Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Medicine

journal homepage:www.elsevier.com/locate/apjtm

Document heading doi:

Protective effect of *Luffa acutangula* extracts on gastric ulceration in NIDDM rats: Role of gastric mucosal glycoproteins and antioxidants

B. P. Pimple[1*], P. V. Kadam[2], M. J. Patil[2]

[1]*P. E. Society's Modern College of Pharmacy, Nigdi, Pune, Maharashtra, India. 411044 and Jawaharlal Nehru Technological University, Hyderabad, Andhra Pradesh, India*

[2]*Marathwada Mitra Mandal's College of Pharmacy, Thergaon, Pune, Maharashtra, India 411033*

ARTICLE INFO

Article history:
Received 10 March 2012
Received in revised form 15 May 2012
Accepted 15 July 2012
Available online 20 August 2012

Keywords:
Aspirin
Glibenclamide
Nicotinamide
Ranitidine
Streptozotocin

ABSTRACT

Objective: To study the comparative gastroprotective effect of *Luffa acutangula* methanolic extract (LAM) and aqueous extract (LAW) on type II diabetes rats. **Methods:** Streptozotocin (65 mg/kg, *i.p.*) along with nicotinamide (120 mg/kg, *i.p.*) was used to induce non insulin dependent diabetes mellitus (NIDDM) in rats. A daily oral dose of aspirin (200 mg/kg, *i.p.*) was administered for initial seven days to induce gastric ulcerations in the diabetic rats. LAM and LAW were administered orally in the doses of 100, 200 and 400 mg/kg once daily for 21 days. Glibenclamide and ranitidine were used as standards for comparing the antidiabetic and antiulcer effect respectively. **Results:** LAM significantly ($P<0.01$) increased mucosal glycoprotein and antioxidant enzyme level in gastric mucosa of diabetic rats than LAW ($P<0.05$). LAM was efficient in reversing the delayed healing of gastric ulcer in diabetic rats close to the normal level. LAM exhibited better ulcer healing effect than glibenclamide and LAW, because of its both antihyperglycemic and mucosal defensive actions. **Conclusions:** Thus, LAM is proved to be a better alternative for treating gastric ulcers co-occurring with diabetes.

1. Introduction

Type II diabetes mellitus is one of the chronic disorders marked by disturbance in metabolism of carbohydrates and fats. It represents a severe setback of public health, as it accounts for substantial portion of national health expenditures globally in most of the countries. Diabetes still continues to amplify in number and significance in the developing countries. According to a study the diabetic population is expected to grow up to 552 million by 2030[1]. Another survey conducted on adult diabetic population (aged 20-79 years), revealed the presence of 285 million diabetic adults in 2010, and will amplify to 439 million by 2030[2]. Inspite of the availability of beneficial treatments to avoid or delay chief complications, diabetes still places a massive burden on both patients and the health care system[3].

Experimental induction of type II diabetes using streptozotocin-nicotinamide has been proved to be an efficient model to evaluate the efficiency of antidiabetic drugs[4]. Research has already proved that the gastric mucosa of diabetic rats is highly susceptible to acute injuries and considerably impairs ulcer healing[5].

Since time immemorial, numerous plants species have been identified worldwide for antidiabetic and antiulcer activity[6]. *Luffa acutangula (L. acutangula)* Linn. var. *amara* Roxb. fruits have recently been proved to be effective in the management of diabetes, stress, gastric complications and respiratory disorders[7,8].

The present study deals with comparative effect of methanolic and aqueous extracts of *L. acutangula* (LA) fruits in the treatment of ulcers in diabetic rats. LA extracts were administered in different doses (100, 200 and 400 mg/kg, *p.o.*) to investigate their ulcer healing potential. Using aspirin for the induction gastric ulcer in streptozotocin (STZ)-induced type II diabetes, our work for the first time demonstrates that the *L. acutangula* stimulates the healing of gastric ulcer in diabetes.

*Corresponding author: B. P. Pimple, P. E. Society's Modern College of Pharmacy, Nigdi, Pune, Maharashtra, India 411044.
Tel: +9199707830030
Fax: +912227661314
E-mail: bhushanppimple@rediffmail.com

2. Material and methods

2.1. Plant material

The dried fruit pulp of *L. acutangula* Linn. var. *amara* Roxb. were obtained from the local markets of Pune and were authenticated by Botanical Survey of India, Western Circle, Pune with voucher specimen No. LABHP-1.

2.2. Preparation of extracts

Methanolic extract (LAM) and aqueous extracts (LAW): The fresh *L. acutangula* fruits were grated, dried in shade and coarsely powdered. The powder was defatted with petroleum ether and then extracted by methanol in a Soxhlet apparatus. The aqueous extract was prepared by maceration with distilled water for 24 h. The extracts were concentrated under reduced pressure and were stored at 8-10 ℃ throughout the study[7]. The yield of LAM and LAW were 2.31% w/w and 7.18% w/w, respectively.

2.3. Chemicals and reagents

STZ and nicotinamide were obtained from Sigma-Aldrich, USA. Analytical grade methanol (99.9% v/v) and other chemicals were procured from Merck, USA.

2.4. Animals

Albino mice (20-22 g) and rats [(200 ± 20) g], of either sex were obtained from the Yash farms, Pune. The animals were kept in polyethylene cages in the departmental animal house at (26±2) ℃ and relative humidity 40%-55% with 12 h light and dark cycles. The animals were fed with regular pellet chow diet (Hind liver) and were allowed free access to water. Every experimental protocol for animal care procedures were approved by the Institutional Animal Ethical Committee (IAEC) with a protocol number MCP/IAEC/02/2009.

2.5. Acute toxicity studies

Swiss albino mice weighing 20-25 g were taken for the study. The animals were divided into three groups. The animals were fasted overnight but were allowed free access to water prior to the day of study. First group served as control and received 1% carboxymethyl cellulose (CMC) in distilled water. While the other two groups received 2 g/kg *p.o.* dose of LAM and LAW, respectively. The extracts were suspended in 1% CMC solution in distilled water as they were not easily soluble in water.

The animals were observed for 5 min every 30 min till 2 h and then at 4, 8 and 24 h to detect any change in the autonomic or behavioral response and also for tremors, convulsion, salivation, diarrhea, lethargy, sleep and coma and then were further observed daily for 14 days for mortality[9].

2.6. Experimental induction of NIDDM and gastric ulcers in rats

Type II diabetes was induced by injecting a single *i.p.* dose of nicotinamide (120 mg/kg) followed by *i.p.* administration of STZ (65 mg/kg) in 0.1 M citrate buffer (pH 4.5). The fasting blood glucose level was estimated after 72 h from STZ administration. Rats exhibiting blood glucose concentration more than 145 mg/dL were considered diabetic and were included in the study. All the diabetic rats included in the study were administered with aspirin (200 mg/kg, *p.o.*) for the initial 7 days[6].

2.7. Experimental procedure

The animals were separated into 10 groups each containing six. A total of 76 rats (6 normal + 54 diabetic) were used. Group 1 was normal untreated rats and received 1% CMC solution. Except the normal group remaining 9 groups contained STZ (65 mg/kg, *i.p.*) induced diabetic rats intoxicated with aspirin (200 mg/kg, *p.o.*). Group 2 represented STZ+aspirin control group and rats housed in this group did not received any treatment. Group 3 animals were treated with glibenclamide (0.25 mg/kg, *p.o.*) whereas group 4 rats were treated with a daily dose of ranitidine (2.5 mg/kg, *p.o.*). Group 5, 6 and 7 were administered with an oral dose of 100, 200 and 400 mg/kg, respectively, *L. acutangula* MeOH extract. The groups 8, 9 and 10 were fed with an oral dose of 100, 200 and 400 mg/kg of *L. acutangula* aqueous extract, respectively[10].

2.8. Scoring of ulcer index

On the 28th day, animals were sacrificed by cervical dislocation and the stomach was isolated and incised along the greater curvature and examined for ulcers. The greatest length of each lesion was measured and the sum of the lengths of all lesions in each stomach was reported as the ulcer index. The % inhibition of ulcer was determined and mean ulcer score for every animal was expressed as ulcer index[10,11]. Entire measurements were made by a person unaware about the treatment the rats were receiving[12]. The % inhibitions of ulcer were calculated and mean ulcer score for each animal was expressed as ulcer index[13].

2.9. Determination of percent protection

% Protection = [(STZ+aspirin control ulcer index) − (drug treated ulcer index)]/(STZ+aspirin control ulcer index) × 100.

The number of ulcers per stomach was recorded and the percent of ulcer incidence of each group as compared to the STZ+aspirin control was calculated[10,11].

2.10. Determination of fasting blood glucose and serum insulin levels

The blood glucose levels were estimated on 0th, 14th and

28th day after induction of NIDDM. Blood was collected from the retro-orbital plexus and glucose was estimated by means of glucostix (One touch Ultra, Johnson and Johnson). Serum insulin was estimated merely on the 28th day to investigate the secretogouge effect of LA extracts[6,7,10,14].

2.11. Determination of mucosal glycoproteins

Glycoproteins were isolated from the samples of gastric mucosal scraping according to the method described by Pimple *et al*, 2012[10]

2.12. Superoxide dismutase (SOD) and catalase (CAT) enzyme assay

SOD and Catalase enzyme estimation was performed according to the established procedure of Kakkar *et al*, 1984[15] and Kaur *et al*, 2006[16], respectively.

2.13. Statistical analysis

The above estimations were analyzed statistically by applying One-way analysis of variance (ANOVA) followed by Dunnet's test for multiple comparisons. The differences were considered significant when $P<0.05$.

3. Results

3.1. Effect of LA extracts on ulcer index and percent protection

The diabetic rats on administration with LA extracts for 21 days showed noticeable protection of the gastric mucosa against the acid attack. LAM at a dose of 200 and 400 mg/kg and LAW at a dose of 400 mg/kg were found to protect the gastric mucosa significantly ($P<001$). The LAM 100, LAW 100 and 200 groups were in efficient in reducing the mucosal damage. The dose level of 100 mg/kg of LAM and LAW were not effective enough to treat the ulcers. Table 1 highlights the curative effect of various LA extracts on gastric ulceration. Glibenclamide was less effective ($P<0.05$) in protection of ulcers as compared to the LAM (Table 1).

3.2. Effect of different LA extracts on fasting blood glucose levels in diabetic rats

The blood glucose was estimated after regular intervals to determine the antidiabetic effect of LA extracts. The LAM extract exhibited dose dependent promising ($P<0.01$) antidiabetic activity whereas the LAW 100 and 200 groups did not respond in depleting the glucose level. But the LAW 400 group showed considerable ($P<0.05$) lowering in fasting blood glucose level. The LAM treated and LAW 400 groups were more efficient than ranitidine group in lowering the elevated glucose level (Table 2).

3.3. Effect of LA extracts on serum insulin and glycosylated hemoglobin

The groups receiving ranitidine, LAW 100 mg/kg and LAW 200 did not show any alteration in STZ-induced depletion of serum insulin. On the other hand each dose of LAM exhibited elevation of serum insulin which was comparable to glibenclamide ($P<0.01$). The LAM 100 and LAW 400 group exhibited considerable ($P<0.05$) rise in serum insulin level and the LAM 200 and 400 group significantly ($P<0.01$) elevated the lowered serum insulin. Similar effects were also observed in case of glycosylaed hemoglobin (HbA_{1C}). Table 3 reveals the comparative effect of LAM and LAW on serum insulin and HbA_{1c} (Table 3).

3.4. Effect of LA extracts on gastric mucosal glycoproteins

The glibenclamide and LAW treated groups were less efficient in restoring the mucosal parameters to their normal values. A dose dependant significant ($P<0.01$) effect of LAM extracts were comparable to ranitidine a well known antiulcer drug in restoring the mucosal glycoproteins to normal levels. Overall the LAM scored well over LAW in treatment of mucosal damage induced by aspirin (Table 4).

Table 1
Effect of LA extract on ulcers index and percent protection of diabetic rats (mean±SEM, $n = 6$).

Groups	Ulcer index	% Protection
Normal	–	–
STZ + aspirin control	62.1±3.3 **	–
Glibenclamide	51.5±2.3 *	17.07
Ranitidine	15.4±1.2 **	75.20
LAM 100	48.8±3.6 *	21.42
LAM 200	27.2±3.5 **	56.20
LAM 400	23.2±1.8 **	62.64
LAW 100	58.3±3.8	6.12
LAW 200	54.1±3.1	12.88
LAW 400	50.2±2.4 *	19.16

LAM 100, LAM 200 and LAM 400 are oral doses of MeOH extract of *L. acutangula* in 100, 200 and 400 mg/kg respectively; LAW 100, LAW 200 and LAW 400 are oral doses aqueous extract of *L. acutangula* in 100, 200 and 400 mg/kg respectively. ** = $P < 0.01$, * = $P < 0.05$ compared to STZ+aspirin control group.

3.5. Effect of LA extracts on antioxidant enzymes SOD and CAT

The SOD enzyme levels were equally but significantly ($P<0.01$) elevated in animals treated with 400 g/kg dose of LAM and LAW. The groups receiving 200 mg/kg dose of LAM was effective ($P<0.05$) in restoring the SOD levels to normal. The LAM 100, LAW 100 and LAW 200 groups were totally inefficient in elevating the depleted SOD levels to normal.

The distinct elevation in the CAT enzymes was observed in the by the groups treated with 200 and 400 mg/kg dose of LAM. The groups receiving LAM at 100 mg/kg and LAW at 100 and 200 mg/kg dose did not exhibit significant rise in CAT enzyme level. In both the cases the LAM extracts superseded the ranitidine treated group. Table 5 highlights the effect of LAM and LAW on antioxidant enzyme level.

Table 2

Effect of different LA extracts on blood glucose level in diabetic rats (mean±SEM, $n = 6$).

Groups	Fasting blood glucose level (mg/dL)	
	0th day	28th day
Normal	78.0±7.6	81.0±6.4 **
STZ + aspirin control	254.0±22.3	266.0±23.1
Glibenclamide	261.0±18.6	85.0±8.2 **
Ranitidine	250.0±20.7	228.0±14.8
LAM 100	255.0±10.3	132.0±12.7 *
LAM 200	262.0±14.6	111.0±15.6 **
LAM 400	253.0±20.1	100.0±11.2 **
LAW 100	258.0±18.4	210.0±10.4
LAW 200	252.0±28.3	165.0±12.4
LAW 400	266.0±23.2	141.0±11.2 *

LAM 100, LAM 200 and LAM 400 are oral doses of MeOH extract of *L. acutangula* in 100, 200 and 400 mg/kg respectively; LAW 100, LAW 200 and LAW 400 are oral doses aqueous extract of *L. acutangula* in 100, 200 and 400 mg/kg respectively. ** = $P < 0.01$, * = $P < 0.05$ compared to STZ+aspirin control group.

Table 3

Effect of LA extracts on serum insulin and glycosylated hemoglobin (HbA1c) level in normal and NIDDM rats(mean±SEM, $n = 6$).

Groups	Serum Insulin(mg/dL)	HbA1c(μ U/mL)
Normal	15.50±0.62 **	0.51±0.02 **
STZ + aspirin control	5.10±0.31	0.88±0.03
Glibenclamide	14.20±0.71 **	0.55±0.02 **
Ranitidine	5.80±0.45	0.79±0.02
LAM 100	8.10±0.45 *	0.74±0.03 *
LAM 200	9.10±0.25 **	0.63±0.03 **
LAM 400	11.20±0.35 **	0.58±0.02 **
LAW 100	5.90±0.35	0.82±0.02
LAW 200	6.10±0.31	0.76±0.03
LAW 400	7.90±0.10 *	0.65±0.02 **

LAM 100, LAM 200 and LAM 400 are oral doses of MeOH extract of *L. acutangula* in 100, 200 and 400 mg/kg respectively; LAW 100, LAW 200 and LAW 400 are oral doses aqueous extract of *L. acutangula* in 100, 200 and 400 mg/kg respectively. ** = $P < 0.01$, * = $P < 0.05$ compared to STZ+aspirin control group.

Table 4

Effect of LA extracts on gastric mucosal glycoproteins (mean±SEM, $n = 6$).

	Total hexose	Hexos-amine	Fucose	Sialic acid	Total carbo-hydrates(TC)	Total proteins(TP)	TC:TP
Normal	2 865±121**	1 755±33**	307±14**	117±9**	4 788±129**	6 112±210**	0.78±0.05**
STZ+aspirin control	2 117±117	1 480±45	202±17	54±8	3 987±248	7 310±220	0.55±0.04
Gliben-clamide	2 675±126*	1 685±63*	274±22	92±5*	4 681±147	6 610±321	0.71±0.03 *
Ranitidine	2 790±154**	1 780±33**	327±29**	113±10**	4 826±185*	6 187±146**	0.78±0.03**
LAM 100	2 640±133*	1 622±51	261±15	89±11	4 533±162	6 370±214*	0.71±0.04*
LAM 200	2 690±120*	1 660±44	289±17*	103±8**	4 581±134	6 310±211*	0.73±0.03**
LAM 400	2 760±131**	1 695±42*	321±12**	123±7**	4 621±120*	6 255±118**	0.74±0.02**
LAW 100	2 626±105*	1 596±65	255±21	81±10	4 644±188*	6 385±210*	0.73±0.03**
LAW 200	2 684±93*	1 632±61	272±28	95±9*	4 660±177*	6 342±166*	0.73±0.04**
LAW 400	2 710±133*	1 680±36*	305±23**	111±12**	4 715±230*	6 280±245**	0.75±0.03**

LAM 100, LAM 200 and LAM 400 are oral doses of *L. acutangula* MeOH extract in 100, 200 and 400 mg/kg respectively whereas; LAW 100, LAW 200 and LAW 400 are oral doses of *L. acutangula* aqueous extract in 100, 200 and 400 mg/kg respectively. ** $P < 0.01$, * $P < 0.05$ compared to respective normal and NIDDM-control groups.

Table 5
Effect of LA extracts on endogenous antioxidant enzyme level (mean±SEM, $n = 6$).

Groups	SOD(U/g of wet tissue)	CAT(U/g of wet tissue)
Normal	97.3±4.2 **	27.3±1.1 **
STZ+aspirin control	48.2±5.1	11.3±0.8
Glibenclamide	66.4±3.2 **	18.2±0.8 **
Ranitidine	52.6±2.5	24.3±0.9
LAM 100	46.6±3.2	16.5±1.3 *
LAM 200	67.3±4.4 *	17.9±1.1 **
LAM 400	84.4±5.1 **	21.5±2.2 **
LAW 100	52.3±2.9	10.2±1.4
LAW 200	57.6±1.8	12.1±1.5
LAW 400	78.4±2.3 **	17.5±0.9 *

LAM 100, LAM 200 and LAM 400 are oral doses of *L. acutangula* MeOH extract in 100, 200 and 400 mg/kg respectively whereas; LAW 100, LAW 200 and LAW 400 are oral doses of *L. acutangula* aqueous extract in 100, 200 and 400 mg/kg respectively. ** $P < 0.01$, * $P < 0.05$ compared to respective normal and NIDDM-control groups.

4. Discussion

Our previous work demonstrates the impact of diabetes on the ulcer healing process[10]. LAM revealed significant reduction in the ulcer index of diabetic rats justifying that it is a step ahead over the drugs which have either only antiulcer (ranitidine) or only antidiabetic (glibenclamide) effects. The potential of LAM in depleting the aspiring induced damage could be attributed to the high amount of phenolics and flavonoids in it as compared to LAW[7].

Diabetes is coupled with decrease in antioxidant status, glycoproteins, mucin secretion and mucosal cell shedding lacking any effect on cell proliferation[17,18]. Hence, in diabetes mucosal defensive factors play a very important role in escalating propensity to gastric ulceration and this may perhaps be one of the reasons for ranitidine to be effective in gastric ulceration in diabetic rats. LAM significantly ($P<0.01$) restored the mucosal glycoprotein in diabetic rats as compared to LAW. The potency of LAM was almost similar to raniditine.

STZ (65 mg/ kg) along with nicotinamide causes fractional destruction of pancreatic β-cells[10]. The antidiabetic action of LAM could be due to motivation of insulin discharge from the normal pancreatic β-cells. This is evident from the fact that LAM significantly uplifted the serum insulin concentration as compared to the LAW. The maintenance of blood glucose level at almost normal level could thus possibly protect the NIDDM patient from peptic ulceration[18]. Earlier reports also reveal that STZ significantly decreases the gastric blood flow[19-21].

Many mechanisms have been postulated for the increased vulnerability of gastric mucosa of diabetic animals to damaged, which includes the destruction of the antioxidative system in the gastric mucosa[22], the inhibition of basic fibroblast growth factor production in the gastric mucosa, impaired duodenal HCO_3^- discharge[23], decreased angiogenesis and the malfunction of capsaicin-sensitive afferent neurons engaged in the protection of gastric mucosa[24].

Insulin is essential for cellular proliferation in devitalized tissue. The main action of insulin is not only to cleanse the wound of exudates and necrotic tissue but also to serve as a hormone by stimulating tissue regeneration[25]. Insulin enhances metabolism leading to mitosis and cellular proliferation of adjacent tissues[26]. Insulin considerably increases gastric mucosal blood flow in diabetic rats[27]. Serum insulin levels in group treated with LAM were significantly higher than LAW treated groups. Thus the elevation was comparable to glibenclamide suggesting the secretogouge role of LAM.

Prolonged elevated blood glucose level may lead in glycosylation of Hb[28]. When LAM was administered for 21 days, an efficient sinking of glycosylated Hb level were seen in diabetic rats, thus signifying its usefulness in correcting the hazardous complications of diabetes.

SOD and CAT play a vital role in detoxification of superoxide anion and H_2O_2 respectively, thus shielding cell against OFRs-induced damage[29]. The reactive superoxide radicals are first converted to H_2O_2 by SOD. Later these H_2O_2 radicals are scavenged by CAT to prevent the lipid peroxidation resulting due to generation of hydroxyl radicals[30]. LAM effectively elevated the concentration of these endogenous antioxidant enzymes as compared to LAW, thereby supporting the process of ulcer healing in diabetic rats.

Throughout the study the LAM superseded LAW in all aspects, which could be attributed to the presence of excess amount of phenolic or polyphenolic groups such as tannins or flavonoids. LA has been previously reported to contain antioxidants like tannins, hydroquinone, phenolic glycosides[7, 8].

Additional investigation on derangement in the state of mucosal defensive factors such as cell shedding and tissue damage due to enhanced lipid peroxidation in diabetes may possibly throw extra light on the precise mechanism of action of LA.

It can be concluded that beneficial effects of *L. acutangula* in diabetic rats with co-existing gastric ulcer might be due to its dual nature *i.e.* antidiabetic (reversing the toxic consequence of diabetes on gastric mucosa) and direct promoting effect on the gastric mucosal protection.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

We would like to thank Principal Dr. P.D. Chaudhari and Prof. Dr. S.L. Badole, P. E. S. Modern College of Pharmacy, Nigdi, Pune; Botanical Survey of India (Western Circle), Pune and University of Pune for providing necessary assistance. This work was supported by Grants-in-Aid for Scientific Research from the Board of College and University Development, University of Pune.

References

[1] Whiting DR, Guariguata L, Weil C, Shaw J. IDF diabetes atlas: Global estimates of the prevalence of diabetes for 2011 and 2030. *Diab Res Clin Pract* 2011. doi:10.1016/j.diabres.2011.10.029.

[2] Shaw JE, Sicree RA, Zimmet PZ. Global estimates of prevalence of diabetes for 2010 and 2030. *Diab Res Clin Pract* 2010; **87**: 4-14.

[3] Berardis GD, D'Ettorre A, Graziano G, Lucisano G, Pellegrini F, Cammarota S, et al. The burden of hospitalization related to diabetes mellitus: A population-based study. *Nutr Metabol Cardiovasc Dis* 2011. doi:10.1016/j.numecd.2010.10.016.

[4] Badole SL, Bodhankar SL. Antidiabetic activity of cycloart-23-ene-3 β , 25 diol (B2) isolated from *Pongamia pinnata* (L.) Pierre in streptozotocin-nicotinamide induced diabetic mice. *Eur J Pharmacol* 2010; **632**: 103-109.

[5] Fouad AA, Al-Sultan AI, Yacoubi MT, Gomaa W. Ameliorative effects of Telmisartan in diabetic rats with indomethacin-induced gastric ulceration. *Eur J Pharmacol* 2010; **637**: 162-170.

[6] Pimple BP, Kadam PV, Patil MJ. Comparative antihyperglycaemic and antihyperlipidemic effect of *Origanum majorana* extracts in NIDDM rats. *Oriental Pharm Experimental Med* 2012; **12**(1): 41-50.

[7] Pimple BP, Kadam PV, Patil MJ. Antidiabetic and antihyperlipidemic activity of *Luffa acutangula* fruit extracts in streptozotocin induced NIDDM rats. *Asian J Pharm Clin Res* 2011; **4**(suppl 2): 156-163.

[8] Kalaskar MG, Surana SJ. Pharmacognostic and phytochemical investigation of *Luffa acutangula* var. *amara* fruits. *Int J Pharm Tech Res* 2010; **2**(2): 1609-1614.

[9] Organization for Economic Co-operation Development. *Guidance document on acute oral toxicity testing environment directorate*. Paris: OECD ; 2001, p. 1-24.

[10] Pimple BP, Kadam PV, Patil MJ. Ulcer healing properties of different extracts of *Origanum majorana* in streptozotocin-nicotinamide induced diabetic rats. *Asian Pac J Trop Biomed* 2012. In Press.

[11] Suhaj M. Spice antioxidants isolation and their antiradical activity: a review. *J Food Composition Analysis* 2006; **19**: 531-537.

[12] Ishihara T, Suemasu S, Asano T, Tanaka K, Mizushima T. Stimulation of gastric ulcer healing by heat shock protein 70. *Biochem Pharmacol* 2011; **82**: 728-736.

[13] Sivaraman D, Muralidharan P. Anti-ulcerogenic evaluation of root extract of *Ficus hispida* Linn. in aspirin ulcerated rats. *Afr J Pharm Pharmacol* 2010; **4**(2): 79-82.

[14] Juvekar AR, Bandawane DD. Antihyperglycaemic and antihyperlipidemic activity of *Aegle marmelos* Linn leaf extract in streptozotocin-induced diabetes rats. *Indian Drugs* 2009; **46**(7): 43-49.

[15] Kakkar P, Das B, Viswanathan PN. A modified spectrophotometric assay of superoxide dismutase. *Indian J Biochem Biophys* 1984; **21**(2): 130-132.

[16] Kaur G, Sarwar AM, Jabbar Z, Javed K, Athar M. Evaluation of antioxidant activity of *Cassia siamea* flowers. *J Ethnopharmacol* 2006; **108**: 340-348.

[17] Dorababu M, Prabha T, Priyambada S, Agrawal VK, Aryya NC, Goel RK. Effect of *Bacopa monniera* and *Azadirachta indica* on gastric ulceration and healing in experimental NIDDM rats. *Indian J Exp Biol* 2004; **42**: 389-397.

[18] Joshi MC, Dorababu M, Prabha T, Kumar MM, Goel RK. Effect of *Pterocarpus marsupium* on NIDDM-induced rat gastric ulceration and mucosal offensive and defensive factors. *Indian J Pharmacol* 2004; **36**(5): 296-302.

[19] Baraka AM, Guemei A, Gawad HA. Role of modulation of vascular endothelial growth factor and tumor necrosis factor-alpha in gastric ulcer healing in diabetic rats. *Biochem Pharmacol* 2010; **79**: 1634-1639.

[20] Gandhi MN, Challa SR, Prasanth P, Gandhi TR. Role of leukotrienes in NSAID induced gastric ulceration and inflammation in wistar rats. *Asian Pac J Trop Dis* 2012; **2**(3): 215-219.

[21] Gupta J, Kumar D, Gupta A. Evaluation of gastric anti-ulcer activity of methanolic extract of *Cayratia trifolia* in experimental animals.*Asian Pac J Trop Dis* 2012; **2**(2): 99-102.

[22] Goldin E, Ardite E, Elizalde JI. Gastric mucosal damage in experimental diabetes in rats: role of endogenous gluthatione. *Gastroenterology* 1997; **112**: 855-863.

[23] Takeuchi K, Takehara K, Tajima K, Kato S, Hirata T. Impaired healing of gastric lesions in streptozotocin-induced diabetic rats: effect of basic fibroblast growth factor. *J Pharm Exp Ther* 1997; **281**: 200-207.

[24] Tashima K, Korolkiewicz R, Kubomi M, Takeuchi K. Increased susceptibility of gastric mucosa to ulcerogenic stimulation in diabetic rats-role of capsaicin-sensitive sensory neurons. *Br J Pharmacol* 1998; **124**(7): 1395-1402.

[25] Naito Y, Takagi T, Oya-Ito T, Okada H, Suzuki T, Hirata I, et al. Impaired gastric ulcer healing in diabetic mice: role of methylglyoxal. *J Physiol Pharmacol* 2009; **60**(7): 123-130.

[26] Belfield WO, Golinsky S, Compton MD. The use of insulin in open- wound healing. Paper presented at: 81st Annual Convention of the California Veterinary Medical Association. 1969; 455-460.

[27] Harsch IA, Brzozowski T, Bazela K, Konturek SJ, Kukharsky V, Pawlik T, et al. Impaired gastric ulcer healing in diabetic rats: role of heat shock protein, growth factors, prostaglandins and proinflammatory cytokines. *Eur J Pharmacol* 2003; **481**(2-3): 249-260.

[28] Chaturvedi A, Bhawani G, Agarwal PK, Goel S, Singh A, Goel RK. Antidiabetic and antiulcer effects of extract of *Eugenia jambolana* seed in mild diabetic rats: study on gastric mucosal offensive acid-pepsin secretion. *Indian J Physiol Pharmacol* 2009; **53**(2): 137-146.

[29] Novak J, Bitsch C, Langbehn J, Pank F, Skoula F, Gotsiou Y, et al. Ratios of *cis*- and *trans*-Sabinene hydrate in *Origanum majorana* L. and *Origanum microphyllum* (Bentham) Vogel. *Biochem Systematics Ecol* 2000; **28**: 697-704.

[30] Jaiswal SK, Rao CV, Sharma B, Mishra P, Das S, Dubey MK. Gastroprotective effect of standardized leaf extract from *Argyreia speciosa* on experimental gastric ulcers in rats. *J Ethnopharmacol* 2011; **137**: 341-344.