2011). Effect of five medicinal plants used in Indian system of medicines on immune function in Wistar rats. *African J. Biotech.* 10(73): 16637-16645.
- Tiwary BK, Goel MC (1985). Contact sensitivity to DNCB in normal and cell-mediated- immunity deficient chickens: *In vivo* detection and correlation with lymphocyte transformation and graft-versus-host reaction. *Vet. Immuno. Immunopathol.* 8(4): 329-339. [http://dx.doi.org/10.1016/0165-2427\(85\)90003-0](http://dx.doi.org/10.1016/0165-2427(85)90003-0)
- Toit KD, Buthelezi S, Bodenstien J (2009). Anti-inflammatory and antibacterial profiles of selected compounds found in South African propolis. *S. Afr. J. Sci.* 105: 470-472.
- Tortora GJ, Funke BR, Case CL (2009). *Microbiology an introduction.* 9th ed. Pearson edu, New Delhi, Chapter 20: 581-608.
- Vimalraj TR, Kumar SS, Vadivel S, Ramesh S, Thejomoorthy P (2009). Antibacterial effect of *Cassia fistula* extract on pathogenic bacteria of veterinary importance. *Tamilnadu J. Vet. Anim. Sci.* 5(3): 109-113.
- Weichert CK (1940). The experimental shortening of delayed pregnancy in the albino rat. *Anat. Rec.* 77(1): 31-47.
- Yadav R, Jain GC (1999). Antifertility effect of aqueous extract of seeds of *Cassia fistula* in female rats. *Adv. Contracept.* 15(4): 293-301. <http://dx.doi.org/10.1023/A:1006784224191>