ANTI DEPRESSANT, ANTI-INFLAMMATORY AND ANALGESIC EFFECT OF *BUTEA FRONDOSA* METHANOLIC SEEDS EXTRACT

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Abstract

The main objective of this study was to investigate the antidepressant, anti-inflammatory and analgesic activities of the extract of seeds of *Butea frondosa*, a medicinal herb. Anti-inflammatory activity was evaluated on mice by formalin test method and significant percentage of inhibition of mice paw edema diameter was recorded at 500 mg/kg (23.5%) whereas Aspirin was used as a standard drug, showed 35.2% of inhibition at $p<0.05$. The extract of *B. frondosa* seeds was found to have some analgesic effects and were also observed in writhing test and formalin induced licking and biting test. The percentage of inhibition of writhes in respect to control was 41% and 30.8% at doses of 300mg/kg and 500mg/kg body weight of mice, respectively, whereas Aspirin (300mg/kg) effects were 71.9 %. Along with the above activities diuretic activity was also studied against Lasix (Furesamide 20mg/kg) and found weak results in comparison to negative control group.

Anti-depressant and anxiolytic properties of *B. frondosa* extract was evaluated at 300 and 500 mg/kg doses in different neuropharmacological tests and Diazepam (2mg/kg) was used as a reference drug. The control group was treated with 0.5 ml saline orally. The results showed moderate anti-depressant activity in open field test, head dip test, rearing test, traction test, summing induced depression test, Y- maize and marble burying test. Present studies revealed that *B. frondosa* seed extract has an anti-inflammatory, analgesic, anti-depressant and anxiolytic properties.

Introduction

In recent time Phytotherapy has getting attention which is a method of treatment and prevention from a disease by using plants and plant parts (Farnsworth and Morris, 1976., De Smet PAGM, 1997, Cragg *et al.*, 1997, Shu 1998). *Butea frondosa* Willd. possesses various pharmacological properties. Devi *et al*., (2000), Lal *et al*. in 1976, Joshi, (1970) and Kaleysaraj and Kurup (1963) reported the anthelmintic activity while David *et al*., (2011) studied the anti ulcer property of this plant species. Silambujanaki *et al*., (2010) concluded that *B. monosperma* exhibits anticonvulsant activity. Mishra *et al*., in 2009 founded that *B. frondosa* possess potent bactericidal action against different strains of bacteria. Chokchaisiri (2009) found that *B. frondosa* has anti-mycobacterial activity. Mengi *et al*., (1995) investigated and found ocular anti inflammatory activity of roots and leaves of *B. frondosa*. Wongkham *et al*., (1994) revealed the human erythrocytes agglutinating activity while Shah *et al*., (1990) observed the antiestrogenic activity of dried flowers of *B. frondosa*. Porwal *et al*., (1989) determined the infertility activity of seeds of *B. monosperma.* Razdan *et al.,* (1971) determined less significant antiestrogenic activity. Present study is based to investigate antidepressant, anti-flammatory and analgesic effect of *Butea frondosa* methanolic seeds extract on mice.

Materials and Methods

Collection and identification: The plant material was collected from local market of Karachi, Pakistan. The plant was identified by the taxonomist and the voucher specimen 2-BFH-08 was deposited in the herbarium of Department of Pharmacognosy, University of Karachi, Karachi-75270, Pakistan.

Plant extraction: The (seeds of *B. frondosa* were produced 1kg) kept for 15 days in methanol at 25±2ºC. The methanol extract was then filtered through a Whatmann filter paper No. 42 (125mm). The extract was concentrated on a rotary evaporator under reduced pressure with the water bath set at 40˚C and a residue was obtained.

Assessment of anti-inflammatory and analgesic activity: Anti-inflammatory and analgesic activities of the extracted material were tested after some modification in the methods described by Koster *et al.,* (1959) and Turner (1965). Animals (mice) were divided in to four groups (n = 5), Group-A for control, Group-B and

Group-C for 300mg/kg and 500mg/kg oral doses of crude extract respectively, and Group-D for standard drug Aspirin (300 mg/kg). In brief inflammation was produced by 20µl of 2% formalin in the right hind paw of mice and the diameter of treated paw was measured by Vernier Calliper. For analgesic activity number of writhes (produced by 0.6% acetic acid) and number of formalin induced licking and biting was counted.

Assessment of diuretic activity: For this activity a modified method of Sripanidkulchai *et al*. (2001) was employed. Animal groups (divided in to four groups; group1: control, group 2: 300 mg/kg of *B. frondosa*, group 3: 500mg/kg and group 4: Furesamide 20mg/kg reference drug) were kept separately in to the metabolic cages and 24 hours urine (ml) was collected in to the tubes fitted to the bottom of the metabolic cage.

Assessment of neuropharmacological activity: Some of the neuropharmacological activities were studied by the methods described by Kennett *et al*., (1985), Turner (1965) (open field test) , Sanchez-Mateo *et al*., (2002) Kasture *et al*., (2002), Debprasad *et al*., (1997) (traction test, head dip test, rearing test, and swimming induced depression test), Prerez *et al*., (1998) (Y-maze test) and Nicolas *et al*., (2006) (Marble burying test). Animals were divided into 4 groups; 1) control, 2) 300 mg/kg *B. frondosa* treated, 3) 500mg/kg *B. frondosa* treated and 4) Diazepam treated groups respectively. Diazepam 2 mg/kg is used as reference standard.

Results

Anti inflammatory activity: The results of anti-inflammatory activity are depicted in Table 1. The Formalin induced inflammation was evaluated by mean paw diameter of mice and measured by Vernier caliper. The results showed a decrease in inflammation of hind paw of drug-treated animals as compared to the control group. This finding suggests that the crude extract of *B. frondosa* possesses some anti-inflammatory activity. The crude extract was tested in two doses that is 300 mg/kg and 500 mg/kg. It was found that the test substance at 500 mg/kg is more effective than 300 mg/kg. The result of drug treated animals when compared with standard drug (Aspirin, 300mg/kg) manifested that anti-inflammatory activity of crude extract of *B. frondosa* is less significant than standard drug.

Analgesic activity: Analgesic activity of *B. frondosa* crude extract was carried by formalin induced licking and biting and writhing test. The results are given in Table 1. The crude extract of *B. frondosa* showed significant dose related inhibition of number of writhes. The control animals produced 146±6.74 number of writhes induced by intra peritoneal injection of acetic acid, which was reduced to 86±7.19 and 101±6.26 with 300 mg/kg and 500 mg/kg oral doses of the test substance respectively. The results of writhing test are significant as compared to control but less than Aspirin (reference standards), which produced 41±3.62 writhes. The % inhibition of writhes of crude extract was 41% (300 mg/kg) and 30.8 % (500 mg/kg) where as with aspirin it was 71.9 %.

Diuretc activity: Diuretic aspect was investigated with standard drug (Lasix 20mg/kg). The crude extract of *Butea frondosa* showed some dose related urination which was 1.7±0.25 and 1.9±0.19 (300 mg/kg and 500 mg/kg respectively) with respect to control which resulted as 1.58 ±0.3 as shown in table-13. The diuretic activity was less significant when compared with positive control 4.92±0.43. This result indicated the weak diuretic effect of *B. frondosa* (Table 1) .

Assessment of neuropharmacological activity: In all neuropharmacological tests Diazepam as 2mg/kg was used as the reference compound. Crude extract of *Butea frondosa* was tested in two doses as 300 mg/kg and 500 mg/kg. The control group was treated with 0.5 ml saline orally.

Open field test: Table 2 and graph 3 exhibits that the mean number of squares crossed by the mice with all the four paws was 376.8 for control group, 286.6 and 298.4 for 300 mg/kg and 500 mg/kg of crude extract of *Butea frondosa* seeds respectively and 165 for Diazepam. This showed that the open field activity is decreased but the effect is less significant, which concludes that the *Butea frondosa* has anxieolytic action.

Head dip test: Exploratory activity is slightly affected. Drug treated animals showed slight decreased activity. Table showed that crude extract of *Butea frondosa*, at 300mg/kg and 500 mg/kg produces less significant anti depressant effect as compare to Diazepam (Table 2).

Rearing test: Like open field and head dip test the exploratory activity was decreased in rearing test. Table 2 indicates that activity was more depressed at 300mg/kg oral dose of *Butea frondosa.* These effects with the sedative drug diazepam as the group treated with 2 mg/kg of diazepam showed an average of 13.8 rearing activities whereas with 300 mg/kg and 500 mg/kg oral doses of crude extract the mean number of rearing activity was 18.62 and 21.1respectively. The mean number of rearing activity for control group was 29.6.

Table 1. Assessment of Biological activities (Anti-inflammatory, analgesic and diuretic activity)

Treatment	Dose mg/kg orally	Licking & biting Test		Paw diameter (mm)		Writhes test		Diuretic activity (ml)	
		Mean±SEM	Inhibition (%)	Mean±SEM	Inhibition (%)	Mean±SEM	Inhibition (%)	Mean ±SEM	% Increase
Control	0.5ml saline	62.2±6.47	00	0.17±0.02	00	146±6.74	00	1.58±0.3	0
Crude extract of *Butea frondosa*	300 mg/kg	31.2±3.48	49.83*	0.14±0.06	21.4*	86±7.19	41*	1.7±0.25	7.59*
	500 mg/kg	25.8±1.09	58.5*	0.13±0.05	23.5*	101±6.26	30.8*	1.9±0.19	20.25*
Aspirin	300 mg/kg	17.44±1.59	71.96**	0.11±0.03	35.2**	41±3.62	71.9**	-	-
Furesamide	40mg/kg	-	-	-	-	-	-	4.92±0.43	211**

* = p<.01 significant level, **= p<.05 significant level.

Table 2. Assessment of neuropharmacological activity of *Butea frondosa*

Treatment	Dose mg/kg orally	Head dip test	Rearing test	Traction test (min.)	Depression test (min.)	Y-maize test	Marble burying test
		Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
Control	0.5ml saline	376.8±20.95	29.6±2.77	3.53±0.21	5.22± 0.34	8.5±1.55	10±0.71
Butea frondosa	300 mg/kg	286.6±32.48*	18.62±1.65*	3.29±0.32*	4.8±0.45*	7.64±1.19*	6.2±0.66*
	500 mg/kg	298.4±21.94*	21.1±1.98*	3.49±3.5*	5.08±0.35*	6.2±0.33*	7.0±0.71*
Diazepam	2 mg/kg	165±7.25**	13.8±1.26**	4.47±0.2**	2.11±0.43**	3.5±0.37**	3.2±0.58**

* = p<.01 significant level, ** = p<.05 significant level.

Traction test: Table 2 showed that time taken to travel iron rod was slightly decreased, which showed muscle relaxant and passive activity of *Butea frondosa.* The mean time taken to travel iron rod by control group was 3.53 minutes, which was decreased by 3.29 and 3.49 minutes by crude extract of *Butea frondosa* at dose 300mg/kg and 500mg/kg respectively. Whereas diazepam taken 4.47 min.

Swimming induced depression test: Table 2 showed that the mean activity time in water tub of animals treated with *Butea frondosa* 3.32 minutes for 300 mg/kg and 4.8 minutes for 500 mg/kg and diazepam 2.11 minutes. In test group mean activity is decreased as compared to the control group (5.22 minutes) and the immobility time was decreased. This finding intimates that *Butea frondosa* can be categorized as less significant anti depressant.

Y-maize test: Table 2 showed that the mean number of times that the mice entered the arm of the maze with all four feet is slightly reduced by crude extract of *Butea frondosa* 7.64 ±1.19 and 6.2 ± 0.73 at doses 300mg/kg and 500mg/kg respectively as compare to control 8.5±1.55 but significant when compared with Diazepam 3.5±1.28. This result indicates that *B. frondosa* possesses slight anxiolytic activity.

***Marble burying* test:** In the marble burying condition 12 glass marbles were evenly spaced in the home cage in the presence of the mouse. After 15 min (standard condition) the number of marbles at least two-thirds covered by sawdust was counted. In control group 10 marbles were found whereas test group should 6.2 and 7 marbles at dosage 300 mg/kg and 500 mg/kg respectively indicated weak anxiolytic activity as compare to Diazepam group which found mean 3.2 marbles (Table 2).

Discussion: The literature survey reveals that *Butea frondosa* is an important medicinal plant. It has various important constituents, which are useful for the treatment of many diseases. As reported in literature it has anti ulcer property anticonvulsant activity (Silambujanaki *et al.*, 2010), osteogenic activity (Maurya 2009), liver protective activity (Sehrawat *et al.*, 2006), anthelmintic (Devi *et al.*, 2000, Lal *et al.*, 1976, Joshi 1970, Kaleysaraj and Kurup, 1962), ocular anti inflammatory activity (Mengi *et al.*, 1995), thyroid inhibitory, anti-peroxidative and hypoglycemic effects (Panda, 2009) , Non-specific treatment for diarrhea (Gunakkunru *et al.*, 2005), and infertility actions. (Porwal *et al.*,1988).

Anti-inflammatory and analgesic activities of seeds of *B. frondosa* were recorded as significant feature at the doses of 300 mg/kg and 500mg/kg. Inflammation is induced in mice by formalin and anti-inflammatory effects were noted by licking and biting and measuring mean paw diameter by venire caliper. In licking and

biting test, results exhibited significant inhibition of inflammation which was 49.83% and 58.5% at doses 300mg/kg and 500mg/kg respectively, but results are less significant than standard drug (Aspirin 300mg/kg) which was 71.96%. Similarly at doses 300mg/kg and 500mg/kg inhibition of inflammation was recorded as 21.4% and 23.5%, respectively whereas standard drug showed 35.2% by measurement of paw diameter. This indicates that *B. frondosa* inhibits the formalin induced inflammation in mice. Similarly investigation of comparative ocular anti inflammatory activity of roots and leaves of *B. frondosa* with Flubiprofen in rabbit revealed significant difference between their activity and found *B. frondosa* more effective than Flubiprofen (Mengi *et al*., 1995).

The results of analgesic activity (writhing test) were also significant as the mean number of writhes in control was 146 and in test group at doses 300mg/kg and 500mg/kg, were recorded to decrease to 86 and 101, respectively.

Diuretic study was also investigated against Lasix 40mg/kg which is loop diuretic, results at doses 300 mg/kg and 500 mg/kg results were slight increase in urination which was 1.7±0.25 and 1.9±0.19 at doses 300 mg/kg and 500 mg/kg respectively with respect to control which resulted as 1.58 ±0.3, while for positive control reading was recorded as 4.92±0.43, showed weak diuretic effect of *B. frondosa.*

In neurophamacological test open field activity of crude extract of *B. frondosa* at doses 300 mg/kg and 500 mg/kg shows that the activity is decreased but the effect is less significant when we compared the test group with standard (Diazepam 2mg/kg), which concludes that the *B. frondosa* has slight anxiolytic activity. Head dip exploratory activity showed slight decline that is 32.8 ± 3.25 and 33.6 ± 1.57 at doses 300 mg/kg and 500 mg/kg respectively. It concluded that crude extract of *B. frondosa,* at 300mg/kg and 500 mg/kg produces less significant anxiolytic activity. In rearing test the exploratory activity was decreased by 18.62±1.65 and 21.1±1.98 respectively. Activity was more decreased at 300mg/kg oral dose of *B. frondosa indicates* anxiolytic activity. Traction test indicates that time taken to travel iron rod was slightly decreased but less significant as compare to Diazepam, which shows less significant anxiolytic activity of *B. frondosa.* Swimming induced depression resulted in decreased activity in test group as compared to the control group and the immobility time was decreased. This finding intimate that *B. frondosa* possesses less significant anti-depressant activity. In Y-maize test table-20 showed that the mean number of times that the mice entered the arm of the maze with all four feet is slightly reduced by crude extract of *B. frondosa* 7.64 ±1.19 and 6.2 ± 0.73 at doses 300mg/kg and 500mg/kg respectively as compare to control 8.5±1.55 but significant when compared with Diazepam 3.5±1.28. This result indicates that *B. frondosa* possesses slight anxiolytic activities. In the marble burying test control group founded 10 marbles whereas test group should 6.2 and 7 marbles at dosage 300 mg/kg and 500 mg/kg respectively indicated weak anxiolytic activity as compared to Diazepam group which found 3.2 marbles.

Conclusion: The evaluation of crude extract of seeds of *Butea frondosa* make us to conclude that it possess significant anti- inflammatory and analgesic activity when we compared the results with negative control but less significant than the standard drug used. Diuretic activity is also weak. CNS effect of *B. frondosa* reveals its anti-depressant and anxiolytic activity significant at the dose of 300mg/kg.

References

Ballant, LP. and Gex-Fabry, M. (2001). Pharmacokinetic optimization in drug research,VHCA Switzerland and Wiley-VCH Germany 16-29.

Bungorn, S., Wongpanch, V., Laupattarakasem, P., Suwansaksri, J. and Jirakulsomchok, D. (2001). Diuretic effects of selected Thai indigenous medicinal plants in rats. *J. Ethnopharmacology* 75: 185-190.

Chokchaisiri, R., Suaisom, C., Sriphota, S., Chindaduang, A., Chuprajob, T., Suksamrarn, A. (2009). Bioactive flavonoids of the flowers of *Butea monosperma*, *Chem Pharm Bull.* 57(4): 428-432.

Cragg, G.M., Newman, D.J. and Snader, K.M. (1997). Natural products in drug discovery and development. *J Nat. Prod.* 60: 52-60.

David, B., Otilia, J.F.B., Meenu, S., Rao, K.N.V. (2011). *Butea frondosa* as a gastro protective against induced gastric lesions, Rev. Bras. Farmacogn 21(4): 691-696.

Debprasad, C., Arunachalam, G., Subhash, C., Mandal, R.B. and Mandal, A.B. (1997). CNS activity of the methanol extract of *Mallotus peltatus* (Geist) Muell Arg. Leaf: an ethnomedicine of Onge. *J. Ethnopharmacol.* 85: 99-105.

De Smet PAGM. (1997). The role of plant-derived drugs and herbal medicines in healthcare. *Drugs* 54: 801-840.

Devi, H.U., Ansari, M.Z., Singh, S.K. and Thakur, D.K. (2000). Comparative efficacy of a herbal anthelmintic and piperazine citrate against natural infection of Toxocara vitulorum in cow and buffalo calves. *Indian J. Anim. Sci.* 70(10) 0367-8318: 1062-1063.

Farnsworth, N.R. and Morris, R.W. (1976). Higher plants - the sleeping giant of drug development. *AMJ Pharm. Educ.* 148: 46-52.

Gunakkunrua, A., Padmanaban, K., Thirumal, P., Pritila, J., Parimala, G., Vengatesan N., Gnanasekar N., Perianayagam James B., Sharma S.K. and Pillai K.K. (2005). Anti-diarrhoeal activity of *Butea monosperma* in experimental animals, *J. Ethanopharmacol.* 98(3): 241-244.

Joshi, H.C. (1970). Some pharmacological studies with the seeds of *Butea frondosa*, *Orrisa Vet J.* 5: 5-8.

Kaleysaraj, R. and Kurup, P.A. (1962). Anthelmintic principle of *Butea frondosa* seeds, *Indian J. Pharmacol.* 24(3): 63-5.

Kasture, V.S., Deshmuk, V.K., Chopde, C.T. (2002). Anxiolytic and anticonvulsive activity of *Sesbania grandiflora* leaves in experimental animals, *Phytother Res.* 16(5): 455-460.

Kennett, G.A., Dickinson, S.L. and Curzon, G. (1985). Enhancement of some 5-HT depressant behavioral responses following repeated immobilization in rats. *Brain Res.* 330: 253-263.

Koster, R., Anderson, M., Debeer, J.M. (1959). Acetic acid used for analgesic screening, Federation Proc. 18: 412.

Lal Jawahar, Chandra, S., Raviprakash, V. and Sabir, M. (1976). Invitro anthelmintic action of some indigenous medicinal plants on *Ascaridia galli* worms. *Indian J. Physiol. Pharmacol.* 20(2): 64-8.

Laurent N.B., Kolb, Y., Eric, P.M., Prinssen, A. (2006). Combined marble burying-locomotor activity test in mice: a practical screening test with sensitivity to different classes of anxiolytics and antidepressants, *Eur. J. Pharmacol.* 547(1-3): 106-115.

Lavhale, M.S. and Mishra, S.H. (2007). Evaluation of free radical scavenging activity of *Butea monosperma* Lam, *Indian J. Exp. Biol.* 45(4): 376-384.

Maurya, R., Dinesh, K., Yadav, Geetu, S., Biju, B., Narayana, Murthy, P.S.., Sahai, M. and Singh, M.M. (2009). Osteogenic activity of constituents from *Butea monosperma*, *Bioorganic Med Chem Lett*. 19:610-613.

Mengi, S.A. and Deshpande, S.G. (1995). Comparative evaluation of *Butea frondosa* and Flubiprofen for ocular anti inflammatory activity in rabbits, *J. Pharmaceut Pharmacol.* 47(12A), 997-1001.

Mishra, U.S., Chakraborty, P., Dasgupta, A., Dastidar, S.G., Martins, M. and Amaral, L. (2009). Potent bactericidal action of a flavanoid fraction isolated from the stem bark of *Butea frondosa.* Int. J. Exp. Clin Pathophysiol Drug Rech. *In vivo* 23(1): 29-32.

Panda, S., Jafri, M., Kar, A. and Meheta, BK. (2009). Thyroid inhibitory, antiperoxidative and hypoglycemic effects of stigmasterol isolated from *Butea monosperma.* Fitoterapia 80: 123-126.

Perez, R.M., Perez, J.A., Garcia, L.M. and Sossa, H. (1998). Neuropharmacological activity of *Solonum nigram* fruit. *J. Ehanopharmacol.* 62(1):43-8.

Porwal, M., Sharma, S. and Mehta, BK. (1988). Isolation of a new triazine and infertility activity of *Butea monosperma*, *Natl Acad Sci. Lett.* 11(3): 81-4.

Razdan, M.K., Kapila, K. and Bhide, N.K. (1970). Antiestrgenic activity of alcoholic extract of petals and seeds of *Butea monosperma*. *Indian J. Physiol Pharmacol.* 14(1): 31-36.

Sakina, M.R. and Dandiya, P.C. (1990). A psycho-neuropharma-cological profile of *Centella asiatica*. *Fitoterapia* 45(4): 291-296.

Sanchez-Mateo, C.C., Prado, B. and Rabanal, R.M. (2002). Antidepressant effects of the methanol extract of several Hypericum species from the Canary Islands. *J. Ethnopharmacol.* 79: 119-127.

Sehrawat, A., Khan, T.H., Prasad, L. and Sultana, S. (2006). *Butea monosperma* and chemomodulation: protective role against thioacetamide-mediated hepatic alterations in wistar rats, Phytomedicine: *Int. J. Phytother & Phytopharmacol* 13 (0944-7113) :157-63.

Shah, K.G. and Baxi, A.J. (1990). Phytochemical studies and antiestrogenic acivity of *Butea frondosa*. *Indian J. Pharm. Sci.* 52(6): 272-5.

Shailendra, S., Gurav, Vijay, D., Gulkari, Nandkishore, J. (2008). Duragkar and Arun T. Patil, Antimicrobial activity of *Butea monosperma* Lam. Gum, *Iran J. Pharmacol. Ther.* 7(1): 21-24.

Shu, YZ. (1998). Recent natural products based drug development: A pharmaceutical industry perspective. *J. Nat Prod* 61: 1053-1071.

Silambujanaki, P., Chitra, V., Kumari, S., Sankari, M., Raju, D., Tejo, B. and Chandra, C.H. (2010). Anticonvulsant activity of methanolic extract of *Butea monosperma* leaves, *Rech J Pharm Biol. Chem. Sci.* 1(2): 431-435.

Sopit, W., Wongkham, C., Trisonthi, C., Boonsiri, P., Simasathiansophon, S. and Atisook, K. (1994). Isolation and properties of a lectin from the seeds of *Butea monosperma*, *Plant Sci.* 103(2): 121-126.

Turner, R.A. (1965). Screening methods in pharmacology. Academic press, London :100.