EVALUATION OF *NEOLAMARCKIA CADAMBA* (ROXB.) BOSSER LEAF EXTRACT ON GLUCOSE TOLERANCE IN GLUCOSE-INDUCED HYPERGLYCEMIC MICE

Firoz Ahmed*, Shahnaz Rahman, Nasir Ahmed, Maraz Hossain, Anup Biswas, Sanjoy Sarkar, Hasanul Banna, Mst. Afsana Khatun*, Majeedul H. Chowdhury, Mohammed Rahmatullah*****

Faculty of Life Sciences, University of Development Alternative, House No. 78, Road No. 11A, Dhanmondi, Dhaka-1205, Bangladesh *Dept. of Pharmacy, Lincoln College, Mayang Plaza, Block A, No 1, Jalan SS 26/2, Taman Mayang Jaya, 47301, Petaling Jaya, Selangor Darul Ehsan, Kuala Lumpur, Malaysia, **New York City College of Technology, The City University of New York 300 Jay Street, Brooklyn, NY 11201, USA.
***Email:** rahamatm@hotmail.com

Abstract

Neolamarckia cadamba (Rubiaceae) leaf is used in folk medicine of Bangladesh for the treatment of diabetes, but so far no scientific study has been done which may support its use in traditional medicine. The present study was carried out to evaluate the possible glucose tolerance efficacy of methanolic extract of *Neolamarckia cadamba* leaf using glucose-induced hyperglycemic mice. The extract at different doses was administered one hour prior to glucose administration and blood glucose level was measured after two hours of glucose administration (p.o.) using glucose oxidase method. The statistical data indicated significant oral hypoglycemic activity on glucose-loaded mice at the two highest doses of 200 and 400 mg extract per kg body weight. Maximum anti-hyperglycemic activity was shown at 400 mg per kg body weight, which was comparable to that of , glibenclamide (10 mg/kg). The methanolic extract of leaf of *Neolamarckia cadamba* had beneficial effects in reducing the elevated blood glucose level of hyperglycemic mice.

Keywords: *Neolamarckia cadamba*; Hypoglycemic activity; Serum glucose level; Glibenclamide

Introduction

Neolamarckia cadamba (Roxb.) Bosser (Rubiaceae), known as Kodom in the Bengali language, is grown commonly in different parts of Bangladesh. It is an evergreen tropical tree found in Bangladesh, Nepal, India, Myanmar, Sri Lanka, the Philippines, Indonesia, and Papua New Guinea (Banerji, 1977; 1978). The tree can grow up to 45 meters high. Various parts of the plant have traditional uses as an anti-diuretic, and for the treatment of fever, anemia and tumor as well as for the improvement of semen quality (Umachigi et al., 2007; Dr. Duke's Phytochemical and Ethnobotanical Databases, 2007). Previous bioactivity studies have revealed its antimicrobial, antioxidant, and wound healing (Umachigi et al., 2007) as well as anti-diarrheal properties (Alam et al., 2008). Phytochemical investigations have resulted in the isolation of saponins, indole and quinoline alkaloids, secoiridoids and triterpenes from this plant (Banerji, 1977; 1978; Brown and Chapple, 1976; Handa et al., 1984; Kitagawa et al., 1996; Sahu et al., 1999). The leaf of this tree is used by the folk medicinal practitioners of Bangladesh for controlling sugar level in diabetic patients. This information suggests that this plant may possess some anti-diabetic activities. No scientific work has been conducted to justify this hypothesis. The present study was carried out to evaluate the glucose lowering potential of methanolic leaf extract of *Neolamarckia cadamba* in glucose-induced hyperglycemic mice.

Materials and methods

Collection of plant material

The leaves of *Neolamarckia cadamba* were collected during June 2009 from Savar, Dhaka, Bangladesh. The leaves were identified by the Bangladesh National Herbarium, Mirpur, Dhaka (Accession No. 34,976) and a sample specimen has been kept there.

Preparation of the test samples

The dried leaves (air-dried for 5 days) of *Neolamarckia cadamba* were pulverized into a fine powder and were mixed with methanol at a ratio of 1:3 (w/v, 100g powder in 300 ml methanol). After 24 hours, the mixtures were filtered; filtrate was collected and the residue was again mixed with methanol at a ratio of 1:2 (w/v, 100g powder in 200 ml methanol) for 24 hrs. After filtration, filtrates were combined and evaporated to dryness (approximate yield 10.1%) using rotary evaporator. Extracts were suspended in 1% Tween 80 in water prior to administration.

Preliminary phytochemical screening

Preliminary phytochemical screening (Kokate, 1994; Harborne, 1998) revealed the presence of presence of saponins, alkaloids, glycosides, and tannins.

Animals

Swiss albino mice (male), weighing 25-30g bred in the animal house of ICDDR,B (International Centre for Diarrheal Disease and Research, Bangladesh) were used for the experiments. All the animals were acclimatized one week prior to the experiments. The animals were housed under standard laboratory conditions (relative humidity 55-65%, room temperature 25.0 ± 2^0C, and 12 hrs light dark cycle). The animals were fed with standard diet from ICDDR,B and had free access to water. The study was approved by the Institutional Animal Ethical Committee of the University of Development Alternative, Dhaka, Bangladesh.

Studied activity

Glucose tolerance test was performed following the procedure as described by Joy and Kuttan (1999) with slight modifications. In brief, fasted mice were divided into six groups of six mice each. Each group received a particular treatment. GroupI served as control and received vehicle (1% Tween 80 in water, 10 $ml.kg^{-1}$ body weight), groupII received standard drug (glibenclamide, 10 $mg.kg^{-1}$ body weight)(Venkatesh et al., 2004) and the four other groups (groups III-VI) received the extract at four different doses of 50, 100, 200 and 400 mg $extract.kg^{-1}$ body weight, respectively. The extract, control, and glibenclamide were given orally. After one hour, all mice were orally treated with 2 $g.kg^{-1}$ of glucose. Blood samples were collected two hours after glucose administration. Serum was separated and blood glucose levels were measured immediately by glucose oxidase method (Venkatesh et al., 2004). The extract up to doses of 1000 $mg.kg^{-1}$ did not show any toxic effects (data not shown).

Statistical analysis

Experimental values are expressed as Mean ± S.E.M. Independent Sample t-test was carried out for statistical comparison. Statistical significance was considered at p value < 0.05 .

Table 1: Effect of methanolic extract of *Neolamarckia cadamba* leaf on serum glucose level in hyperglycemic mice.

Group (n=6)	Treatment	Serum Glucose level (mg/dl)	% inhibition
I	Control	103.5 ± 4.2	-
II	Standard	67.1 ± 2.3*	35.2
III	Extract (50 mg/kg)	100.4 ± 3.8	3.0
IV	Extract (100 mg/kg)	93.4 ± 8.2	9.8
V	Extract (200 mg/kg)	79.8 ± 6.6*	22.9
VI	Extract (400 mg/kg)	78.5 ± 2.5*	24.2

Extracts and drug were given orally one hour before glucose administration and serum glucose level was measured two hours after glucose administration. Values are given as Mean ± S.E.M. from six mice in each group. * $P < 0.05$ is significant compared to hyperglycemic control animals.

Results and Discussion

The results obtained from this study indicate that the methanol extract of the leaf of *Neolamarckia cadamba* lowered serum glucose levels when compared to control (group-I) in a dose dependent manner. The lowering of glucose, however, was

found to be statistically significant only at the two highest doses of 200 and 400 mg extract.kg^{-1} body weight. Maximum hypoglycemic activity of methanol extract of *Neolamarckia cadamba* leaves in glucose-induced hyperglycemic mice was observed with a 400 mg.kg^{-1} dose (24.2%), while the standard drug, glibenclamide produced 35.2 % inhibitory activity at 10 mg.kg^{-1} dose (Table 1). The present preliminary experimental results indicated that *Neolamarckia cadamba* exhibited significant blood glucose lowering property in glucose-induced hyperglycemic mice. Saponins present in the crude extract may be responsible for the observed pharmacological activity (Xi et al., 2010; Bhavsar et al., 2009). A further exploration of the bioactive molecules exactly responsible for this activity is currently under investigation. The mechanism underlying the glucose lowering efficacy of *Neolamarckia cadamba* is yet to be established but we can speculate that the glucose lowering activity of the extract may be by potentiating the pancreatic secretions of insulin or increasing the glucose uptake. The obtained results provide pharmacological evidence and validate the folklore claim of *Neolamarckia cadamba* leaves as an antidiabetic agent.

Acknowledgements

The authors are thankful to the University of Development Alternative for funding this study and to Bangladesh National Herbarium for plant identification.

References

1. Alam, M.A., Akter, R., Subhan, N., Rahman, M.M., Majumder, M.M., Nahar, L., and Sarker, S.D. (2008). Antidiarrhoeal property of the hydroethanolic extract of the flowering tops of *Anthocephalus cadamba*. Revista Brasileira de Farmacognosia. **18:**155-159.
2. Banerji, N. (1977). New saponins from stem bark of *Anthocephalus cadamba* MIQ. Indian Journal of Chemistry B. **15:**654-655.
3. Banerji, N. (1978). Structure of 2 new saponins from stem bark of *Anthocephalus cadamba* MIQ. Journal of Indian Chemical Society. **55:**275-278.
4. Bhavsar, S.K., Föller, M., Gu, S., Vir, S., Shah, M.B., Bhutani, K.K., Santani, D.D., and Lang, F. (2009). Involvement of the PI3/AKT pathway in the hypoglycemic effects of saponins from *Helicteres isora*. J Ethnopharmacol. **126:**386-396.
5. Brown, R.T., and Chapple, C.L. (1976). *Anthocephalus* alkaloids: cadamine and isocadamine. Tetrahedron Letters. **19:**629-630.
6. Dr. Duke's Phytochemical and Ethnobotanical Database (2007). ARS, National Genetic Resources program. Germplasm Resources Information Network – (GRIN), National Germplasm Resources Laboratory, Beltsville, Maryland. Available online at http://www.ars-grin.gov/cgi-bin/duke/ethnobot.pl
7. Harborne, J.B. (1998). Phytochemical Methods, Chapman & Hall, London (1998): pp. 60.
8. Joy, K.L., and Kuttan, R.J. (1999). Anti-diabetic activity of *Picrorrhiza kurroa* extract. Journal of Ethnopharmacology. **67:**143-148.
9. Handa, S.S., Gupta, S.K., Vasisht, K., Keene, A.T., and Phillipson, J.D. (1984). Quinoline alkaloids from *Anthocephalus chinensis*. Planta Medica. **50:**358.
10. Kitagawa, I., Wei, H., Nagao, S., Mahmud, T., Hori, K., Kobayashi, M., Uji, T., and Shibuya, H. (1996). Indonesian medicinal plants. XIV. Characterization of 3′-O-caffeoylsweroside, a new secoiridoid glucoside, and kelampayosides A and B, two new phenolic apioglucosides, from the bark of *Anthocephalus chinensis* (Rubiaceae). Chemical & Pharmaceutical Bulletin (Tokyo). 44:1162-1167.
11. Kokate, C.K. (1994). Practical Pharmacognosy, Vallabh Prakashan, New Delhi, pp. 105–107.
12. Sahu, N.P., Koike, K., Jia, Z.H., Achari, B., Banerjee, S., and Nikaido, T. (1999). Structure of two novel isomeric triterpene saponins from *Anthocephalus cadamba*. Magnetic Resonance in Chemistry. **37:**737-742.
13. Umachigi, S.P., Kumar, G.S., Jayaveera, K.N., Kishore, D.V.K., Ashok-Kumar, C.K., and Dhanpal, R. (2007). Antimicrobial, wound healing and antioxidant activities of *Anthocephalus cadamba*. Afr J Tradit. Complement Altern. Med. **4:**481-487.
14. Venkatesh, S., Reddy, G.D., Reddy, Y.S.R., Sathyavathy, D., Reddy, B.M. (2004). Effect of *Helicteres isora* root extracts on glucose tolerance in glucose-induced hyperglycemic rats. Fitoterapia. **75:**364-367.
15. Xi, M., Hai, C., Tang, H., Wen, A., Chen, H., Liu, R., Liang, X., and Chen, M. (2010). Antioxidant and antiglycation properties of triterpenoid saponins from *Aralia taibaiensis* traditionally used for treating diabetes mellitus. Redox Report. 15:20-28.