doi.org/10.36721/PJPS.2020.33.5.SUP.2341-2345.1

Evaluation of effect of *Acacia jacquemontii* Benth. on blood pressure in normotensive and fructose induced hypertensive sprague dawley rats: An ethnopharmacological approach

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Abstract: *Acacia jacquemontii* Benth. is used traditionally to treat hypertension but no scientific literature supports this claim. So, this study was aimed at validating this claim. This was done by injecting various doses of crude extract of *Acacia jacquemontii*, AJC (5, 10, 20, 30mg/kg) and all fractions (hexane, ethyl acetate, n-butanol and aqueous) (3, 5, 10, 20mg/kg) intravenously in anaesthetized rat. Based on the results, butanol fraction (AJB) at 20mg/kg was found to be the most potent, so it was selected for exploring mechanisms of action. For this purpose, different groups were injected with various pharmacological inhibitors (L-NAME, atropine, captopril, propranolol and hexamethonium) prior to AJB administration. Also, AJB at 20mg/kg was evaluated for prolonged hypotensive effect for the period of 40 min. Results showed a significant dose dependent reduction in BP in normotensive and in hypertensive rats. AJC and AJB produced a decline in SBP, DBP and MAP with $p<0.05$ - $p<0.001$ and $p<0.001$ respectively in normotensive animals. Whereas in hypertensive animals, AJC showed significant reduction at 5mg/kg with $p<0.01$ and at 10, 20 and 30 mg/kg with $p<0.001$. AJB produced a decline in hypertensive animals at all tested doses with $p<0.001$. AJB resulted in hypotensive effect mediated by β receptors, ganglionic block operating central sympathetic neural responses and renin angiotensin aldosterone system (RAAS). This study supports the ethnomedicinal claim of *Acacia jacquemontii* Benth. in treating hypertension.

Keywords: *Acacia jacquemontii*, hypotension, cardiovascular diseases, RAAS, β receptors.

INTRODUCTION

Hypertension is a major risk factor for cardiovascular diseases (CVDs) that contributes greatly to increasing rates of mortality. During 2000, 26.4% adults suffered from hypertension, which is expected to increase up to 29.2% by 2025. High prevalence of hypertension is so alarming that, according to a survey, heart diseases would become the leading cause of mortality in the coming years (Benjamin *et al*., 2017). It is treated with ACE inhibitors, calcium channels blockers, diuretics and beta-blockers. Also, plants are being used traditionally to treat cardiovascular ailments. Scientific evidence supports the importance of nutrients and dietary supplements in the management of hypertension (Reddy and Katan, 2004). Pakistan is a land of medicinal plants which are used traditionally by the native people. *Acacia jacquemontii* Benth., member of Fabaceaae (Sankhla *et al*., 2017), is the plant native to Pakistan (Khan *et al.,* 2012) and is used traditionally to treat various CVDs including hypertension (Ahmad *et al.,* 2014). Although used traditionally but no scientific data supports its claim. Therefore, this study was undertaken to explore the hypotensive effects of *Acacia jacquemontii* and to delineate the hypotensive mechanism of the most potent fraction of this plant i.e. butanol fraction.

MATERIALS AND METHODS

Drugs and Chemicals

Ethanol, n-hexane, ethyl acetate, n-butanol, dichloromethane, fructose, heparin, thiopental sodium, N(G)-Nitro-L-arginine methyl ester (L-NAME), atropine, captopril, propranolol and hexamethonium were obtained from Sigma–Aldrich Chemical Co. (St- Louis, MO,USA). All chemicals were of analytical grade.

Collection, extraction and fractionation of the plant

Aerial parts of the plant were collected from Shekhupura (Punjab), Pakistan in March 2016. Plant was authenticated by Prof. Dr. Zaheer-ur-Din, Department of Botany, Government College University, Lahore, Pakistan (Identification No.GC.Herb.Bot. 3476). Dried plant (5 kg) was comminuted to fine powder and crude hydro-ethanolic extract (AJC) was obtained by cold maceration process (Cifuentes *et al*., 2016). Percentage yield of AJC was 29%. Sequential fractions were obtained by dissolving 100 gm AJC into solvent of increasing polarity; 12gm n-hexane (AJH), 14gm ethyl acetate

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(AJE), 25gm butanol (AJB) and 23gm aqueous (AJA) fractions were obtained (Khan *et al*., 2017). Dichloromethane fraction (AJD) was obtained in a negligible amount so this fraction was not used further.

Experimental Animals

Local strain of adult Sprague-Dawley rats (180-300gm) were kept under standard conditions of temperature (25±1°C) and humidity (40-60%). Standard diet was provided with water *ad libitum*. Guide for the Care and Use of Laboratory Animals (NIH publication # 85-23, revised in 1985) was followed. Study design was approved by Animal Ethical Committee of University of Sargodha (No. IAEC/UOS/ 2016/47).

Pharmacological Investigations

Direct measurement of BP in anaesthetized rats

Procedure recommended by Khan and Kumar, 2017 was followed with some revisions. Briefly, animals were anesthetized by injecting thiopental sodium (i.p; 80 mg/kg). First, trachea was exposed and cannulated to facilitate respiration. Next, jugular vein and carotid artery were cannulated with PE-50 catheter for drug administration and for recording BP, respectively. Catheter inserted into carotid artery was connected to power-lab data acquisition system and an application program (Chart, V, 4.1; ADI Instruments, Australia). Following surgery, animals were allowed to stabilize for 30-45 minutes before taking any readings (Tolouei *et al*., 2019).

Hypotensive dose response effect of A. jacquemontii

Rats were divided into seven groups (n=6) in the following order: Group I: 0.9% NaCl; Group II: captopril (2.5mg/kg); Group III: AJC (5, 10, 20, 30mg/kg); Group IV: AJH (3, 5, 10, 20mg/kg); Group V: AJE (3, 5, 10, 20mg/kg); Group VI: AJB (3, 5, 10, 20mg/kg); Group VII: AJA (3, 5, 10, 20mg/kg). SBP, DBP, MAP and HR were recorded after extract administration (i.v; 1 ml/kg). Interval of 15-20 min was given in between the doses. All the doses were prepared freshly and sterilized with a sterile syringe filter (0.22 µm pore size) before dosing. After experiment, rats were euthanized with the overdose of thiopental sodium. Based on results, AJB was found to have most pronounced effect. So, this fraction (AJB) at 20mg/kg was selected for further experimentations. In a separate set of experiment, delayed effect of a selected single dose of AJC and AJB was recorded for 40 min.

Anti-hypertensive dose response effect of A. jacquemontii

Hypertension was induced by treating rats orally with 10% fructose for 6 consecutive weeks. Procedure described earlier was adopted and same grouping schedules were made. Changes in SBP, DBP, MAP and HR were measured after extract administration.

Mechanism underlying hypotensive effect of A. jacquemontii

Rats were divided into seven groups (n=6); Group I: 0.9% NaCl; Group II: AJB (20mg/kg); Group III: Atropine (1mg/kg) + AJB (20mg/kg); Group IV: Captopril (2.5mg/kg) + AJB (20mg/kg), Group V: L-NAME (20mg/kg) + AJB (20mg/kg); Group VI: Propranolol (100mg/kg) + AJB (20mg/kg); Group VII: Hexamethonium (30mg/kg) + AJB (20mg/kg).

Procedure described above was adopted, different groups received iv injection of pharmacological antagonists 10 min before AJB administration. Changes in SBP, DBP, MAP and HR were recorded for 45 minutes post treatment (Shih *et al*., 2008).

STATISTICAL ANALYSIS

Values were expressed as mean ± S.E.M. One-way ANOVA was applied to calculate changes in baseline and in the presence of agonist/antagonist followed by Bonferroni's posthoc test. Effects of extract and fraction were assessed using two-way ANOVA and Dunnett's posthoc test. $P<0.05$ was considered significant. Statistics was applied using Graph pad prism 5.0.

RESULTS

Acacia jacquemontii produced acute hypotensive effect in anesthetized rats

Baseline values of SBP, DBP, MAP and HR were 125±5.30, 115±5.18, 118±5.13 mm-Hg and 265±7.11 bpm respectively. Administration of vehicle (NaCl) did not affect the basal values. Compared to vehicle control, treatment with AJC ($p<0.05$ - p0.001) and AJB ($p<0.001$) produced a significant reduction in SBP, DBP and MAP (fig. 1a,b). Non-significant changes were produced in heart rate. AJE, AJH, AJA did not produce any significant changes.

AJC and AJB produced persistent hypotensive effect in anesthetized normotensive rats

AJC and AJB at 20mg/kg produced a persistent hypotensive effect and shifted the baseline down as shown in fig. 2 (a,b).

AJC and AJB produced acute anti-hypertensive effect in anesthetized rats

Average baseline value of SBP, DBP, MAP and HR were 162.52±4.97, 152.84±4.86, 156.37±4.88 mm-Hg and 355.35±26.91 bpm respectively. NaCl did not produce any significant changes. Compared to NaCl, AJC and AJB showed significant anti-hypertensive effect; AJC at 5mg/kg ($p<0.01$) and at 10, 20 and 30 mg/kg ($p<0.001$) (fig 3a), AJB produced a decline in BP with $p<0.001$ (fig 3b). HR remained unchanged. AJE, AJH and AJA did not produce any significant changes.

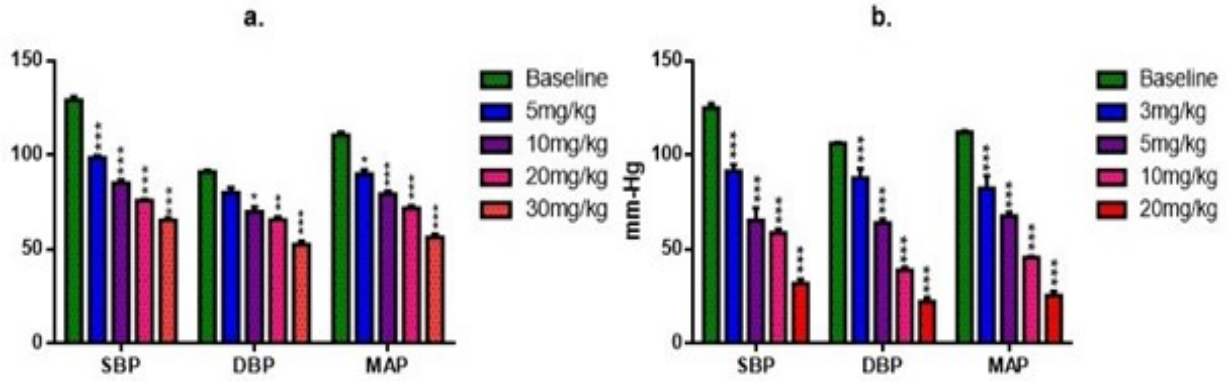

Fig. 1: Effect of various doses of a) AJC b) AJB in anesthetized normotensive rats on SBP, DBP and MAP. Results are expressed as mean ± S.E.M (n=6). whereas, *= p<0.05, **= p<0.01 and *** =p<0.001, as compared to baseline (normal saline treated) group.

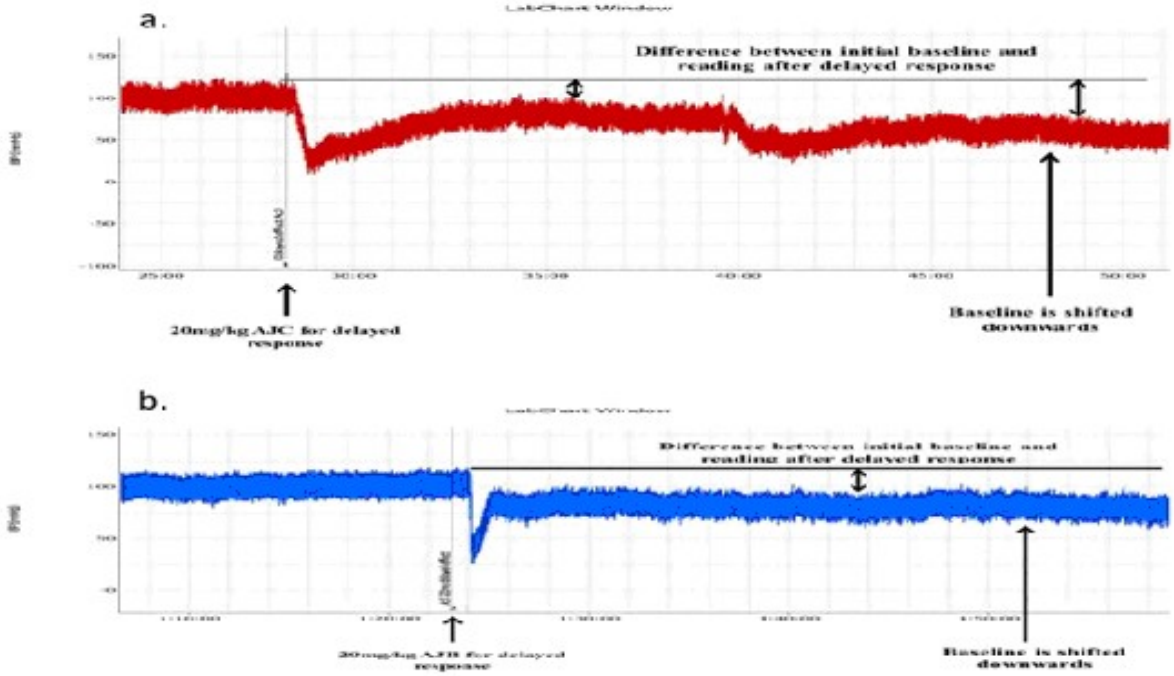

Fig. 2: Original tracing of persistent response of AJC and AJB at 20mg/kg over 45 minutes.

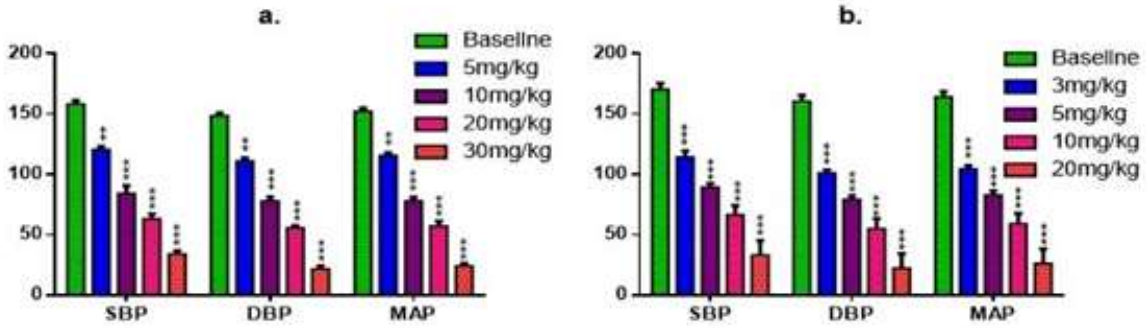

Fig. 3: Effect of various doses of a) AJC b) AJB in hypertensive rats on SBP, DBP and MAP. Results are expressed as mean ±S.E.M. whereas, **=p<0.01 and ***=p<0.001 compared to baseline normal saline treated group).

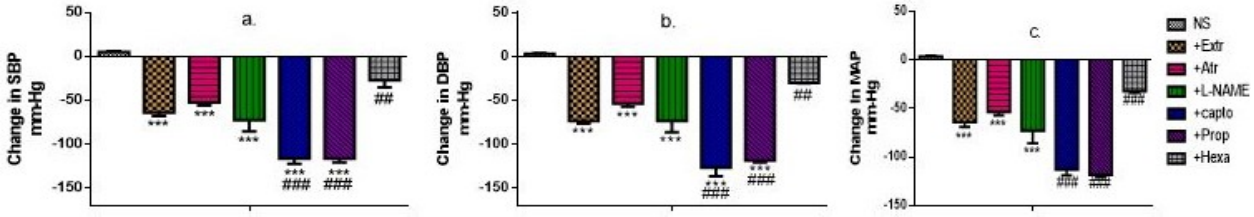

Fig. 4: Effect of AJB at 20 mg/kg on (a) systolic BP, (b) diastolic BP and (c) Mean arterial BP in anesthetized rats pre-treated with different antagonists. Results are expressed as mean ± S.E.M. ***= p<0.001 compared to NaCl treated group, while ###= p<0.001 compared to AJB at 20 mg/kg.

AJB resulted in hypotensive effect mediated by various mechanisms

AJB resulted in hypotensive response that was significantly potentiated in the presence of propranolol and captopril; diminished in the presence of hexamethonium and remained unaltered in the presence of L-NAME and atropine (fig. 4).

DISCUSSION

Since prehistoric civilization, plants are used to treat several ailments (Sharif *et al*., 2018). Owing to this, indigenous medicinal plant of Pakistan was selected for scientific validation based on its traditional use. Results of this study confirmed that crude extract of *Acacia jacquemontii* (AJC) lowers blood pressure significantly in normotensive and in fructose fed hypertensive rats. Various fractions were evaluated for their hypotensive and anti-hypertensive effects and butanol fraction (AJB) was found to be the most active. Therefore, this fraction was tested to delineate mechanisms underlying hypotensive effects. Literature shows that various mechanisms can be involved in hypotensive effect of plants (Katoriand Majima, 2014). To find the role of various mediators in producing hypotensive effect, active fractions (AJB) was assessed in the presence of captopril (RAAS inhibitor), propranolol (β-blocker), hexamethonium (ganglionic blocker), atropine (muscarinic blocker) and L-NAME (nitric oxide synthesis inhibitor). Effect of AJB was not altered in the presence of atropine and L-NAME but in the presence of hexamethonium it was diminished suggesting that muscarinic pathway and NO has no involvement while ganglionic block operating central sympathetic neural responses might be accountable for hypotensive effect of AJB. This behavior of AJB against muscarinic receptors were also documented by Gilani *et al*., 1999 for another species of acacia, *Acacia nilotica*. Propranolol, however, boosted the hypotensive effect of AJB. Augmentation of the response in the presence of propranolol may be due to synergistic effect suggesting the involvement of β-adrenoceptor in producing vasodilatory responses. RAAS has a vital role in maintaining renal and cardiovascular homeostasis. In this study effect of AJB was potentiated in the presence of captopril suggesting the effect may be due to ACE inhibition (Miyazaki and Takai, 2006). Numerous studies on species of acacia confirmed the presence of quercetins (Jain *et al*., 2007; Gulzar *et al*., 2015; Roy *et al*., 2012; Al-Nour, Ibrahim, & Elsaman, 2019). Also, Abigail *et al*., 2010 validated ACE inhibition effects of quercetins against hypertension. So, it can be said that ACE inhibition effect of AJB might be due to the presence of quercetins. Flavonoids is an important class of phyto-constituents that possesses many pharmacological activities. Phytochemical investigation of other species of Acacia showed the presence of many flavonoid (Raghavendra *et al*., 2006). We can say that hypotensive effect of AJB might also be due to the presence of flavonoids. Nobutomo *et al.,* 2018 studied hypotensive effects of polyphenols found in acacia. We can say that this phytoconstituent might also be responsible in the hypotensive potential of AJB.

CONCLUSION

Data presented in this study provides scientific background to the hypotensive and antihypertensive role of butanolic fraction of *Acacia jacquemontii* Benth. Probable hypotensive mechanisms are also proposed in this research work.

REFERENCES

Ahmad M, Sultana S, Fazl-I-Hadi S, Ben Hadda T, Rashid S, Zafar M, Khan MA, Khan MPZ and Yaseen G (2014). An Ethnobotanical study of Medicinal Plants in high mountainous region of Chail valley (District Swat-Pakistan). *J. Ethnobiol. Ethnomed*., **10**(1): 36.

Al-Nour MY, Ibrahim MM and Elsaman T (2019). Ellagic acid, Kaempferol, and Quercetin from Acacia nilotica: Promising combined drug with multiple mechanisms of action. *Curr. Pharmacol. Rep*., **5**(4): 255-280.

Ayoub, SMH (1984). Polyphenolic molluscicides from Acacia nilotica. *Planta medica*., **50**(06): 532-532.

Benjamin EJ, Blaha MJ and Chiuve SE (2017). Cardiovascular disease remains leading cause of death in U.S. In: CASEY, T. (ed.) Heart Disease and Stroke Statistics Update. United States: American Heart Association.

Gilani AH, Shaheen F, Zaman M, Janbaz KH, Shah BH and Akhtar MS (1999). Studies on antihypertensive and antispasmodic activities of methanol extract of Acacia nilotica pods. *Phytotherapy Research: Intl. J. Dev. Pharmacol. Toxicol. Eval. Nat. Prod. Der*., **13**(8): 665-669.

Gulzar T, Adeel S, Hanif I, Rehman F, Hanif R, Zuber M and Akhtar N (2015). Eco friendly dyeing of gamma ray induced cotton using natural quercetin extracted from acacia bark (*A. nilotica*). *J. Nat. Fibers*., **12**(5): 494-504.

Gulzar T, Adeel S, Hanif I, Rehman F, Hanif R, Zuber M, and Akhtar N (2015). Eco-friendly dyeing of gamma ray induced cotton using natural quercetin extracted from acacia bark (*A. nilotica*). *J. Natural Fibers.*, **12**(5): 494-504.

Jain R, Patni V and Arora DK (2007). Isolation and identification of flavonoid quercetin from *Acacia catechu* (Lf) Willd-A Katha yielding plant. *J. Phytol. Res.*, **20**(1): 43-45.

Kalsom YU, Khairudddin HI and Zakri MM (2001). Flavonol glycoside from the leaves of *Acacia mangium* and related species. *Malaysian J. Anal. Sci*., **17**(1): 109-112.

Katori M and Majima M (2014). Renal (tissue) kallikrein-kinin system in the kidney and novel potential drugs for salt sensitive hypertension. *Prog Drug Res*., **69**: 59-109.

Khan MY and Kumar V (2017). Mechanism of antihypertensive effect of *Mucuna pruriens* L. seed extract and its isolated compounds. *J Complement Integr Med*., **21**(4):14-4.

Khan R, Srivastava R, Khan MA, Alam P and Abdin MZ (2012). Variation in oil content and fatty acid composition of the seed oil of Acacia species collected from the northwest zone of India. *J. Sci. Food. Agr*., **92**(11): 2310-2315.

Leela V, Saraswathy A and Ravikumar R (2011). Estimation of quercetin in *Acacia nilotica* Linn. (Flowers) using HPTLC. *J. Pharm. Res*., **4**(3): 818.

Majewska-Wierzbicka M and Czeczot H (2012). Flavonoids in the prevention and treatment of cardiovascular diseases. *Pol. Merkur. Lekarski*., **32**(187): 50-54.

Miyazaki M and Takai S (2006). Tissue angiotensin II generating system by angiotensin converting enzyme and chymase. *J. Pharmacol. Sci*., **100**(5): 391-397.

Patwardhan B, Vaidya AD and Chorghade M (2004). Ayurveda and natural products drug discovery. *Cur Sci.*, **86**(6): 789-799.

Raghavendra MP, Satish S and Raveesha KA (2006). *In vitro* evaluation of anti-bacterial spectrum and phytochemical analysis of *Acacia nilotica. Agr Sci Tech-Iran*., **2**(1): 77-88.

Raj V, Rai A and Singh M (2017). Detection of bioflavonoids from methanol bark extracts of Acacia and their role as antidiarrhoeal agents. *J. Anal. Pharm Res*., **5**(4): 1-7.

Rather LJ and Mohammad F (2015). Study on the application of *Acacia nilotica* natural dye to wool using fluorescence and FT-IR spectroscopy. *Fiber Polym*., **16**(7): 1497-1505.

Reddy KS and Katan MB (2004). Diet, nutrition and the prevention of hypertension and cardiovascular diseases. *Public Health Nutr*., **7**(1a): 167-186.

Roy A, Lakshmi T and Geetha RV (2012). Estimation of Quercetin in *Acacia catechu* ethanolic bark extract by HPLC method. *Int. J. Pharmtech Res.,* **4**(1): 501-505.

Sankhla IS, Tak N, Meghwal RR, Choudhary S, Tak A, Rathi S, Sprent JI, James EK and Gehlot HS (2017). Molecular characterization of nitrogen fixing microsymbionts from root nodules of Vachellia (Acacia) jacquemontii, a native legume from the Thar Desert of India. *Plant and Soil*., **410**(1-2): 21-40.

Sharif A, Asif H, Younis W, Riaz H, Bukhari IA and Assiri AM (2018). Indigenous medicinal plants of Pakistan used to treat skin diseases: A review. *Chin Med*., **13**(1): 52.

Shih CD, Kuo DH, Huang CW, Gu YH and Chen FA (2008). Autonomic nervous system mediates the cardiovascular effects of *Rhodiola sacra* radix in rats. *J Ethnopharmacol*., **119**(2): 284-290.

Tolouei SEL, Palozi RAC, Tirloni CAS, Marques AAM, Schaedler MI, Guarnier LP and dos Santos AC (2019). Ethnopharmacological approaches to *Talinum paniculatum* (Jacq.) Gaertn.-Exploring cardiorenal effects from the Brazilian Cerrado. *J. Ethnopharmacol*., **238**(2019): 111873.