# Synthesis and Antidepressant Activity of Pyrazoline Derivatives

**B.C. Revanasiddappa, M. Vijay Kumar and Hemanth Kumar**

Department of Pharmaceutical Chemistry, NGSM Institute of Pharmaceutical Sciences of Nitte - Deemed to be University, Paneer, Deralakatte Mangalore-575 018, Karnataka, India

**(Received: April 07, 2020; Accepted: November 27, 2020; Published (web): December 10, 2020)**

**ABSTRACT:** In the present study a series of substituted pyrazolines **(3a-f)** has been synthesized by reacting chalcones **(2a-f)** and semicarbazide **(1)** in methanol medium. All the title compounds were assessed for their in-*vivo* anti-depressant activity by tail suspension test (TST) and forced swimming test (FST) methods. Compound **3a** was found to exhibit moderate antidepressant activity in comparison to standard Imipramine. Newly synthesized compounds were characterized by mass (MS), $^{1}$H-NMR and infrared (IR) spectral analytical data.

**Key words:** Chalcones, pyrazolines, semicarbazide, tail suspension test, forced swimming test.

## INTRODUCTION

Nitrogen containing heterocyclic compounds are gaining lot of interest in the last decade because of their prominent and diverse pharmacological activities. The fascination towards the nitrogen containing heterocyclic compounds attributes due to their diversified biological activities. Pyrazole ring has attracted much attention due to its presence in antipyrine. Basically, pyrazolines are the dihydro derivative of pyrazole. For so many years, pyrazolines have attracted the researchers due to their various biological and pharmacological activities.[1] Pyrazoline nucleus is present in so many currently available drugs like metamizole or dipyrone (analgesic and antipyretic), phenylbutazone (anti-inflammatory) and sulfinpyrazone (chronic gout). Pyrazolines are reported to possess antimicrobial[3], anticancer[4], anti-inflammatory[5], antimalarial[6], antioxidant[7], antifungal[8], antidepressant[9] activities.

Chalcones are used as one of the common intermediates in the synthesis of so many heterocyclic compounds. Chalcones are composed of highly reactive α,β-unsaturated keto functional group, which is responsible for so many bioactivities. The key intermediate chalcones are prepared as per the well-known Claisen-Schmidt condensation reaction between aromatic aldehydes and ketones in an alcohol medium. Chalcones constitute an outstanding group of compounds and reported to possess antiviral[10], antimicrobial[11], antitubercular[12], and anti-inflammatory[13] activities.

Based on the above biological and pharmacological profile exhibited by chalcones and pyrazolines and in the continuing process of our work on pyrazolines[14-17], it was thought worthwhile to synthesize a new series of pyrazoline derivatives and evaluate their antidepressant activity.

## MATERIALS AND METHODS

IR spectra were recorded as KBr pellets in an Alpha Bruker IR spectrometer and the values are expressed in $cm^{-1}$. Mass spectra were recorded on Perkin Elmar Clarus GC-MS spectrometer. $^{1}$H-NMR spectra were acquired by utilizing a 300 MHz Bruker Avance-II NMR spectrometer using DMSO as solvent and TMS as internal standard. Melting points have been determined by open capillary method and were uncorrected. The completion of the reactions was checked by TLC over silica G plates. UV lamp was used to visualize the spots on TLS plates. Ethyl acetate-acetone (9:1) was used as the mobile phase.

**Correspondence to:** B.C. Revanasiddappa
Email: revan@nitte.edu.in

Dhaka Univ. J. Pharm. Sci. **19**(2): 179-184, 2020 (December)

**DOI: https://doi.org/10.3329/dujps.v19i2.50634**

**General procedure for the synthesis of Pyrazoline derivatives (3a-f).** A mixture of chalcones **(2a-f)** (0.01 mol), NaOH (0.02 mol) and semicarbazide **(1)** (0.0 1mol) was dissolved in 30 ml of methanol. The reaction mixture was refluxed for 16-28 hrs and cooled to room temperature overnight. The solution was added to crushed ice with slow stirring and the compound was precipitated, filtered, dried and recrystallized from alcohol to give compounds **(3a-f)** (Table 1).

**Table 1. Physical data of pyrazoline derivatives (3a-f).**

| Comp. | Ar-CHO | $Ar^1$-$COCH_3$ | M.P ($^0$C) | Yield (%) |
|---|---|---|---|---|
| 3a | 4-$NO_2$ | 4-Cl | 103-05 | 69 |
| 3b | 2-Cl | 4-$CH_3$ | 134-36 | 66 |
| 3c | 2-Cl | 4-OH | 183-85 | 60 |
| 3d | 4-Cl | 4-OH | 141-43 | 62 |
| 3e | 4-F | 2,4-$(Cl)_2$ | 163-65 | 61 |
| 3f | 4-Cl | 4-$NO_2$ | 171-73 | 66 |

**5-(4-Chlorophenyl)-3-(4-nitrophenyl)-4, 5-dihydro-*1H*-pyrazole-1-carboxamide (3a):** IR (KBr) *v* ($cm^{-1}$): 807 (Cl), 1521 (C=C), 1581 (C=N), 1647 (C=O), 3084 (C-H), 3637 (NH); $^1$H-NMR (300 MHz, $CDCl_3$): δ 3.51 (s, $NH_2$, 2H), 6.54-6.56 (dd, 2H, $H_A$, $H_B$,, *J*= 6.0 Hz), 7.16-718 (dd, 1H, $H_X$, *J*= 6.0 Hz), 7.20-8.18 (m, Ar-H, 8H). MS (*m/z*): 344.75($M^+$).

**3-(4-Chlorophenyl)-5-p-tolyl-4, 5-dihydro-*1H*-pyrazole-1-carboxamide (3b):** IR (KBr) *v* ($cm^{-1}$): 830 (Cl), 1482 (C=C), 1599 (C=N), 1652 (C=O), 3082 (C-H), 3143 (NH); $^1$H-NMR (300 MHz, $CDCl_3$): δ 2.40 (s, $CH_3$, 3H), 3.31 (s, $NH_2$, 2H), 7.27-7.32 (dd, 2H, $H_A$, $H_B$' *J*= 15.0 Hz,), 7.37-7.39 (dd, 1H, $H_X$, *J*= 6.0 Hz), 7.70-8.07 (m, Ar-H, 8H). MS (*m/z*): 313.78 ($M^+$).

**3-(4-Chlorophenyl)-5-(4-hydroxyphenyl)-4, 5-dihydro-*1H*-pyrazole-1-carboxamide (3d):** IR (KBr) *v* ($cm^{-1}$): 813 (Cl), 1647 (C=C), 1581 (C=N), 1656 (C=O), 2891 (C-H), 3748 (OH); $^1$H-NMR (300 MHz, $CDCl_3$): δ 3.31 (s, $NH_2$, 2H), 6.81-6.85 (dd, 2H, $H_A$, $H_B$, *J*= 12.0 Hz,), 7.13-7.15 (dd, 1H, $H_X$, *J*= 6.0 Hz), 7.35-8.20 (m, Ar-H, 8H), 11.46 (s, OH, 1H), MS (*m/z*): 315.75 ($M^+$).

**5-(2,4-Dichlorophenyl)-3-(4-fluorophenyl)-4,5-dihydro-*1H*-pyrazole-1-carboxamide (3e):** IR (KBr) *v* ($cm^{-1}$): 852 (Cl), 1494 (C=C), 1598 (C=N), 1650 (C=O), 3058 (C-H), 3243 (NH); $^1$H-NMR (300 MHz, $CDCl_3$): δ 3.44-3.46 (dd, 1H, $H_A$, *J*= 9.0 Hz), 4.03-4.07 (dd, 1H, $H_B$, *J*= 12.0 Hz), 6.80-6.82 (dd, 1H, $H_X$, *J*= 6.0 Hz), 6.86 (s, $NH_2$, 2H), 6.97-8.00 (m, Ar-H, 7H). MS (*m/z*): 352.19 ($M^+$).

**Acute toxicity studies.** Acute toxicity studies were conducted to determine the median lethal dose ($LD_{50}$) of the newly synthesized compounds. It was conducted in adult female albino mice by "up and down method" (OECD guidelines 425). Different dose levels of the compounds were administered orally to different groups of mice consisting of an adequate number of animals. Following the administration, the animals were observed continuously for 2-3 hours for general behavioral, neurological, autonomic profiles and death for 24 hours. The test allowed the observation of signs and symptoms of toxicity.[18] Each group consisted of six animals. The animal experiments were conducted after the protocol was approved by the Institutional Animal Ethical Committee, Registration No.: NGSMIPS/IAEC/May-2017/55**.**

**Forced swimming test (FST).** Imipramine 10 mg/kg was used as standard antidepressant drug. The test compounds (100 mg/kg) and standard drug were suspended in 1% aqueous solution of Tween 80 and administered (p.o.) to mice at a volume of 0.5 ml/ body weight. A control was also maintained. The mice were individually forced to swim in an open cylindrical container (25 cm height), 10 cm diameter

containing water to a height of 18 cm and observed for 6 min. Treatment was given before one hour of the study. The animal showed initial vigorous struggling in the first 2 min. The immobility time was recorded during the last 4 min. Each group consisted of six animals. The percentage change from control was calculated by using the following formula[19]:

% Change of immobility = [(test/control) ×100) - 100]

**Tail suspension test (TST).** The total duration of immobility by FST was measured accordingly as per the above procedure. Treatment was given before one hour of the study. After one hour, mice were suspended 50 cm above the floor with the help of adhesive tape and placed 1cm from the tip of the tail. Animals were allowed to hang for 6 min and the duration of immobility was recorded. A decrease in the immobility period indicates the antidepressant activity. Each group consists of six animals. The percentage change from control was calculated by using the following formula[19]:

% Change of immobility = [(test/control) ×100) - 100]

**Statistical analysis.** Data were analyzed using Graphpad Prism 6 software. All the results were expressed as mean ± SEM. The data which was obtained from the pharmacological experiments were analyzed with one-way analysis of variance (ANOVA) followed by Tukey Kramer's multiple comparison tests. A p-value of less than 0.05 was considered statistically significant.

## RESULTS AND DISCUSSION

A series of new pyrazoline derivatives were prepared by the reaction of semicarbazide and chalcones with methanol as the solvent in the presence of a base. The key intermediates chalcones **(2a-f)** were synthesized by the well-known Claisen-Schmidt-condensation reaction. The synthetic route for the preparation of new compounds is depicted in Scheme-01. The stepwise procedures and reaction conditions are explicated in the experimental section. The synthesized compounds were yielded in good yields to inaugurate as new antidepressant agents, and were identified by the spectroscopic data. *In-vivo* antidepressant activity was carried out for all the new compounds. Imipramine was used as a standard drug for comparison purpose. The compounds were tested at 100 mg/kg body weight.

The $^1$H-NMR spectrum of compound **3d** showed signals δ 6.81-6.92 and 7.13-7.15 corresponding to three protons of pyrazoline in $H_A$, $H_B$, $H_X$ pattern. The signals for aromatic protons were observed in the region δ 7.35-8.20. A broad singlet was observed for OH at δ 11.46. The IR spectrum of compound **3d** showed the appearance of C=O band at 1652 $cm^{-1}$

**Table-2. Antidepressant activity of pyrazoline derivatives by forced swimming test (FST)**

| Compound[a] | Duration of immobility (s) | % Change in immobility |
|---|---|---|
| 3a | 119.5±10.49*** | -52.92 |
| 3b | 203.33±6.22 | -19.89 |
| 3c | 175.16±22* | -30.99 |
| 3d | 214.83±43.89 | -15.36 |
| 3e | 231.83±27.63 | -8.66 |
| 3f | 132.33±16.58** | -47.86 |
| Control | 253.83±26.02 | ---- |
| Imipramine | 94±9.2 | -62.96 |

Values represent the mean ± S.E.M. (n= 6). [a]Compounds and imipramine were tested at 100 and 10 mg/kg dose level (ip), respectively.

**Table 3. Antidepressant activity of pyrazolines derivatives by tail suspension test (TST).**

| Compound[a] | Duration of immobility (s) | % Change in immobility |
|---|---|---|
| 3a | 223.33±26.09* | -33.3 |
| 3b | 248.33±11.3 | -25.83 |
| 3c | 265.33±12.59 | -20.75 |
| 3d | 223.33±24.2* | -33.3 |
| 3e | 185.83±5.7** | -44.49 |
| 3f | 233±13.08* | -30.41 |
| Control | 334.83±2.95 | --- |
| Imipramine | 119±9.88 | -64.45 |

Values represent the mean ± S.E.M. (n= 6). [a]Compounds and imipramine were tested at 100 and 10 mg/kg dose level (ip), respectively.

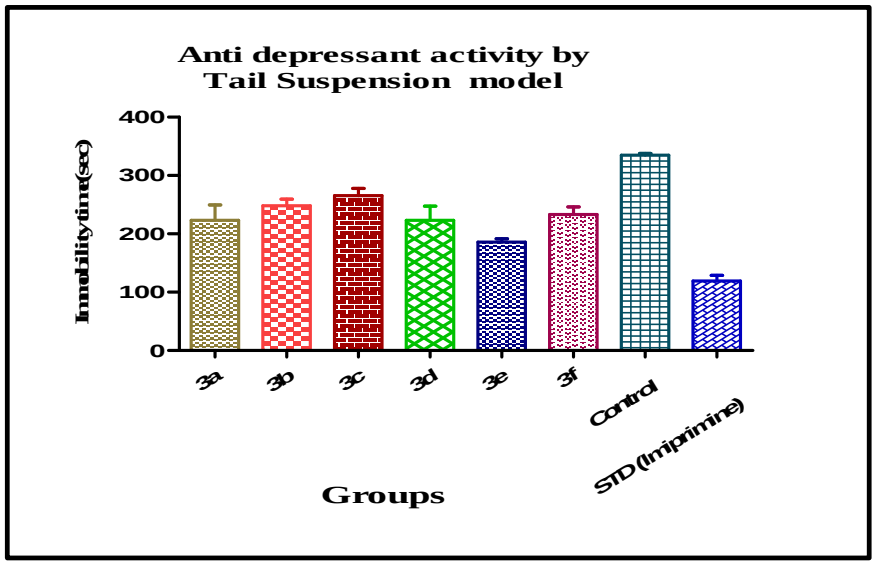



Figure 1. Antidepressant activity by tail suspension test (TST).

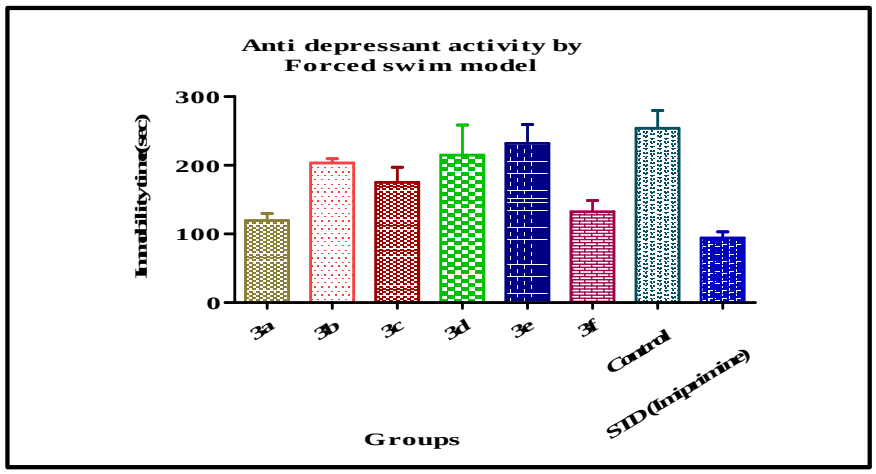
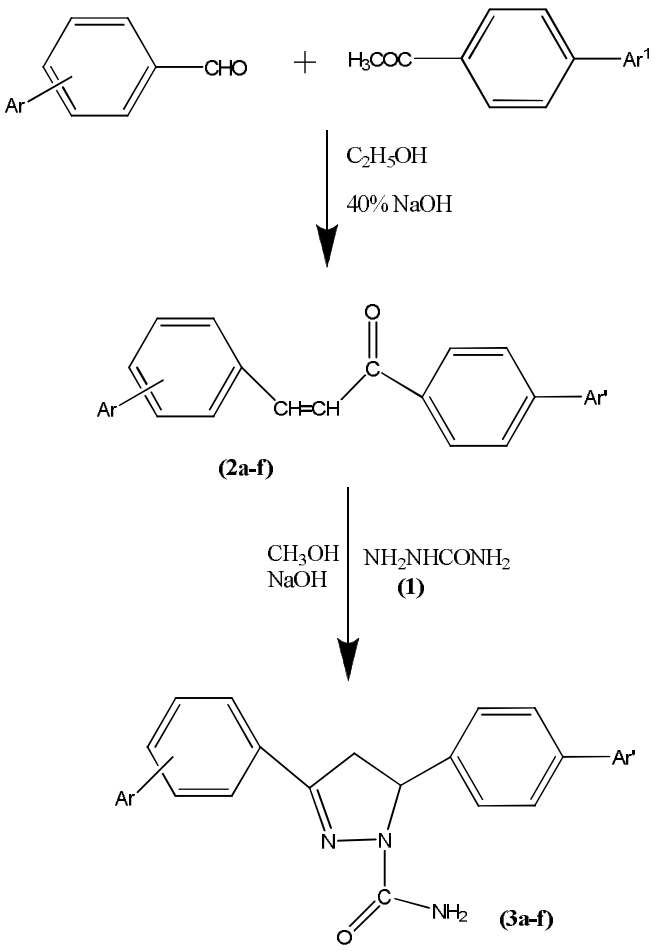



Figure 2. Antidepressant activity by forced swimming test (FST).




**Scheme 1**

and the other bands at 3082 and 1599 $cm^{-1}$ corresponded to the C-H and C=N, respectively. Finally, the compound **3d** showed a stable molecular ion peak at *m/z* = 315.75 ($M^+$), which is in consistent with the molecular formula $C_{16}H_{14}N_3O_2Cl$. The mass spectrum of the pyrazoline derivative was in good agreement with their suggested structure.

FST and TST are the widely used common *in-vivo* animal models to screen the antidepressant activity and in the present investigation these two models were employed to screen the antidepressant activity of the title compounds. These models are quite sensitive and relatively specific to MAO inhibitors and typical antidepressants. Antidepressant activity was determined as mean immobility time in seconds and data of the compounds is showed in table 2 and 3 (Figures 1 and 2). The reduction in the duration of immobility time when compared to the control was significantly showed by the tested compounds.

*In-vivo* anti-depressant activity was carried out using FST and TST methods. FST results revealed that the compounds **3a** and **3f** showed moderate activity when compared to the standard and also the compound **3e** demonstrated moderate activity. In TST, the compound **3e** showed moderate activity when compared with to the standard drug, imipramine. The other tested compounds **3d** and **3f** also exhibited moderate activity. In TST model, the mechanism of depressive stress was due to the inverted hanging posture of the animal, while in FST, it was due to forced swim, so both mechanisms are different and the tested compounds exhibited a difference in the immobility time.

## CONCLUSIONS

In conclusion, a series of substituted pyrazolines were synthesized via the formation of chalcones and semicarbazide in methanol medium. The compounds which were evaluated for the antidepressant activity showed only moderate activity when compared to the standard imipramine. None of the tested compounds were found to show significant activity in both the models. The nature of substituents has a strong influence on the extent of antidepressant action. The presence of electron withdrawing groups on both sides of the phenyl ring may be responsible for the antidepressant activity. The synthesized novel pyrazoline derivatives might be helpful in the development of new antidepressant agents which requires further modifications in the structure of the compounds to get a better pharmacological profile.

## ACKNOWLEDGEMENTS

Authors are indebted to authorities of Nitte-Deemed to be University, Mangalore for providing financial help (Grant No.: NUFR1/2016/18-04). The authors also thankful to Vellore Institute of Technology, Vellore, for their immense help in recording the NMR and mass spectral data.

## REFERENCES

1. Das, B.C., Bhowmilk, D., Chiranjib, B. and Mariappan, G. 2010. Synthesis and biological evaluation of some pyrazoline derivatives. *J. Pharm. Res.* **3,** 1345-1348.
2. Bardalai, D and Panneerselvam, P. 2012. Pyrazole and 2-pyrazoline derivatives: potential anti-inflammatory and analgesic agents. *Int. Res. J. Pharm. App Sci.* **2**, 1-8
3. Venkataraman, S., Jain S., Shah, K. and Upmanyu N. 2010. Synthesis and biological activity of some novel pyrazolines. *Acta Poloniae. Pharmaceut. Drug. Res.* **67**, 361-366,
4. Lv, P.C., Li, D.D., Li, Q.S., Lu, X., Xiao, Z.P. and Zhu, H.L. 2011. Synthesis, molecular docking and evaluation of thiazolyl-pyrazoline derivatives as EGFR TK inhibitors and potential anticancer agents. *Bioorg. Med. Chem. Lett.* **21**, 5374-5377.
5. Bano, S., Javed, K., Ahmad, S., Rathish, I.G., Singh, S. and Alam M.S. 2011. Synthesis and biological evaluation of some new 2-pyrazolines bearing benzene sulfonamide moiety as potential anti-inflammatory and anti-cancer agents. *Eur. J. Med. Chem.* **46**, 5763-5768.
6. Insuasty, B., Montoya, A., Becerra, D., Quiroga, J., Abonia, R., Robledo, S., Darío Vélez, I., Upegui, Y., Nogueras, M. and Cobo, J. 2013. Synthesis of novel analogs of 2-pyrazoline obtained from [(7-chloroquinolin-4-yl) amino]chalcones and hydrazine as potential antitumor and antimalarial agents. *Eur. J. Med. Chem.* **67**, 252-262.
7. Nagarajan, G., Suthakaran, R., Somashekar, G. and Marikannan, M. 2007. Synthesis, anti-inflammatory, antioxidant and antibacterial activities of 7-methoxy benzofuran pyrazoline derivatives. *Asian. J. Chem.* **19**, 3353-3362.
8. Chetan, B.P., Sreenivas, M.T. and Bhat, A.R. 2004. Synthesis and evaluation of certain pyrazolines and related compounds for their antitubercular, antibacterial and antifungal activities. *Ind. J. Het. Chem.* **13**, 225-228.
9. Ozdemir, A., Altıntop, M.D., Kaplancıklı, Z.A., Can, O.D.; Demir-Ozkay, U. and Turan-Zitouni, G. 2015. Synthesis and evaluation of new 1,5-Diaryl-3-[4-(methylsulfonyl) phenyl]-4,5-dihydro-1H-pyrazole derivatives as potential antidepressant agents. *Molecules*, **20**, 2668-2684.
10. Yu, D., Churkin., Panfilova, L.V. and Boreko, E.I. 1982. Biologically active thiophene derivatives IV: Synthesis and antiviral activity of unsaturated ketones of thiophene series. *Pharm. Chem.* **16**, 103-105
11. Rajendra, P.Y., Praveen Kumar, P. and Ravi Kumar, P. 2008. Synthesis and antimicrobial activity of some new chalcones of 2-acetyl pyridine. *E- J. Chem.* **5,** 144-148.
12. Shivakumar, P.M., Geetha Babu, S.M. and Mukesh, D. 2005. QSAR studies on chalcones and flavonoids as antitubercular agents using genetic function approximation (GFA) method. *Chem. Pharm. Bull.* **55**, 44-49.
13. Shen Jevwon., Chang., Tsung., Liv., Loti, T. and Sao.2005.Synthetic chalcones as potential anti-inflammatory and cancer chemo preventive agents. *Eur. J. Med. Chem.* **40**, 103-112.
14. Revanasiddappa, B.C., Subrahmanyam, E.V.S. and Lakshmi, T.N. 2011. Synthesis and biological evaluation of pyrazolines. *Ind. J. Pharm. Edu. Res.* **45**, 164-167.
15. Satyanarayana, D., Revanasiddappa, B.C. and Neema, K.V. 2013. Synthesis and biological evaluation of some novel pyrazolines. *Ind. J. Het. Chem.* **22**, 353-356.
16. Revanasiddappa, B.C., Jisha, M.S., Varghese, S.S., Kalsi, J. and Jose, N. 2005. Synthesis and biological evaluation of novel 1,3,5-trisubstituted pyrazolines. *Ind. J. Het. Chem.* **24**, 51-54.
17. Revanasiddappa, B.C., Vijay Kumar, M., Nayak, P., Ajmal Roshan Ali and Jisha, M.S. 2017. Synthesis, antibacterial and antifungal evaluation of novel pyrazoline derivatives. *Res. J. Pharm. Tech*. **10**, 1481-1484.
18. OECD, Acute oral toxicity: up and down procedures, Guidelines for testing of chemicals. 200. OECD, Paris. No. 425.
19. Vijay Kumar, M. and Revanasiddappa, B.C. 2016. Synthesis and antidepressant evaluation of novel pyrazolone derivatives. *Bangladesh. J. Pharmacol.* **11**, 558-563.