WORLD JOURNAL OF PHARMACY AND PHARMACEUTICAL SCIENCES
SJIF Impact Factor 6.647
Volume 6, Issue 8, 135-145 Research Article ISSN 2278 – 4357

PRELIMINARY PHYTOCHEMICAL SCREENING AND EVALUATION OF ANTIHYPERGLYCEMIC AND ANTINOCICEPTIVE EFFECTS OF A COMBINATION OF *LEUCAS ASPERA* AERIAL PARTS AND *ZINGIBER OFFICINALE* RHIZOMES

Akmal Hosain Nipu, Sahara Akter and Mohammed Rahmatullah*

Department of Pharmacy, University of Development Alternative, Lalmatia, Dhaka-1207, Bangladesh.

Article Received on
06 June 2017,
Revised on 27 June 2017,
Accepted on 18 July 2017
DOI: 10.20959/wjpps20178-9813

***Corresponding Author**
Prof. Dr. Mohammed Rahmatullah
Department of Pharmacy, University of Development Alternative, Lalmatia, Dhaka-1207, Bangladesh.

ABSTRACT

Background: *Leucas aspera* and *Zingiber officinale* are plant species commonly available in Bangladesh. Aerial parts of the first plant is consumed by the local people following cooking for alleviating pain, while the rhizomes of the second are both consumed and applied topically for blood glucose lowering and pain alleviating effects, respectively. It was thus of interest to evaluate the antihyperglycemic and antinociceptive potentials of a combination of *Leucas aspera* aerial parts and *Zingiber officinale* rhizomes. **Methods:** Antihyperglycemic activity was determined through oral glucose tolerance tests (OGTT) in mice. Antinociceptive activity was determined through reductions in number of writhings caused by intraperitoneally injected acetic acid-induced abdominal pain in mice. **Results:** Administration of methanol extract of a 1:1 (w/w) combination of aerial parts of *Leucas aspera* and rhizomes of *Zingiber officinale* led to significant and dose dependent reductions of blood glucose in mice in OGTT. At the highest dose of the combination (400 mg each of the two extracts per kg body weight) blood glucose was decreased by 54.0% versus control mice. A standard antihyperglycemic drug, glibenclamide, when administered at a dose of 10 mg per kg, decreased blood glucose by 61.2%. In antinociceptive activity tests, the combination of the two extracts at the highest dose tested (400 mg each extract per kg) reduced abdominal writhings by 65.4%. A standard antinociceptive drug, aspirin reduced abdominal writhings by 50.0%, when administered at a dose of 400 mg per kg. **Conclusion:** The two extract combination demonstrated excellent antihyperglycemic and antinociceptive properties.

KEYWORDS: Antihyperglycemic, *Leucas aspera*, *Zingiber offinale*, antinociceptive, OGTT

BACKGROUND

Diabetes mellitus is characterized by elevated blood glucose levels. The disease cannot be cured totally and progresses with time to more complications like cardiovascular, eye, and kidney disorders. Existing allopathic drugs like metformin or glibenclamide (both administered orally), as well as insulin injections can lower blood glucose but are costly and not properly available to the rural people of Bangladesh. The disease is reaching endemic proportions in Bangladesh and the average cost for treatment of merely diabetes without added complications is beyond the capability of most people.[1,2]

Pain is also a common feature of most Bangladesh people because of the hard labor involved in agricultural work, construction work or work like manually pulling rickshaws or vans with heavy loads. Acute pain also arises from other factors like sprains or even minor injuries. In addition, diseases like cancer, Chikungunya or arthritis are common in Bangladesh and these diseases can cause chronic pain.[3,5] Pain is usually treated with drugs like aspirin or paracetamol; chronic and severe pain may need opioid drugs for treatment.

Metformin is contraindicated in renal diseases or renal dysfunction; the adverse effects of metformin with other antidiabetic drugs like glimepiride or glibenclamide have been noted.[6] Pain relieving drugs like aspirin, paracetamol, or opioids can with prolonged use or overdosage lead to complications like gastric ulceration, hepatotoxicity or addiction, respectively. As such, new drugs are necessary, which can combine efficacy with affordability, availability, and preferably no adverse effects.

Since plants have always proved to be good sources for effective new drugs.[7] we had been investigating various floral species of Bangladesh individually or in combination for their blood glucose lowering and/or pain-relieving capabilities.[8,33] *Leucas aspera* (Willd.) Link (Lamiaceae) is a common plant in Bangladesh (local name – dondo kolosh), sometimes cultivated and consumed following cooking to alleviate pain, but more often found growing in the wild. We have previously reported on antinociceptive activity of *Leucas* whole plants,[34] and antihyperglycemic activity of *Leucas* leaf and stems.[35] *Zingiber officinale* Roscoe (Zingiberaceae family, local name – ada) is commonly cultivated in Bangladesh both for its medicinal value and use as a spice. In a previous report, we have shown that an herbal formulation containing rhizomes of *Zingiber officinale* and cloves of *Allium sativum* can

improve oral glucose tolerance in mice.[36] Antiinflammatory and analgesic properties have been observed with ethanol extract of rhizomes of *Z. officinale*.[37] As such, it was the objective of the present study to determine whether the combination of two commonly available and affordable plant parts in Bangladesh can lower blood glucose levels in glucose-challenged mice (oral glucose tolerance test or OGTT) and also alleviate pain (in acetic acid induced writhing test).

METHODS

Plant material collection

Rhizomes of *Z. officinale* and aerial parts of *L. aspera* were collected from a local market in Chandra, Dhaka city, Bangladesh. The rhizomes were peeled and were thoroughly dried and finely powdered prior to extraction with methanol. Aerial parts of L. aspera were dried in the shade and finely powdered prior to extraction.

Preparation of methanolic extract of plant parts

Methanolic extraction was carried out separately for rhizomes of *Z. officinale* and aerial parts of *L. aspera*. Briefly, 100g of dried powdered plant part was extracted with 500 ml methanol at room temperature with constant stirring for 48 hours. The suspension was then filtered, and methanol evaporated at 50^{o}C. Extracts were stored at -20^{o}C.

Chemicals and Drugs

Glibenclamide, glucose and aspirin were obtained from Square Pharmaceuticals Ltd., Bangladesh. All other chemicals were of analytical grade.

Animals

Swiss albino mice, which weighed between 15-18g were used in the present study. The animals were obtained from International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR, B). The animals were acclimatized for three days prior to actual experiments. The study was conducted following approval by the Institutional Animal Ethical Committee of University of Development Alternative, Dhaka, Bangladesh.

Oral glucose tolerance tests (OGTT) for evaluation of antihyperglycemic activity

Oral glucose tolerance tests were carried out as per the procedure previously described by Joy and Kuttan (1999).[38] with minor modifications. Briefly, fasted mice were grouped into six groups of five mice each. The various groups received different treatments like Group 1

received vehicle (1% Tween 20 in water, 10 ml/kg body weight) and served as control, Group 2 received standard drug (glibenclamide, 10 mg/kg body weight). Groups 3-6 received a 1:1 (w/w) combination of the two extracts (MEZO + MELA – extracts of rhizomes of *Z. officinale* and aerial parts of *L. aspera*, respectively) at doses of 50, 100, 200, and 400 mg each extract per kg body weight, respectively. All substances were orally administered. Following a period of one hour, all mice were orally administered 2g glucose/kg of body weight. Blood samples were collected 120 minutes after the glucose administration through puncturing heart. Blood glucose levels were measured by glucose oxidase method. [39] The percent lowering of blood glucose levels were calculated according to the formula described below.

Percent lowering of blood glucose level = $(1 – W_e/W_c)$ X 100, where W_e and W_c represents the blood glucose concentration in glibenclamide or (MEZO + MELA) administered mice (Groups 2-6), and control mice (Group 1), respectively.

Antinociceptive activity

Antinociceptive activity of a 1:1 (w/w) combination of MEZO and MELA was examined using previously described procedures.[40] Briefly, mice were divided into six groups of five mice each. Group 1 served as control and was administered vehicle only. Group 2 was orally administered the standard antinociceptive drug aspirin at a dose of 400 mg per kg body weight. Groups 3-6 were administered (MEZO + MELA) at doses of 50, 100, 200 and 400 mg each extract per kg body weight, respectively. Following a period of 60 minutes after oral administration of standard drug or extract, all mice were intraperitoneally injected with 1% acetic acid at a dose of 10 ml per kg body weight, which leads to pain and writhings. A period of 5 minutes was given to each animal to ensure bio-availability of acetic acid, following which period the number of abdominal constrictions (writhings) was counted for 10 min. The following formula was used for calculation of percent inhibition of the number of writhings in aspirin and (MEZO + MELA) administered animals compared to control mice,

$$\text{Percent inhibition} = (1 – W_e/ W_c) \text{ X } 100$$

where W_e and W_c represents the number of writhings in aspirin or (MEZO + MELA) administered mice (Groups 2-6), and control mice (Group 1), respectively.

Statistical analysis

Experimental values are expressed as mean ± SEM. Independent Sample t-test was carried out for statistical comparison. Statistical significance was considered to be indicated by a p value < 0.05 in all cases.[41]

Phytochemical screening of the extracts

Preliminary phytochemical screening of the two extracts, MELA and MEZO, were conducted as described previously.[42]

RESULTS

(MEZO + MELA), when administered at doses of 50, 100, 200 and 400 mg each extract showed significant and dose-dependent reductions in blood glucose levels in glucose-administered mice. At the afore-mentioned four doses, the percent reductions in blood glucose levels were, respectively, 32.3, 45.0, 51.2, and 54.0%, respectively. Glibenclamide, a standard antihyperglycemic drug, when administered at a dose of 10 mg per kg body weight, lowered blood glucose by 61.2%. The results are shown in Table 1, and suggest that the two extract combination has substantial blood glucose lowering capacity.

At doses of 50, 100, 200 and 400 mg extract each of MEZO and MELA per kg body weight, the combination reduced dose-dependently and significantly the number of writhings by 26.9, 34.6, 53.8, and 65.4%, respectively versus the 50.0% reduction in the number of writhings obtained with aspirin at 400 mg per kg. The results are shown in Table 2 and demonstrate that the extract combination possess more potent antinociceptive activity than aspirin.

Phytochemical analysis of MEZO indicated the presence of saponins, alkaloids and flavonoids. Tannins, alkaloids and flavonoids were present in MELA.

Table 1: Effect of (MEZO + MELA) on blood glucose level in hyperglycemic mice following 120 minutes of glucose loading.

Treatment	Dose (mg/kg body weight)	Blood glucose level (mmol/l)	% lowering of blood glucose level
Control	10 ml	5.82 ± 0.25	-
Glibenclamide	10 mg	2.26 ± 0.18	61.2*
(MEZO + MELA)	50 mg each	3.94 ± 0.39	32.3*
(MEZO + MELA)	100 mg each	3.20 ± 0.54	45.0*
(MEZO + MELA)	200 mg each	2.84 ± 0.37	51.2*
(MEZO + MELA)	400 mg each	2.68 ± 0.27	54.0*

All administrations were made orally. Values represented as mean ± SEM, (n=5); *P < 0.05; significant compared to hyperglycemic control animals.

Table 2: Antinociceptive effect of (MEZO + MELA) in the acetic acid-induced pain model in mice.

Treatment	Dose (mg/kg body weight)	Mean number of writhings	% inhibition
Control (Group 1)	10 ml	5.2 ± 0.20	-
Aspirin (Group 2)	400 mg	2.6 ± 0.24	50.0*
MEZO + MELA (Group 3)	50 mg	3.8 ± 0.37	26.9*
MEZO + MELA (Group 4)	100 mg	3.4 ± 0.40	34.6*
MEZO + MELA (Group 5)	200 mg	2.4 ± 0.24	53.8*
MEZO + MELA (Group 6)	400 mg	1.8 ± 0.49	65.4*

All administrations (aspirin and extract) were made orally. Values represented as mean ± SEM, (n=5); *P < 0.05; significant compared to control.

DISCUSSION

Diabetes is a disease, which in a large number of cases needs continuous monitoring of blood glucose and adjustments in drug doses or units of insulin injected. Blood glucose can be conveniently monitored at home with a glucometer, but such equipment plus glucose measuring strips are costly, needs some training to be used properly, and considered to be inconvenient by many people because of pricking with a needle. As a result oral drugs which are safe (less toxic), and more affordable and readily available will be preferred by most people and certainly the rural people of countries like Bangladesh, people who are not able to afford allopathic drugs. In the present instance, since both plant parts are consumed, it is very much possible that simple consumption of plant parts can lead to the desired effects of lowering elevated blood glucose levels and alleviation pain. However, this possibility needs to be investigated.

From these practical considerations, the combination of MEZO and MELA has all the advantages of affordability, availability and possibly no toxicity. Both plants are readily available in Bangladesh and aerial parts of *Leucas aspera* and rhizomes of *Zingiber officinale* are consumed without any reported problems of adverse effects.

6-gingerol, a phytocomponent of Zingiber officinale, can be responsible for the observed antinociceptive effect of MEZO.[43] Both 6-gingerol and 8-gingerol have been reported to increase glucose uptake and so can be beneficial during diabetes.[44] Alkaloids and flavonoids

present in Leucas aspera may be responsible for the observed antihyperglycemic and antinociceptive effects as has been previously reported with other plants.[45, 46]

CONCLUSION

The results suggest that methanolic extract of aerial parts of *Leucas aspera* and rhizomes of *Zingiber officinale* can be used for lowering blood glucose and alleviate pain when used in combination.

Conflicts of interest

The author(s) declare that they have no competing interests.

REFERENCES

1. Zaman MM, Choudhury SR, Ahmed J, Talukder MH, Rahman AH: Blood glucose and cholesterol levels in adult population of Bangladesh: Results from STEPS 2006 survey. Indian Heart J, 2016; 68(1): 52-56.
2. Afroz A, Chowdhury HA, Shahjahan M, Hafez MA, Hassan MN, Ali L: Association of good glycemic control and cost of diabetes care: Experience from a tertiary care hospital in Bangladesh. Diabetes Res Clin Pract, 2016; 120: 142-148.
3. Azam S, Dipti T, Rahman S: Prevalence and clinical pattern of juvenile idiopathic arthritis in a semi-urban area of Bangladesh. Int J Rheum Dis, 2012; 15(1): 116-120.
4. Latter S, Hopkinson JB, Lowson E, Hughes JA, Hughes J, Duke S, Anstey S, Bennett MI, May C, Smith P, Richardson A: Supporting carers to manage pain medication in cancer patients at the end of life: A feasibility trial. Palliat Med 2017, 269216317715197. doi: 10.1177/0269216317715197.
5. Brito CA, Sohsten AK, Leitão CC, Brito RC, Valadares LD, Fonte CA, Mesquita ZB, Cunha RV, Luz K, Leão HM, Brito CM, Frutuoso LC: Pharmacologic management of pain in patients with Chikungunya: a guideline. Rev Soc Bras Med Trop, 2016; 49(6): 668-679.
6. Ingle Pravinkumar V, Talele Gokul S: Adverse effects of metformin in combination with glimepiride and glibenclamide in patients with Type 2 diabetes mellitus. Asian J Pharmaceut Clin Res, 2012; 5(1): 108-110.
7. Rates SMK: Plants as source of drugs. Toxicon, 2001; 39: 603-613.
8. Shaha SR, Rahmatullah M: Oral glucose tolerance and analgesic studies with methanol extract of *Brassica alba* seeds. World J Pharm Pharmaceut Sci, 2015; 4(9): 207-215.

9. Sayeed MSR, Ahmed H, Rahman S, Ahmad I, Rahman MM, Hossan MS, Rahmatullah M: Polyherbal formulation for lowering blood glucose levels: Evaluation of a combination of *Foeniculum vulgare* and *Brassica alba* seeds. World J Pharm Pharmaceut Sci, 2015; 4(10): 79-85.
10. Mazhar J, Mazumder A: Evaluation of antidiabetic activity of methanolic leaf extract of *Coriandrum sativum* in alloxan induced diabetic rats. Res J Pharmaceut Biol Chem Sci (RJPBCS), 2013; 4(3): 500-507.
11. Widodo GP, Handayani SR, Herowati R: Antihyperglycemic, antioxidant, and pancreas protective effects of Coriandrum sativum seed in alloxan-induced diabetic rats. Indonesian J Pharm, 2015; 26(3): 129-133.
12. Rahman M, Hasan N, Das AK, Hossain T, Jahan R, Khatun A, Rahmatullah M: Effect of *Delonix regia* leaf extract on glucose tolerance in glucose-induced hyperglycemic mice. Afr J Tradit Complement Altern Med, 2011; 8(1): 34-36.
13. Hasan MY, Al-Mahamud R, Rahman S, Ahmad I, Rahmatullah M: A preliminary report on antihyperglycemic and analgesic properties of methanol extract of *Brassica oleracea* L. var. *italica* sprouts. World J Pharm Pharmaceut Sci, 2015; 4(9): 225-234.
14. Ahmed M, Trisha UK, Shaha SR, Dey AK, Rahmatullah M: An initial report on the antihyperglycemic and antinociceptive potential of *Lablab purpureus* beans. World J Pharm Pharmaceut Sci, 2015; 4(10): 95-105.
15. Rahmatullah M, Sultan S, Toma TT, Lucky SS, Chowdhury MH, Haque WM, Annay MEA, Jahan R: Effect of *Cuscuta reflexa* stem and *Calotropis procera* leaf extracts on glucose tolerance in glucose-induced hyperglycemic rats and mice. Afr J Trad Complement Altern Med, 2010; 7(2): 109-12.
16. Ahmed F, Rahman S, Ahmed N, Hossain M, Biswas A, Sarkar S, Banna H, Khatun MA, Chowdhury MH, Rahmatullah M: Evaluation of *Neolamarckia cadamba* (Roxb.) Bosser leaf extract on glucose tolerance in glucose-induced hyhperglycemic mice. Afr J Trad Complement Altern Med, 2011; 8(1): 79-81.
17. Shahreen S, Banik J, Hafiz A, Rahman S, Zaman AT, Shoyeb MA, Chowdhury MH, Rahmatullah M: Antihyperglycemic activities of leaves of three edible fruit plants (*Averrhoa carambola*, *Ficus hispida* and *Syzygium samarangense*) of Bangladesh. Afr J Trad Complement Altern Med, 2012; 9(2): 287-91.
18. Rahmatullah M, Hosain M, Rahman S, Rahman S, Akter M, Rahman F, Rehana F, Munmun M, Kalpana MA: Antihyperglycaemic and antinociceptive activity evaluation of

methanolic extract of whole plant of *Amaranthus tricolour* L. (Amaranthaceae). Afr J Trad Complement Altern Med, 2013; 10(5): 408-11.

19. Rahmatullah M, Hossain M, Mahmud A, Sultana N, Rahman SM, Islam MR, Khatoon MS, Jahan S, Islam F: Antihyperglycemic and antinociceptive activity evaluation of 'khoyer' prepared from boiling the wood of *Acacia catechu* in water. Afr J Trad Complement Altern Med, 2013; 10(4): 1-5.
20. Haque ME, Rahman S, Rahmatullah M, Jahan R: Evaluation of antihyperglycemic and antinociceptive activity of *Xanthium indicum* stem extract in Swiss albino mice. BMC Complement Alternat Med, 2013; 13: 296-299.
21. Hossain AI, Faisal M, Rahman S, Jahan R, Rahmatullah M: A preliminary evaluation of antihyperglycemic and analgesic activity of *Alternanthera sessilis* aerial parts. BMC Complement Alternat Med, 2014; 14: 169-173.
22. Tazin TQ, Rumi JF, Rahman S, Al-Nahain A, Jahan R, Rahmatullah M: Oral glucose tolerance and antinociceptive activity evaluation of methanolic extract of *Vigna unguiculata* ssp. *unguiculata* beans. World J Pharm Pharmaceut Sci, 2014; 3(8): 28-37.
23. Rahman S, Jahan R, Rahmatullah M: Effect of paddy husk extracts on glucose tolerance in glucose-induced hyperglycemic mice. World J Pharm Pharmaceut Sci, 2014; 3(8): 111-120.
24. Jahan S, Rahmatullah M: Methanolic extract of aerial parts of *Raphanus sativus* var. *hortensis* shows antihyperglycemic and antinociceptive potential. World J Pharm Pharmaceut Sci, 2014; 3(8): 193-202.
25. Ghosh D, Mandal I, Rumi JF, Trisha UK, Jannat H, Ahmed M, Rahmatullah M: Effect of *Allium sativum* leaf extracts on glucose tolerance in glucose-induced hyperglycemic mice. Adv Nat Appl Sci, 2014; 8(8): 66-69.
26. Haque ME, Rahmatullah M: *Elephantopus spicatus*: a plant with hitherto unreported antihyperglycemic and antinociceptive potential. World J Pharm Pharmaceut Sci, 2014; 3(9): 71-80.
27. Hasan MN, Ferdoushi A, Ara N, Rahman S, Hossan MS, Rahmatullah M: Preliminary phytochemical screening, toxicity, antihyperglycemic and analgesic activity studies with *Curcuma longa* leaves. World J Pharm Pharmaceut Sci, 2014; 3(9): 81-91.
28. Sultana S, Nandi JK, Rahman S, Jahan R, Rahmatullah M: Preliminary antihyperglycemic and analgesic activity studies with *Angiopteris evecta* leaves in Swiss albino mice. World J Pharm Pharmaceut Sci, 2014; 3(10): 1-12.

29. Rahman KMH, Nandi JK, Sultana S, Rahman S, Hossan S, Rahmatullah M: Phytochemical screening, antihyperglycemic and analgesic activity studies with methanol extract of *Trevesia palmata* leaves. World J Pharm Pharmaceut Sci, 2014; 3(10): 91-101.
30. Syeda S, Rahman S, Afsana NA, Mahal MJ, Swarna A, Rahmatullah M: Antihyperglycemic activity evaluation of a formulation consisting of *Phyllanthus emblica, Terminalia bellirica* and *Terminalia chebula* fruits and *Trigonella foenum graecum* seeds. Adv Nat Appl Sci, 2014; 8(1): 12-15.
31. Monalisa MN, Rahmatullah M: Antihyperglycemis, analgesic activity, and acute toxicity studies with methanol extract of *Foeniculum vulgare* seeds. World J Pharm Pharmaceut Sci, 2015; 4(9): 198-206.
32. Parvin S, Marzan M, Rahman S, Das AK, Haque S, Rahmatullah M: Preliminary phytochemical screening, antihyperglycemic, analgesic and toxicity studies on methanolic extract of aerial parts of *Corchorus olitorius* L. J Appl Pharmaceut Sci, 2015; 5(9): 68-71.
33. Akther M, Islam E, Islam MT, Das PR, Haque ME, Jahan R, Al-Nahain A, Rahman S, Rahmatullah M: A preliminary study on significant antihyperglycemic activity as determined through oral glucose tolerance tests of three common plants belonging to the Brassicaceae family. World J Pharm Pharmaceut Sci, 2016; 5(8): 159-172.
34. Hossan ANMF, Zaman F, Barman MR, Khatoon S, Zaman M, Khatun F, Mosaiab T, Mostafa F, Sintaha M, Jamal F, Rahmatullah M: Antinociceptive activity of *Xanthium indicum* J. Koenig ex Roxb. (Asteraceae) leaves and Leucas aspera (Willd.) Link (Lamiaceae) whole plants. Adv Nat Appl Sci, 2011; 5(2): 214-217.
35. Mannan A, Das H, Rahman M, Jesmin J, Siddika A, Rahman M, Rahman S, Chowdhury MH, Rahmatullah M: Antihyperglycemic activity evaluation of *Leucas aspera* (Willd.) Link leaf and stem and *Lannea coromandelica* (Houtt.) Merr. bark extract in mice. Adv Nat Appl Sci, 2010; 4(3): 385-388.
36. Saha M, Rohani S, Rayhana N, Toma IJ, Rana S, Rahmatullah M: An herbal formulation containing *Zingiber officinale* rhizomes and *Allium sativum* cloves can increase oral glucose tolerance in mice. Biology, Engineering and Medicine, 2017; 2(1): 1-3.
37. Raji Y, Udoh US, Oluwadara OO, Akinsomisoye OS, Awobajo O, Adeshoga K: Anti-inflammatory and analgesic properties of the rhizome extract of *Zingiber offinale*. Afr J Biomed Res, 2002; 5: 121-124.
38. Joy KL, Kuttan RJ: Anti-diabetic activity of *Picrorrhiza kurroa* extract. J Ethnopharmacol, 1999; 67(2): 143-148.

39. Venkatesh S, Reddy GD, Reddy YSR, Sathyavathy D, Reddy B: Effect *of Helicteres isora* root extracts on glucose tolerance in glucose-induced hyperglycemic rats. Fitoterapia, 2004; 75(3-4): 364-367.
40. Shanmugasundaram P, Venkataraman S: Anti-nociceptive activity of *Hygrophilous auriculata* (Schum) Heine. Afr J Trad Complement Altern Med, 2005; 2(1): 62- 69.
41. Akter A, Rahman S, Morshed MT, Hossain S, Jahan S, Swarna A, Rahmatullah M: Evaluation of antihyperglycemic and antinociceptive potential of *Colocasia esculenta* (L.) Schott (Araceae) leaves. Adv Nat Appl Sci, 2013; 7(2): 143-148.
42. Kumar C, Kumar R, Nehar S: Phytochemical properties, total antioxidant status of acetone and methanol extract of *Terminalia arjuna* Roxb. bark and its hypoglycemic effect on Type-II diabetic albino rats. J Pharmacogn Phytochem, 2013; 2: 199-208.
43. Al-Nahain A, Jahan R, Rahmatullah M: *Zingiber officinale*: A potential plant against rheumatoid arthritis. Arthritis Article ID 159089, 2014.
44. Roufogalis BD: Zingiber officinale (ginger): A future outlook on its potential in prevention and treatment of diabetes and prediabetic states. New J Science, Article ID 674684, 2014.
45. Jan S, Khan MR: Antipyretic, analgesic and anti-inflammatory effects of *Kickxia ramosissima*. J Ethnopharmacol, 2016; 182: 90-100.
46. Cok A, Plaisier C, Salie MJ, Oram DS, Chenge J, Louters LL: Berberine acutely activates the glucose transport activity of GLUT1. Biochimie, 2011; 93(7): 1187-1192.