



International Journal of Pharmacology

ISSN 1811-7775

science
alert

ansinet
Asian Network for Scientific Information

Anti-Inflammatory, Diuretic and Antibacterial Activities of Aerial Parts of *Mucuna pruriens* Linn.

¹V. Bala, ¹A. Debnath, ¹A.K. Shill and ^{1,2}U. Bose

¹Pharmacology Discipline, Khulna University, Khulna-9028, Bangladesh

²School of Pharmacy, University of Queensland 4102, Australia

Abstract: The ethanolic extract of the dried aerial parts of *Mucuna pruriens* Linn. (Fabaceae) was investigated for its possible anti-inflammatory, diuretic and antibacterial activities in animal models. Anti-inflammatory activity was tested using carrageenin induced rat paw edema model and cotton pellet implantation method in rat. Diuretic activity was measured by the comparison of control group, standard drug group (furosemide) at a dose of 0.5 mg kg⁻¹ and the sample extracts 200 and 400 mg kg⁻¹, respectively. The antibacterial property was studied by the disc diffusion method using extract 200 mg disc⁻¹. The extracts significantly (p<0.001) reduced carrageenin induced paw edema in rats. Cotton pellet implantation model for anti-inflammatory activity, the extract showed a significant reduction in the weight of the cotton pellet in test animal compared to control (p<0.001). At the doses of 200 and 400 mg kg⁻¹, the extract exhibited 24.59 and 33.41% reduction of the weight of the cotton pellets. Diuretic activity was proved by the electrolyte loss ratio (Na⁺/K⁺ excretion ratio was 1.48 and 1.45 at the doses of 200 and 400 mg kg⁻¹, respectively) as that of the standard diuretic furosemide (1.47). The extracts also showed the antibacterial activity against eight species of bacteria. The results obtained shows that the extracts of *Mucuna pruriens* Linn. has anti-inflammatory, diuretic and antibacterial properties. This finding provides a basis for the traditional use of the plant material.

Key words: *Mucuna pruriens* Linn, carrageenin, cotton pellet granuloma, furosemide, disc diffusion, ethanolic extract

INTRODUCTION

Mucuna pruriens Linn. (Fabaceae), commonly known as cowage plant or kapikacho, is the most popular drug in the Ayurvedic and Unani medicine system in Bangladesh. The plant is being cultivated in Bangladesh, India, Srilanka, South East Asia and Malaysia (Suresh *et al.*, 2010). *Mucuna pruriens* seeds extract was used to manage several free radical mediated diseases, for instance rheumatoid arthritis, diabetes, atherosclerosis, nervous disorders, analgesic, procoagulant activity, antipyretic, use in the management of Parkinson's disease and male infertility (Rajeshwar *et al.*, 2005; Suresh *et al.*, 2009; Guerranti *et al.*, 2001; Aguiyi *et al.*, 1999; Manyam, 1995). In plateau state, Nigeria, the seed is prescribed as a prophylactic oral anti-snake remedy by traditional practitioners and it is claimed that when seeds are swallowed intact, the individual is protected for one full year against snake bite (Guerranti *et al.*, 2001). Another study have revealed that the ethanol extract of *Mucuna pruriens* seeds showed significant in-vitro anti-oxidant activity while it has also been indicated that the ethanol extract of *Mucuna pruriens* can be

potential source of natural anti-oxidant agent (Ahmad *et al.*, 2008). It also restores antioxidant levels and reduces lipid peroxide content (Shukla *et al.*, 2010). Preliminary phytochemical investigation showed the presence of fairly high concentration of phytosterols e.g., β -sitosterol (Murthy and Mishra, 2009). Different types of compounds also reported from *Mucuna pruriens* seeds, for example, L-Dopa, alkaloidal constituents, epoxy fatty acids and multiform glycoprotein (Tan *et al.*, 2009). In view of this and evidence from the existing information showed that this plant also may have possessed some other important biological activities. The present study was carried out to evaluate the anti-inflammatory, diuretic and antimicrobial activities of the ethanolic extract of the dried aerial parts of *Mucuna pruriens* (MP).

MATERIALS AND METHODS

Plant material collection and extraction: The aerial parts of MP were collected from Paikgacha, Khulna, Bangladesh in June 2009 and were taxonomically identified by Mrs. Rabeya Akter (Senior Officer) at the Bangladesh National

Herbarium (accession no: 33654). The voucher specimens were deposited in the Bangladesh National Herbarium, Dhaka. About 400 g of powdered sample were taken in a clean, flat-bottomed glass container and soaked in 1,300 mL of 80% ethanol. The container with its contents was sealed and kept for a period of 7 days accompanying occasional shaking and stirring. The whole mixture then underwent a coarse filtration by a piece of cotton followed by a filtration through Whatmann filter paper and the filtrate thus obtained was concentrated using a rotary evaporator (Bibby RE200, Sterilin Ltd., U.K.) to get the crude extract.

Animals: Animal studies were performed in accordance with the declaration of Helsinki guidelines (WMO, 1996) for the ethical handling and the use of laboratory animals. Ethical approval was obtained from the Institutional Animal Ethics Committee (IAEC, approval no. 343/07/ab/KURS) of Khulna University ethical review committee. Young Swiss-albino mice of either sex, weighing 20-25 g, purchased from the Animal Research Branch of the International Centre for Diarrhoeal Disease and Research, Bangladesh (ICDDR, B) were used for diuretic activity test. Wistar rats of either sex, weighing 180-200 g, were purchased from the Animal Resources Branch of (ICDDR, B) and were used for anti-inflammatory activity tests. The animals were kept at animal house (Pharmacy Discipline, Khulna University) for adaptation after their purchase under standard laboratory conditions (relative humidity 55-65%, room temperature $25.0 \pm 2.0^\circ\text{C}$ and 12 h light-dark cycle) and fed with standard diets (ICDDR, B formulated) and had free access to tap water.

Drugs: Carrageenin (Sigma Chemicals, USA), Aspirin (Square Pharmaceuticals Ltd, Bangladesh), Furosemide (Square Pharmaceuticals Ltd, Bangladesh).

Pharmacological studies

Anti-inflammatory activity

Carrageenin induced paw edema in rats: Anti-inflammatory activity of MP was tested using the carrageenin-induced rat paw edema model (Winter *et al.*, 1962). Experimental animals (Wistar rats) were randomly divided into four groups with six animals in each group. Control group received vehicle (1% Tween 80 in water) at the dose of 10 mL kg⁻¹ body weight. Positive control group received aspirin (standard drug) at the dose of 150 mg kg⁻¹ and the test groups were treated with MP extract at the doses of 200 and 400 mg kg⁻¹. The drugs were administered orally 1h prior to the injection of 0.1 mL of 1% freshly prepared suspension of carrageenin into the left hind paw of each rat. The paw volume was

measured by using a plethysmometer (Ugo Basile 7140, Italy) every hour for 5 h after the carrageenin injection.

Cotton pellet implantation: Wistar rats were anesthetized and 10 mg of the sterile cotton pellets were inserted in each axilla of rats. MP extracts (200 and 400 mg kg⁻¹), aspirin (150 mg kg⁻¹) and control vehicle (1% Tween 80 in water, 10 mL kg⁻¹) were administered orally for seven consecutive days starting from the day of cotton pellet implantation. The animals were anesthetized again on the 8th day and cotton pellets were removed surgically, freed from extraneous tissue. These pellets were incubated at 37°C for 24 h and dried at 60°C to constant weight (D'Arcy *et al.*, 1960).

Diuretic activity: Diuretic activity of the extract was investigated using the method (Lipschitz *et al.*, 1943). The test animals were randomly chosen and divided into five groups having ten mice in each. Twenty-four hours prior to the experiment, the test animals were placed in to metallic cages with the withdrawal of food and water. Group-1 or the control group received vehicle (1% Tween 80 in water) at a dose of 10 mL kg⁻¹ body weight orally. Group-2 was provided with urea solution at a dose of 500 mg kg⁻¹. Group-3 was provided with standard diuretic drug furosemide at a dose of 0.5 mg kg⁻¹. Group-4 and group-5, the test groups were treated with the ethanol extract of MP at the doses of 200 and 400 mg kg⁻¹ respectively. From the graduated urine chamber of metabolic cage, the urinary output of each group was recorded 5 h after the above treatments. Collected urine was centrifuged and then estimated for sodium and potassium by using digital flame photometer (Elico Pvt. Ltd., model CL 22D). Chloride was estimated by the Schales and Schales method (Godkar, 1994).

Antibacterial activity: The antibacterial activity of MP extract was studied against *Staphylococcus saprophyticus*, *Shigella sonnie*, *Salmonella typhi*, *Vibrio cholera*, *Streptococcus epidermidis*, *Shigella flexneri* and *Staphylococcus aureus* clinical isolates. All bacterial strains were kindly provided by IMTECH, Chandigarh (India). Cultures of these bacteria were grown in a nutrient broth at 37°C and maintained on nutrient agar (Himedia, India) slants at 40°C. The antibacterial property was studied by the disc diffusion method (Chattopadhyay *et al.*, 2002) using extract 200 mg disc⁻¹. Blank disks contained solvents only (50% aqueous ethanol). Gentamycin was used as positive controls. Minimum Inhibitory Concentration (MIC) was

evaluated by the micro dilution method using 5 mL of liquid broth with different concentrations of extract.

Statistical analysis: Student's t-test was used to determine a significant difference between the control group and experimental groups.

RESULTS

Anti-inflammatory activity

Carrageenin induced rat paw edema: In the carrageenin induced rat paw edema model of anti-inflammatory activity, the ethanolic extract of aerial parts of MP showed a significant inhibitory effect on the edema formation from the first hour to fifth hour. The highest inhibitory effect was found during the third hour where the inhibition was 24.42% (p<0.001) and 39.86% (p<0.001) at the doses of 200 and 400 mg kg⁻¹, respectively. These findings were comparable to standard drug aspirin where the inhibition was 49.23% (Table 1).

Cotton pellet implantation: In the cotton pellet implantation model for antiinflammatory activity, the extract showed a significant reduction in the weight of

the cotton pellet in test animal compared to control (Table 2). At the doses of 200 and 400 mg kg⁻¹, the extract exhibited 24.59 and 33.41% reduction of the weight of the cotton pellets, respectively which was comparable to that of the standard drug aspirin where the reduction was 40.79%. These results were statistically significant (p<0.001).

Diuretic activity: The effect of the ethanolic extract of MP aerial parts on the urination of mice was observed for 5 h which revealed that the extract has a marked diuretic effect in the test animals. This was comparable to that of standard drug furosemide and diuretic agent urea. Electrolyte loss showed similar ratio (Na⁺/K⁺ excretion ratio was 1.48 and 1.45 at the doses of 200 and 400 mg kg⁻¹, respectively) as that of the loop diuretic furosemide (1.47) (Table 3).

In vitro antibacterial activities: The extract of MP exhibited significant *in vitro* antibacterial activity against *Staphylococcus saprophyticus*, *Shigella sonnie*, *Salmonella typhi*, *Vibrio cholera*, *Streptococcus epidermidis*, *Shigella flexneri* and *Staphylococcus aureus* with the zones of inhibition ranging from 10.76 to 15.55 mm (Table 4).

Table 1: Effect of ethanolic extract of *Mucuna pruriens* Linn. on carrageenin-induced rat paw edema

Animal group/treatment	Time after carrageenin injection				
	1 h	2 h	3 h	4 h	5 h
Edema volume × 1000 (mL) (Percent inhibition)					
Control	14.0±0.47	163.5±1.61	245.5±1.82	276.0±1.92	229.0±1.23
1% Tween 80 10 mL kg ⁻¹ ; p.o.	11.54±1.23**	100.5±2.24*	119.25±1.82*	172.8±1.62*	155.4±2.11*
Positive control (Aspirin 150 mg kg ⁻¹ ; p.o.)	(29.71)	(38.91)	(49.23)	(36.47)	(32.72)
Test group-1 (MP extract, 200 mg kg ⁻¹ ; p.o.)	12.86±0.52*	132.8±0.98*	183.38±1.43*	224.4±1.66*	194.7±1.76*
	(14.26)	(18.68)	(24.42)	(17.51)	(15.71)
Test group-2 (MP extract 400 mg kg ⁻¹ ; p.o.)	11.43±1.04*	112.3±1.10*	145.30±2.21*	193.6±1.72*	171.5±1.27*
	(23.80)	(31.13)	(39.86)	(29.18)	(26.18)

Values are expressed as Mean±SEM (Number of animals, n = 6); *indicates p<0.001, **indicates p<0.05 vs. control; MP: *Mucuna pruriens* Linn.; p.o.: per oral. The drugs were administered orally 1 h prior to the injection of 0.1 mL of 1% freshly prepared suspension of carrageenin into the left hind paw of each rat. The paw volume was measured every hour for 5 h after the carrageenin injection

Table 2: Effect of ethanolic extract of *Mucuna pruriens* Linn. (MP) on cotton pellet-induced granuloma pouch in albino rat

Animal group/Treatment	Mean weight of granuloma pouch (mg)	Inhibition (%)
Control (1% tween-80 solution in water 10 mL kg ⁻¹ ; p.o.)	18.84±0.75	-
Positive control (Aspirin 150 mg kg ⁻¹ ; p.o.)	12.54±0.24*	40.79
Test group-1 (MP extract 200 mg kg ⁻¹ ; p.o.)	14.21±0.31*	24.59
Test group-2 (MP extract 400 mg kg ⁻¹ ; p.o.)	13.05±0.26*	33.41

Values are expressed as Mean±SEM (Number of animals, n = 6); * indicates p<0.001 vs. control; p.o.: per oral; MP extracts, aspirin and control vehicle, were administered orally for seven consecutive days starting from the day of cotton pellet implantation pellet followed by incubation at 37°C for 24 h

Table 3: Effect of ethanolic extract of *Mucuna pruriens* Linn. on urine excretion parameters in mice

Treatment	(Dose mg kg ⁻¹ ; p.o.)	Volume of urine (mL)	Concentrations of ions (meq L ⁻¹)			
			Na ⁺	K ⁺	Cl ⁻	Na ⁺ /K ⁺
Group-1 (Control)	-	2.55±0.08	76.67±1.24	48.75±1.18	77.54±1.24	1.52
Group-2 (Urea)	500	3.84±0.09	112.67±1.35**	76.56±1.27**	88.72±1.38*	1.48
Group-3 (Furosemide)	0.5	4.10±0.13	122.86±1.74**	85.46±1.67**	93.36±1.49*	1.47
Group-4 (ME)	200	4.34±0.07	118.50±1.19**	79.34±1.87**	90.73±1.68*	1.48
Group-5 (ME)	400	4.94±0.05	133.75±1.52**	91.23±1.79**	98.59±1.87*	1.45

ME: Ethanolic extract of *Mucuna pruriens* Linn.; Values are expressed as Mean±SEM (Number of animals, n = 10); *indicates p<0.01, **indicates p<0.001 vs. control, Urine was collected for 5 h after treatment

Table 4: *In vitro* antibacterial activity of the ethanolic extract (EE) of *Mucuna pruriens* Linn. (MP) aerial parts extract

Bacterial stains	EE of MP (mm)	Blank	Control(mm)
<i>Staphylococcus saprophyticus</i>	12.22	0	30.00
<i>Shigella sonnie</i>	15.55	0	44.88
<i>Salmonella typhi</i>	14.43	0	36.88
<i>Vibrio cholera</i>	14.58	0	32.44
<i>Streptococcus epidermidis</i>	12.31	0	34.28
<i>Shigella flexneri</i>	10.76	0	28.90
<i>Staphylococcus aureus</i>	10.90	0	30.50

Diameter of inhibition zones (mm). The antibacterial property was studied by the disc diffusion method using extract 200 mg disc⁻¹. Blank disks contained solvents only (50% aqueous ethanol). Gentamycin was used as positive controls

DISCUSSION

Carrageenin induced rat paw edema model is one of the most widely used primary test for the screening of new anti-inflammatory agents (Winter *et al.*, 1962). MP significantly inhibited carrageenin-induced paw edema and cotton pellet granuloma in rats. The MP extract produced a dose-dependent inhibition of carrageenin edema and the effect of 200 and 400 mg kg⁻¹ dose of MP extract was comparable with that of aspirin (150 mg kg⁻¹). Similarly, in the cotton pellet study, MP dose-dependently inhibited granuloma formation and the doses of 200 and 400 mg kg⁻¹ MP were found to be comparable to that of aspirin.

Inflammatory responses occur in three distinct phases and are apparently mediated by different mechanisms: Acute phase by local vasodilation and increased capillary permeability, subacute phase by infiltration of leukocytes and phagocytic cells and chronic proliferative phases, where tissue degeneration and fibrosis occur (Ramadori and Meyerzum, 1989; Di Rosa *et al.*, 1971; Ramadori and Meyerzum, 1990; Emery and Salmon, 1991). Accordingly, anti-inflammatory tests are divided into those measuring acute inflammation, subacute process and chronic repair processes (Salmon and Emery, 1991; Damas and Remacle-Volon, 1992; Penn and Ashford, 1963).

Carrageenin-induced edema falls in the category of acute inflammation which involves the synthesis or release of inflammatory mediators at the injured site which further cause pain and fever (Brooks *et al.*, 1991). On the other hand, the proliferative phase or chronic inflammation is measured by methods for testing granuloma formation such as cotton pellet granuloma (Bush and Alexander, 1960). MP was effective in both carrageenin-induced paw edema as well as cotton pellet granuloma and it can be assumed that it is effective in all the phases of inflammation i.e., acute, subacute and proliferative phases. Two doses 200 and 400 mg kg⁻¹ of MP were effective in carrageenin induced edema and the other two doses was also effective against cotton pellet granuloma method. The results of the both methods for anti-inflammatory activity further support the anti-

inflammatory activity of the crude extract. It can also assume that the doses variations are also responsible for the acute and chronic inflammation.

These results tend to suggest that the inhibitory activity of the extracts observed in the first phase of carrageenin induced inflammation may be due to inhibition of early mediators, such as histamine and serotonin. The action on the second phase may be due to the inhibition of bradykinin and prostaglandins.

Diuretic activity may be very useful in a number of conditions like hypertension, hypercalciuria, cirrhosis of liver. Furosemide, used as the standard drug in this experiment belongs to the loop or high-ceiling diuretics, which act by inhibiting Na⁺/K⁺/Cl⁻ co-transport of the luminal membrane in the ascending limb of the loop of Henle and have the highest efficacy in mobilizing Na⁺ and Cl⁻ from the body. The extract was able to increase the volume of urine with statistical significance along with a considerable Na⁺ and Cl⁻ load which was comparable to that of furosemide. The diuretic action of the extract may be due to its action on the kidney. The extract may also contain a high proportion of osmotically active compounds or their metabolites that lead to an increased urine volume. There was an increase in the ratio of concentration of excreted sodium and potassium ions after plant extract treatment. This also indicates that the extract increases sodium ion excretion to a greater extent than potassium, which is a very essential quality of a good diuretic with lesser hyperkalaemic side effect. This observed diuretic effect may be due to the effect of extract on the glomerular filtration rate and the direct inhibitory effect on the reabsorption mechanism of salt. Further studies may be carried out to identify whether these actions are associated with the same agent or a number of agents that are responsible for such activities.

In this experiment, ethanolic extract of *Mucuna pruriens* Linn. showed moderate sensitivity to the seven of the test organisms both gram positive and gram negative type of bacteria. The highest zone on inhibition (15.55 mm) was recorded against *Shigella sonnie*. MP was previously reported effective on *Ichthyophthirius multifiliis* pathogenic parasite (Ekanem *et al.*, 2004). Present experiment was only conducted with seven

species of bacteria as test samples. Therefore further research is essential to evaluate the sensitivity of the plant extract against other species of bacteria, fungi, virus of other microorganisms.

CONCLUSION

Thus, in the present investigation, it could be suggested that the ethanol extract of *Mucuna pruriens* Linn. aerial parts showed potent anti-inflammatory and diuretic activities. However, it was found to be effective as antibacterial activities in some specific organisms. These facts indicate the scientific basis of *Mucuna pruriens* Linn. being used as a traditional medicine. However, further experiments may help to determine the pharmaceutical potentialities of the plant as a medicine.

ACKNOWLEDGMENTS

The authors are thankful to Prof. Dr. Samir Kumar Sadhu, Head, Pharmacy Discipline, Khulna University; Ahmed Ayedur Rahman, Assistant professor, Pharmacy Discipline, Khulna University; Dr. Mahiuddin Alamgir, Research Scientist, National Measurement Institute (NMI), Australia for their encouragement during the research time. All the informants of the study area are cordially acknowledged for their valuable cooperation.

REFERENCES

- Aguiyi, J.C., A.C. Igweh, U.G. Egesie and R. Leoncini, 1999. Studies on possible protection against snake venom using *Mucuna pruriens* protein immunization. *Fitoterapia*, 70: 21-24.
- Ahmad, M.K., A.A. Mahdi, K.K. Shukla, N. Islam, S.P. Jaiswar and S. Ahmad, 2008. Effect of *Mucuna pruriens* on semen profile and biochemical parameters in seminal plasma of infertile men. *Fertil. Steril.*, 90: 627-635.
- Brooks, R.R., J.F. Carpenter, S.M. Jones, T.C. Ziegler and S.F. Pong, 1991. Canine carrageenin induced acute paw inflammation model and its response to nonsteroidal antiinflammatory drugs. *J. Pharmacol. Methods*, 25: 275-283.
- Bush, I.E. and R.W. Alexander, 1960. An improved method for the assay of antiinflammatory substances in rats. *Acta. Endocrinol. (Copenh)*, 35: 268-276.
- Chattopadhyay, D., G. Arunachalam, A.B. Mandal, T.K. Sur, S.C. Mandal and S.K. Bhattacharya, 2002. Antimicrobial and anti-inflammatory activity of folklore: *Mellotus peltatus* leaf extract. *J. Ethnopharmacol.*, 82: 229-237.
- D'Arcy, P.F., E.M. Howard, P.W. Muggleton and S.B. Townsend, 1960. The anti-inflammatory action of griseofulvin in experimental animals. *J. Pharm. Pharmacol.*, 12: 659-665.
- Damas, J. and G. Remacle-Volon, 1992. Influence of a long-acting bradykinin antagonist, Hoe 140, on some acute inflammatory reactions in the rat. *Eur. J. Pharmacol.*, 211: 81-86.
- Di Rosa, M., P.J. Giroud and D.A. Willoughby, 1971. Studies of the mediators of acute inflammatory response induced in rats in different sites by carrageenan and turpene. *J. Pathol.*, 101: 15-29.
- Ekanem, A.P., A. Obiekezie, W. Kloas and K. Knopf, 2004. Effects of crude extracts of *Mucuna pruriens* (Fabaceae) and *Carica papaya* (Caricaceae) against the protozoan fish parasite *Ichthyophthirius multifiliis*. *Parasitol. Res.*, 92: 361-366.
- Emery, P. and M. Salmon, 1991. The immune response 2, Systemic mediators inflammation. *Br. J. Hosp. Med.*, 45: 164-168.
- Godkar, P.B., 1994. Text Book of Medical Laboratory Technology. Bhalani Publishing House, Mumbai, India, pp: 77-79.
- Guerranti, R., J.C. Aguiyi, E. Errico, R. Pagani and E. Marinello, 2001. Effects of *Mucuna pruriens* extract on activation of prothrombin by *Echis carinatus* venom. *J. Ethnopharmacol.*, 75: 175-180.
- Lipschitz, W.L., Z. Hadidian and A. Kerpesar, 1943. Bioassay of diuretics. *J. Pharmacol. Exp. Therap.*, 79: 97-110.
- Manyam, B.V., 1995. An alternative medicine treatment for Parkinson's disease: Results of a multi centre clinical trial. HP-200 in Parkinson. HP-200 in Parkinson *J. Alternat. Comp. Med.*, 3: 249-255.
- Murthy, K. and S. Mishra, 2009. Quantification of β -sitosterol from *Mucuna pruriens* by TLC. *Chromatographia*, 69: 183-186.
- Penn, G.B. and A. Ashford, 1963. The inflammatory response to implantation of cotton pellets in the rat. *J. Pharm. Pharmacol.*, 15: 798-803.
- Rajeshwar, Y., K.G.P. Senthil, M. and Gupta, U. Mazumdar, 2005. Studies on *in vitro* antioxidant activities of methanol extracts of *M. Prurines* seeds. *Eur. Bull. Drug Res.*, 12: 31-36.
- Ramadori, G. and B.K.H. Meyerzum, 1989. Acute phase reaction and its mediators, 1: Interleukin-1. *Z. Gastroenterol.*, 12: 746-750.
- Ramadori, G. and B.K.H. Meyerzum, 1990. The acute phase reaction and its mediators, II: a tumor necrosis factor and interleukin 6. *Z. Gastroenterol.*, 28: 14-21.
- Salmon, M. and P. Emery, 1991. The immune response 1, the local response and understanding T cell specificity. *Br. J. Hosp. Med.*, 45: 89-93.

- Shukla, K.K., A.A. Mahdi, M.K. Ahmad, S.P. Jaiswar, S.N. Shankwar and S.C. Tiwari, 2010. *Mucuna pruriens* reduces stress and improves the quality of semen in infertile men. eCAM Advance Access., 7: 137-144.
- Suresh, S., E. Prithiviraj and S. Prakash, 2009. Dose-and time-dependant effects of ethanolic extract of *Mucuna pruriens* Linn. seed on sexual behaviour of normal male rats. J. Ethnopharmacol., 122: 497-501.
- Suresh, S., E. Prithiviraj and S. Prakash, 2010. Effect of *Mucuna pruriens* on oxidative stress mediated damage in aged rat sperm. Int. J. Androl., 33: 22-32.
- Tan, N.H., S.Y. Fung, S.M. Sim, E. Marinello, R. Guerranti and J.C. Aguiyi, 2009. The protective effect of *Mucuna pruriens* seeds against snake venom poisoning. J. Ethnopharmacol., 125: 356-358.
- WMO, 1996. Declaration of Helsinki. Br. Med. J., 313: 1448-1449.
- Winter, C.A., E.A. Risley and G.W. Nuss, 1962. Carrageenan induced oedema in hind paw of the rats as an assay of anti-inflammatory drug. Proc. Soc. Exp. Biol. Med., 111: 544-547.