International Journal of Pharmacology

ISSN 1811-7775

science alert

ansinet
Asian Network for Scientific Information

OPEN ACCESS International Journal of Pharmacology

ISSN 1811-7775
DOI: 10.3923/ijp.2018.1066.1071

Research Article
In vivo Pharmacodynamics Studies of *Acacia tortilis* Found in Kingdom of Saudi Arabia on Cardiovascular System of Rats

[1]Abdulhalim S. Serafi, [1]Aisha Azmat, [2]Muhammad Ahmed, [1]Mohammed Bafail and [1]Zahir Hussain

[1]Department of Physiology, Faculty of Medicine, Umm Al-Qura University, Makkah, Saudi Arabia
[2]Department of Pharmacology and Toxicology, Faculty of Pharmacy, Umm Al-Qura University, Makkah, Saudi Arabia

Abstract

Background and Objectives: Herbal medicines are recommended as remedy for different cardiac diseases. Acacia possessing significant pharmacological activities, widely found in KSA. The effect of *Acacia tortilis* leaves decoction (ATLD) on cardiac hemodynamics was evaluated in rats. **Methods:** Different hemodynamic parameters (systolic, diastolic, mean arterial blood pressure and heart rate) were recorded in normotensive rats before and after the intravenous administration of ATLD. **Results:** The different doses showed a dose dependent significant hypotensive effect. However the highest dose (30 mg kg^{-1}) showed maximum activity and lowered the systolic pressure (17.4±2.95%), diastolic blood pressure (15.2±1.8%) and mean arterial blood pressure (17.1±2.4%). Intravenous administration of ATLD also showed a dose-dependent reduction in heart rate ($p<0.001$). **Conclusion:** The hypotensive and bradycardia might had been due to presence of N,N Dimethyltryptamine in extract that reduced the blood pressure and heart rate. Thus, the authors conclude that the ATLD possesses potent hypotensive activity and sought to be useful for anti hypertensive drug development and worth much merit to be investigated.

Key words: *Acacia tortilis* leaves decoction (ATLD), systolic pressure, diastolic pressure, mean arterial pressure (MAP), heart rate

Received: April 01, 2018 Accepted: July 16, 2018 Published: October 15, 2018

Citation: Abdulhalim S. Serafi, Aisha Azmat, Muhammad Ahmed, Mohammed Bafail and Zahir Hussain, 2018. *In vivo* pharmacodynamics studies of *Acacia tortilis* found in Kingdom of Saudi Arabia on cardiovascular system of rats. Int. J. Pharmacol., 14: 1066-1071.

Corresponding Author: Abdulhalim S. Serafi, Department of Physiology, Faculty of Medicine, Umm Al-Qura University, Makkah, Saudi Arabia
Tel: 00966556611957

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Blood pressure is the normal force produced by heart, exerted by blood against the walls of the arteries. Blood pressure varies normally throughout the day[1], but continuous blood pressure elevation can cause health problems. Pathophysiology of hypertension in perspective to pharmacological studies have been carried out in recent years[2-5]. The British Hypertension Society guidelines proposed that changes in lifestyle can be helpful for the primary prevention of hypertension[6]. However, hypertensive patients need proper treatment if systolic/diastolic blood pressure is greater than 160/90 mmHg[7]. Hypertension is one of the most common chronic medical problem that requires adequate management. Hospital appointments, diagnostic laboratory tests and treatment charge about $420 per year[8]. During 2010, the mean expenditure per person for treatment for hypertension was somewhat higher (for women: $751 and men: $713) as reported[9]. Hypertension is continually increasing in Kingdom of Saudi Arabia (KSA)[10]. The Global Burden of Disease estimates that hypertension was the leading risk factor for death in KSA[11]. In KSA, 81% of the population is under the age of 40[10]. The burden of hypertension in future will become major health problem to the health system. Thus searching for new low cost hypertensive drug is necessary[12]. Herbal medicines are recommended for the treatment of different diseases especially in Europe, Middle East and Asia. Scientists agree with some aspects of traditional use of herbal drugs and plants as remedy for different cardiac diseases (hypertension). Few of these are *Hibiscus sabdariffa* L.[13], Tribulus terrestris[14], Persea Americana[15], *Tropaeolum majus* L.[16]. Search for new anti hypertensive drugs continue that can counter the currently available high cost medicines and other drugs with little efficacy. Acacia possessing significant pharmacological activities is one of the 86 different medicinal species reported more than a decade ago in KSA[17]. The literature reveals anti diarrhoeal and antimicrobial activity of *Acacia nilocita*[18,19], antioxidant activity of *Acacia confusa*, *Acacia seyal* and *Acacia laeta*[20,21], antidiabetic activity of *Acacia ataxacantha*[22]. Fortunately, the species of acacia (*Acacia tortilis*) are widely found in Saudi Arabia especially in Makkah region. However, acacia plant in KSA is only contributing to form a good habitat for the honey bee that produces good quality honey: marketably known as acacia honey. Acacias widely spread in KSA have numerous uses and values. Now a days home remedies and use of plants to cure different diseases are becoming more popular in people beside pharmaceutical market. Hypoglycemic and hypocholesterolemic activity[23], diazepam like activity[24], neuroprotective and anticonvulsant activity[25] of *Acacia tortilis* has recently been investigated in KSA. It is quite interesting to mention that *Acacia tortilis* have not yet been scientifically screened for hypotensive and anti hypertensive activity and mechanism of action and safety. Hence, it is quite fascinating to search thoroughly the influences and efficacy of this plant especially in cardiology/cardiovascular system disorders. The present part of this project using relevant methods, procedures and instruments mentioned in the project comprised mainly the collection and authentication of Acacia species, preparation of standard rat model for studying the pharmacodynamic cardiovascular efficacy of plants extracts available in Makkah, KSA.

MATERIALS AND METHODS

Collection and authentication of plant: *Acacia tortilis* plant was plucked from Al-Abdiyah region, Makkah, KSA, in December, 2016 and verified by Dr. Ibrahim Abd-Elhady, Associate Professor, Department of Pharmacognosy, Faculty of Pharmacy, Umm Al-Qura University. A voucher specimen number PHG128 was deposited. *Acacia tortilis* leaves were separated and washed by using tap water.

Preparation of *Acacia tortilis* leave decoction (ATLD): For the preparation of ATLD, leaves (100 g) were grinded and macerated in distilled water for 1 h after that boiled in same distilled water as reported by Chinese medicinal herbs[26]. At the end a bright green color extract was obtained and filtered. This filtrate was used for intravenous administration in rats.

Oral toxicity test (acute): Acute oral toxicity test was performed in healthy rats and mice, following OECD (Organization of Economic Cooperation and Development) Guideline 423. In rats and mice (3 each), 2000 mg kg^{-1} (maximum dose) of ATLD was administered orally. Animals were monitored for the next 48 h[27] for any sign of toxicity and mortality.

Experimental animals: In the current study ten albino Sprague-dawley rats (200-250 g: either sex) were used for the recording of data. The rats were fed on a standard pellet diet and tap water *ad libitum*. The experimental protocol followed was permitted by the Animal Ethics Committee, Faculty of Medicine, Umm Al-Qura University, Makkah, Saudi Arabia (Ethical approval number HAPO-02-K-012-2015-11-125).

Measurement of normal blood pressure of rats (*In vivo* activity): Sprague-Dawley rats (normotensive: 200-250 g) were anaesthetized with thiopental sodium (40 mg kg^{-1}, i.p)[28]. Once animal was anaesthetized, it was positioned on dissection bench (dorsal decubitus), under an overhead lamp to maintain body temperature. Afterward the trachea of a rat was intubated with polyethylene cannula to promote extempore respiration. A mid trachea longitudinal incision (20 mm) exposed the right jugular vein and both common carotid arteries. The right jugular vein was catheterized with a scalp vein set (BROMED) filled with a saline solution to administer drugs. The left carotid artery was catheterized and reusable blood pressure transducer (Millar Micro tip Pressure volume catheter SPR 838, Powerlab, AD Instruments) was inserted. Transducer was used for monitoring the systolic and diastolic pressure (mm Hg), as well as heart rate (beats min^{-1}, bpm) continuously during the experiment and calculated internally in the Powerlab using the Chart software (labChart Pro v8.1.5, Powerlab AD instrument). After surgery, rat and all parameters were equilibrated for at least 30 min before starting the tests. For control reading saline was administered (0.20-0.25 mL) through a scalp vein set introduced into the external jugular vein. After normal Data (Control), Ach (10^{-6} M) and extract were administered one by one and their activity was recorded. These records were used for the calculation of arterial blood pressure (Systole and Diastole: mmHg), cardiac cycles (s) and Heart rate (BPM).

Statistical analysis: A software Minitab 17 was used for statistical analysis. A power analysis suggested that the sample size be set at N = 3 to achieve a power of 0.8 at an alpha of 0.05 calculated by G*Power[29]. However, in the present study number of animals per group were N = 10. All hypotheses were tested using one way analysis of variance (ANOVA) tests. Alpha was set at (0.05). Significant effects were subjected to Tukey's multiple-range tests for comparison of treatment means with a control.

RESULTS

The blood pressure recording and parameters before (normal) and after the administration of ATLD are presented in Table 1. ATLD doses (10, 30 mg kg^{-1}) lowered the systolic, diastolic and the mean arterial pressure of normotensive rats significantly ($p<0.05$) and the effect of ATLD was more pronounced as compared to Ach in normotensive rats. Fall in Blood pressure was dose dependent. Greater effect was observed at 30 mg kg^{-1} with reductions of 17.4±2.9 and 15.2±1.8% in systolic and diastolic blood pressures respectively. Decreases in various blood pressure parameters were statistically significant ($p<0.005$).

Effect of Acacia on heart rate and cardiac cycle: There was a significant decrease in the heart rate in rats following the administration of the Acacia extract. Dose of 10 mg kg^{-1} caused significant reduction in heart rate ($p<0.01$), initially 361.72±26.13 beats/min, to 238.91±65.5 (Table 1). Intravenous administration of ATLD at 10 and 30 mg kg^{-1} dose led to decrease in heart rate at (34 and 42%, respectively, $p = 0.0001$) and increase in the duration of cardiac cycle significantly ($p<0.001$) as shown in Table 1.

Table 1: Effect of *Acacia tortilis* on various blood pressure parameters

	Before	After	Reduction (%)	p-value
10 mg kg^{-1}				
Systolic pressure	149.50±6.10	129.53±10.7	13.5±2.7	0.01
Diastolic pressure	121.90±7.50	112.80±6.20	7.32±1.95	0.05
Mean arterial pressure	135.46±5.20	121.20±8.10	10.0±3.5	0.01
Heart rate	361.72±26.13	238.91±65.5	34.0±6.0	0.01
Cardiac cycle	0.17±0.02	0.29±0.019	Increased	0.01
30 mg kg^{-1}				
Systolic pressure	126.60±9.08	104.80±6.90	17.4±2.9	0.01
Diastolic pressure	109.86±6.24	92.80±7.19	15.2±1.8	0.05
Mean arterial pressure	118.23±8.30	98.68±8.50	17.1±2.4	0.01
Heart rate	358.00±40.9	206.97±98.5	42.4±5.8	0.01
Cardiac cycle	0.169±0.02	0.47±0.06	Increased	0.01
Ach				
Systolic pressure	121.25±5.40	90.01±14.20	25.6±6.4	0.01
Diastolic pressure	86.29±1.60	66.24±10.70	23.2±8.5	0.05
Mean arterial pressure	105.80±3.30	78.67±12.60	25.7±7.3	0.01
Heart rate	305.19±5.67	223.59±12.95	26.8±5.60	0.05
Cardiac cycle	0.17±0.05	0.41±0.030	Increased	0.01

n = 10, Each value represents the Mean±STDEV

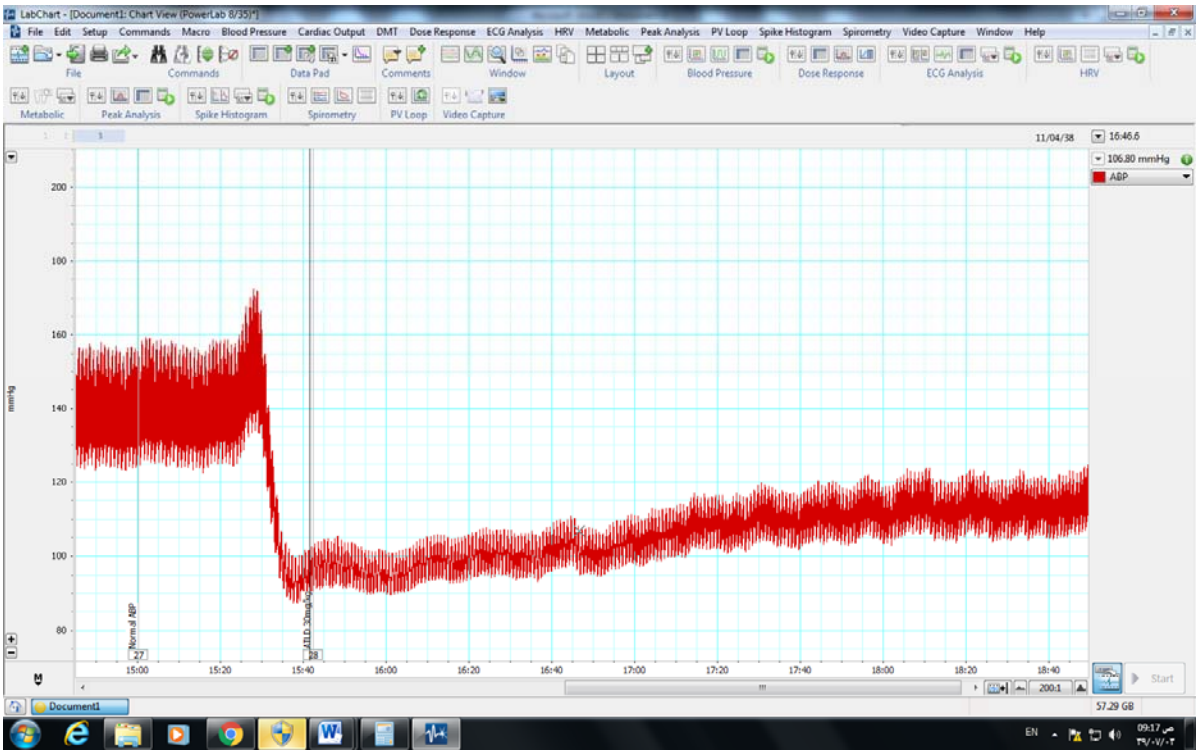

Fig. 1: Tracing from a typical experiment showing the ATLD for its effects on blood pressure (BP) in the anesthetized rats

Toxicological studies in mice and rats: Toxicological studies of ATLD were carried out in mice and rats. Administration of ATLD (P.O. 2000 mg kg^{-1}) did not show any significant change in physical behavior of mice and rats. None of the groups showed any significant change.

DISCUSSION

This present study is related to *in vivo* pharmacodynamics cardiovascular efficacy of KSA *Acacia tortilis* using rat model. *Acacia tortilis* have scientifically been screened for hypotensive activity. The results obtained from the acute oral toxicity study showed that ATLD demonstrated high safety margin when the animals tolerated ATLD up to 2000 mg kg^{-1} b.wt., orally. Previously Mukhtar *et al.*[30] has also reported that the margin of ethanolic extract of *Acacia tortilis* leaves safety up to 2000 mg kg^{-1}.

In the present study the i.v. administration of ATLD caused a transient fall in blood pressure parameters (Systolic, Diastolic, MABP) and heart rate significantly as shown in Fig. 1. These hypotensive effects were brief returning to normal within 5 min. The significant hypotensive effect after ATLD administration depends on the *Acacia tortilis* components ratios. Previously another species (Acacia catechu) was reported to produce a dose dependent decrease in blood pressure in both anaesthetized dogs and rats[31]. Acacia Nilotica[32] and Acacia Phenol[33] were also considered to exhibit anti-hypertensive effects.

It was suggested that serum sodium was dropped after the administration with gum arabic[33]. This might be the cause of hypotensive activity and drop of blood pressure. The presence of N, NDimethyltryptamine (DMT) has been confirmed previously in Acacia leaves water extract[34] belong to tryptamine family (psychedelic compound), tend to reduced systolic blood pressure and heart rate[35]. DMT (inhaled or injected), the effects last a short period of time (about 5-15 min), our results are in accordance with this study that after the i.v. administration of ATLD effect persist for only 5 min[36].

Crowch and Okello[37] demonstrated acetylcholinesterase antagonist activity of Acacia nilotica and Acacia auriculiformis in experimental models[37,38]. Inhibition of acetylcholinesterase amplifies and prolongs the cholinergic response. Acetylcholine binds and stimulates the M2 receptor in heart cause bradycardia (negative chronotropic effect)[39]. However the stimulation of M3 receptors causes vasodilation and decreased peripheral resistance which lowers blood pressure[40].

CONCLUSION ANS FUTURE RECOMMENDATION

Conclusively, ATLD exhibits profound hypotensive and heart rate lowering (Bradycardia) activities. It is proposed that ATLD might causes vasodilation and decreased peripheral resistance which lowers blood pressure. However, further studies are needed to be carried out for understanding the mechanism of action of ATLD.

SIGNIFICANCE STATEMENTS

In present study oral administration of ATLD (2000 mg kg^{-1}) was safe and did not cause any mortality or side effect in rats.

In this research work, an intravenous administration of different doses of ATLD produced hypotensive activity significantly comparable to Ach.

ATLD not only produced profound hypotensive effect in experimental rats, but also appeared to have a heart rate lowering (Bradycardia) activities.

It was a preliminary study, due to increase in the rate of hypertension in Saudi Arabia. This study will open the doors for further research and will provide an alternative low cost therapy for hypertensive patients.

For future it is recommended to carry out further studies on understanding the mechanism of action of ATLD.

ACKNOWLEDGMENT

The authors are thankful to the institute Deanship of Scientific Research for providing financial support through grant no.15-MED-3-1-0064, Umm Al Qura University, Makkah, KSA.

REFERENCES

1. Mancia, G., G. Parati, M. Di Rienzo and A. Zanchetti, 1997. Blood Pressure Variability. In: Pathophysiology of Hypertension, (Handbook of Hypertension, Vol. 17), Zanchetti, A. and G. Mancia (Eds.)., Elsevier Science, New York, pp: 117-169.
2. Akcilar, R., S. Turgut, V. Caner, A. Akcilar and C. Ayada *et al*., 2013. Apelin effects on blood pressure and RAS in DOCA-salt-induced hypertensive rats. Clin. Exp. Hypertens., 35: 550-557.
3. Trott, D.W. and D.G. Harrison, 2014. The immune system in hypertension. Adv. Physiol. Educ., 38: 20-24.
4. Vaneckova, I., L. Maletinska, M. Behuliak, V. Nagelova, J. Zicha and J. Kunes, 2014. Obesity-related hypertension: Possible pathophysiological mechanisms. J. Endocrinol., 223: R63-R78.
5. Fernandez-Vallinas, S., M. Miguel and A. Aleixandre, 2016. Long-term antihypertensive effect of a soluble cocoa fiber product in spontaneously hypertensive rats. Food Nutr. Res., Vol. 60, No. 1. 10.3402/fnr.v60.29418.
6. Williams, B., N.R. Poulter, M.J. Brown, M. Davis and G.T. McInnes *et al*., 2004. Guidelines for management of hypertension: Report of the fourth working party of the British hypertension society, 2004-BHS IV. J. Hum. Hypertens., 18: 139-185.
7. Poulter, N.R. D. Prabhakaran and M. Caulfield, 2015. Hypertension. Lancet, 386: 801-812.
8. Odell, T.W. and M.C. Gregory, 1995. Cost of hypertension treatment. J. Gen. Internal Med., 10: 686-688.
9. Davis, K., 2013. Expenditures for hypertension among adults age 18 and older, 2010: Estimates for the U.S. civilian noninstitutionalized population. Statistical Brief No. 404. April, 2013. Agency for Healthcare Research and Quality, Rockville, MD.
10. Al-Nozha, M.M., M. Abdullah, M.R. Arafah, M.Z. Khalil and N.B. Khan *et al*., 2007. Hypertension in Saudi Arabia. Saudi Med. J., 28: 77-84.
11. IHME., 2013. GBD arrow diagram, Saudi Arabia. Risk of deaths 1990-2010. Institute for Health Metrics and Evaluation, University of Washington, Seattle, Wash, USA.
12. Krishnaraju, A.V., T.V.N. Rao, D. Sundararaju, M. Vanisree, H.S. Tsay and G.V. Subbaraju, 2006. Biological screening of medicinal plants collected from Eastern Ghats of India using *Artemia salina* (Brine Shrimp Test). Int. J. Applied Sci. Eng., 4: 115-125.
13. Hopkins, A.L., M.G. Lamm, J.L. Funk and C. Ritenbaugh, 2013. *Hibiscus sabdariffa* L. in the treatment of hypertension and hyperlipidemia: A comprehensive review of animal and human studies. Fitoterapia, 85: 84-94.
14. Mossa, J.S., M.A. Al Yahys, I.A. Al Meshal and M. Tariq, 1983. Phytochemical and biological screening of Saudi medicinal plants. Part 5. Fitoterapia, 54: 147-152.
15. Olaniyan, M.F., 2014. Effect of liquid extract of pear avocado leaf (*Persea Americana*) on plasma levels of aminotransferases, cholesterol and total bile acids in hypertensive patients. Am. J. Med. Med. Sci., 4: 87-91.
16. Gasparotto, Jr. A., F.M. Gasparotto, E.L.B. Lourenco, S. Crestani and M.E.A. Stefanello *et al*., 2011. Antihypertensive effects of isoquercitrin and extracts from *Tropaeolum majus* L.: Evidence for the inhibition of angiotensin converting enzyme. J. Ethnopharmacol., 134: 363-372.
17. Rahman, M.A., J.S. Mossa, M.S. Al-Said and M.A. Al-Yahya, 2004. Medicinal plant diversity in the flora of Saudi Arabia 1: A report on seven plant families. Fitoterapia, 75: 149-161.
18. Misar, A., R. Bhagat and A.M. Mujumdar, 2007. Antidiarrhoeal activity of *Acacia nilotica* Willd. bark methanol extract. Hindustan Antibiot. Bull., 49: 14-20.

19. Sharma, A., M. Shetty, A. Parida, S. Adiga and S. Kamath, 2014. Effect of ethanolic extract of *Acacia auriculiformis* leaves on learning and memory in rats. Pharmacogn. Res., 6: 246-250.
20. Chang, S.T., J.H. Wu, S.Y. Wang, P.L. Kang, N.S. Yang and L.F. Shyur, 2001. Antioxidant activity of extracts from *Acacia confusa* bark and heartwood. J. Agric. Food Chem., 49: 3420-3424.
21. Abdel-Farid, I.B., M.G. Sheded and E.A. Mohamed, 2014. Metabolomic profiling and antioxidant activity of some *Acacia* species. Saudi J. Biol. Sci., 21: 400-408.
22. Arise, R.O., A.I. Ganiyu and O.O. Oguntibeju, 2014. Lipid Profile, Antidiabetic and Antioxidant Activity of *Acacia ataxacantha* Bark Extract in Streptozotocin Induced Diabetic Rats. In: Antioxidant-Antidiabetic Agents and Human Health, Oguntibeju, O. (Ed.)., InTech, London, ISBN: 978-953-51-1215-0.
23. Alharbi, W.D.M. and A. Azmat, 2011. Hypoglycemic and hypocholesterolemic effects of *Acacia tortilis* (Fabaceae) growing in Makkah. Pak. J. Pharmacol., 28: 1-8.
24. Alharbi, W.D.M. and A. Azmat, 2014. Diazepam like effects of herbal extract acacia tortilis growing in KSA on locomotor activity in mice. World J. Pharm. Res., 3: 1216-1222.
25. Alharbi, W.D.M. and A. Azmat, 2015. Anticonvulsant and neuroprotective effects of the *Acacia tortilis* growing in KSA. Pak. J. Pharm. Sci., 28: 531-534.
26. Ayinde, B.A., E.K. Omogbai and F.C. Amaechina, 2007. Pharmacognosy and hypotensive evaluation of *Ficus exasperata* Vahl (Moraceae) leaf. Acta Pol. Pharm., 64: 543-546.
27. Nath, P. and A.K. Yadav, 2015. Acute and sub-acute oral toxicity assessment of the methanolic extract from leaves of *Hibiscus rosa-sinensis* L. in mice. J. Int. Ethnopharmacol., 4: 70-73.
28. Ahmed, M., A. Azmat and S. Khan, 2017. Acute hypotensive and diuretic activities of *Berberis vulgaris* root bark aqueous extract in normal rats. Braz. J. Pharm. Sci., Vol. 53, No. 4. 10.1590/s2175-97902017000417077.
29. Erdfelder, E., F. Faul and A. Buchner, 1996. GPOWER: A general power analysis program. Behav. Res. Methods Instrum. Comput., 28: 1-11.
30. Mukhtar, M.H., W.H. Almalki, A. Azmat, M.R. Abdalla and M. Ahmed, 2017. Evaluation of anti-diabetic activity of *Acacia tortilis* (Forssk.) hayne leaf extract in streptozotocin-induced diabetic rats. Int. J. Pharmacol., 13: 438-447.
31. Sham, J.S.K., K.W. Chiu and P.K.T. Pang, 1984. Hypotensive action of *Acacia catechu*. Planta Med., 50: 177-180.
32. Gilani, A.H., F. Shaheen, M. Zaman, K.H. Janbaz, B.H. Shah and M.S. Akhtar, 1999. Studies on antihypertensive and antispasmodic activities of methanol extract of *Acacia nilotica* pods. Phytother. Res., 13: 665-669.
33. Alkarib, S.Y., A.M. Saeed, S.A. Khalid, E.A. Groun and M.B. Ghalib, 2016. Gum Arabic role in high blood pressure among patients with stage III chronic kidney disease. J. Pharmacol. Clin. Res., 1: 1-6.
34. Alharbi, W.D.M. and A. Azmat, 2016. Pharmacological evidence of neuro-pharmacological activity of *Acacia tortilis* leaves in mice. Metab. Brain Dis., 31: 881-885.
35. Dos Santos, R.G., E. Grasa, M. Valle, M.R. Ballester and J.C. Bouso *et al*., 2012. Pharmacology of ayahuasca administered in two repeated doses. Psychopharmacology, 219: 1039-1053.
36. Greene, S.L., 2013. Tryptamines. In: Novel Psychoactive Substances Classification, Pharmacology and Toxicology, Dargan, P.I. and D.M. Wood (Eds.)., Chapter 15. Academic Press, UK., pp: 363-381.
37. Crowch, C.M. and E.J. Okello, 2009. Kinetics of acetylcholinesterase inhibitory activities by aqueous extracts of *Acacia nilotica* (L.) and *Rhamnus prinoides* (LHr.). Afr. J. Pharm. Pharmacol., 3: 469-475.
38. Sharma, C., K.R. Aneja, P. Surain, R. Dhiman, P. Jiloha, V. Meashi and M. Kaur, 2014. *In vitro* evaluation of anti-microbial spectrum of *Acacia nilotica* leaves and bark extracts against pathogens causing otitis infection. J. Innovative Biol., 1: 51-56.
39. Lefkowitz, R.J., B.B. Hoffman and P. Taylor, 1998. Neurotransmission: The Autonomic and Somatic Motor Nervous Systems. In: Goodman and Gilman's the Pharmacological Basis of Therapeutics, Hardman, J.G. and L.E. Limbird (Eds.)., McGraw-Hill, New York, pp: 105-139.
40. Brown, J.H. and P. Taylor, 1998. Muscarinic Receptor Agonists and Antagonists. In: Goodman and Gilman's the Pharmacological Basis of Therapeutics, Hardman, J.G. and L.E. Limbird (Eds.)., McGraw-Hill, New York, pp: 141-160.