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# Status of mucosal offensive and defensive factors in pylorus ligated-induced gastric ulceration in NIDDM rats *vis-à-vis* plantain banana

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**Abstract**

Objective: The aim of the present study was to evaluate the status of gastric mucosal defense in pylorus-ligated (PL)-induced gastric ulceration (GU) and secretion in normal and non-insulin dependent diabetes mellitus (NIDDM) rats and to study the effect of methanolic extract of dried powder of unripe plantain banana pulp (*Musa sapientum var. paradisiaca*, MSE) shown to have both ulcer protective action and hypoglycemic principle in normal and NIDDM rats with concurrent gastroduodenal ulceration. Methods: NIDDM was induced in 5 days old rat pups by administering 70 mg/kg of streptozotocin intraperitoneally and blood glucose estimation was done after 12 weeks (blood glucose level >140 mg/dL and stable). Gastric ulcers were induced both in normal (NR) and NIDDM rats by 4 h PL. MSE was used in a dose of 100 mg/kg, orally (po), once daily (od) for 6 days. Standard ulcer protective drug sucralfate (SFT) and standard oral hypoglycemic agent glibenclamide (GLC) were used in a dose of 500mg/kg and 0.6 mg/kg respectively, po, od for 6 days. Results: MSE but not SFT tended to decrease blood glucose level at the ulcer protective dose used, while GLC significantly reduced it both in normal as well as in NIDDM rats. There was an increased propensity to gastric ulceration in NIDDM-PL rats compared to the normal PL rats. NIDDM rats showed a tendency to increase in acid-pepsin secretion and decrease in mucin secretion and life span of mucosal cells. Both SFT and MSE showed significant antiulcer activity against PL-GU in NR and NIDDM rats, where as GLC showed a significant effect only against PL-GU in NIDDM rats. Both MSE (having little or no effect on offensive acid-pepsin secretion) and SFT (caused decrease in pepsin secretion) significantly increased the defensive factors like mucin secretion and life span of mucosal cells both in NR-PL and NIDDM-PL rats while, GLC reversed the above parameters only in NIDDM-PL rats near to the normal control level. Conclusion: The study thus indicated that diabetes do affect both the offensive and defensive gastric mucosal factors and correction of either blood sugar level or promotion of defensive mucosal factors do overcome the damage induced by diabetes vis a vis gastric ulceration. Plantain banana by virtue of its having both ulcer protective activity and hypoglycemic principle showed better effect than SFT or GLC alone in NIDDM rats.

**Key words:** NIDDM, Gastric ulcer, Acid-pepsin, Mucin, Cell shedding, plantain banana

## 1. Introduction

Experimental diabetes mellitus has been reported to have deleterious effect on gastric ulceration and healing in rats [1-3]. There have been reports of an increase in acid secretion in diabetes with concurrent peptic ulceration. The status of defensive mucosal factors like mucin

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secretion and life span of mucosal cells has not been studied in detail earlier. Both offensive acid-pepsin secretion and defensive mucosal factors like mucin secretion, mucosal glycoproteins, PGs, cell proliferation and life span of cell etc. have been reported to play an important role in the causation of peptic ulceration [4].

Plantain banana (*Musa sapientum Var. paradisiaca*, MSE) has been reported to possess anti-ulcer and ulcer healing activities by augmenting the mucosal defensive factors without any effect on offensive factors [5-11]. Leucocyanidin, a flavonoid has been isolated from dried powder of unripe plantain banana pulp gifted by our laboratory earlier and was reported to have anti ulcer activitiy [12]. A dimethoxy derivative of leucocyanidin 3-O-β-D-galactosyl cellobioside and ether of leucocyanidin 3-O-α-D-galactosyl cellobioside isolated from *F. bengalensis* were reported to possess antidiabetic and antioxidant activity respectively [13,14].

The present study was undertaken to examine the susceptibility of streptozotocin-induced non-insulin dependent diabetes mellitus (NIDDM) rat gastric mucosa to ulceration induced by 4 hr pylorus ligation (PL) and changes in various parameters of both gastric mucosal offensive acid-pepsin secretion and defensive mucin secretion and life span of mucosal cells in PL normal and NIDDM rats. Further the effects of sucralfate (SFT, 500mg/kg) and *Musa sapientum Var. paradisiaca* (MSE, 100 mg/kg), a standard and herbal ulcer protective drugs respectively and standard oral hypoglycemic drug, glibenclamide (GLC 0.6 mg/kg) were studied on the above ulcer model and gastric secretion parameters in both normal and NIDDM rats to assess the status of both mucosal offensive and defensive factors in diabetes with concurrent gastric ulceration vis-à-vis effects of both standard drugs GLC (hypoglycemic) and SFT (ulcer protective) and MSE on the above parameters.

## 2. Materials and Methods

### *2.1 Drug treatment*

Reported ulcer protective doses of SFT (500 mg/kg) [3] and MSE (100 mg/kg) [10] and standard antidiabetic dose of GLC (0.6 mg/kg) [3] were given orally both to normal as well as to NIDDM rats for 6 days prior to PL. Drugs were suspended in 1% carboxymethyl cellulose (CMC) in distilled water. Normal and NIDDM control groups of rats received suspension of 1% CMC in distilled water.

### *2.2 Animals*

The protocol was approved by the Institutional Animal Ethical Committee. Albino rats (CF strain) of either sex weighing between (160-200 g), obtained from the central animal house of Institute. They were kept in the departmental animal house at 26 ± 2°C, relative humidity 44-56%, under 10/14 light / dark cycles. Animals were provided with standard rodent pellet diet (Hind liver). Five days old pups, thus obtained, were either allowed to grow normal or used for production of non-insulin dependent diabetes mellitus (NIDDM) by administering streptozotocin (STZ, 70 mg/kg, ip,). After 12 weeks of STZ administration, diabetes was confirmed as detailed below. Food was withdrawn 18-24 h before the experiment, though water was allowed *ad libitum*. 'Principles of laboratory animal care' (NIH publication no. 82-23, received 1985) guidelines were followed.

### *2.3 Induction of NIDDM*

NIDDM was produced in 5 days old rat pups [15] by an intraperitoneal injection of STZ (70 mg/kg) dissolved in saline. The control pups received saline alone. The pups were weaned till one month. Twelve weeks after injection of

STZ, animals were checked for fasting glucose level and those showing glucose level greater than 140 mg/dL were considered as NIDDM rats. Blood was collected from the retro-orbital plexus of the rat and the blood glucose levels were estimated by glucose GOD-POD method (Ranbaxy diagnostic kits).

*2.4 Anti-ulcer study*

Acute gastric ulcers were produced in rats by 4 hr pylorus ligation both in normal as well as in NIDDM rats and ulcer index were calculated following the method as reported earlier [16].

*2.5 Gastric secretion study*

Gastric juice was collected 4 hr after PL and centrifuged for 5 min at 2000 rpm and the volume of the supernatant was expressed as ml/100g body weight. Total acid output was determined by titrating with 0.01*N* NaOH, using phenolphthalein as indicator and was expressed as µEq/ml as concentration and µEq/4h as output. Peptic activity was determined using hemoglobin as substrate and was expressed as µmol of tyrosine/ml as concentration and µmol/4h as output [17]. Dissolved mucosubstances were estimated in the 90% alcoholic precipitate of the gastric juice. The precipitate, thus obtained was either dissolved in 1 ml of 0.1 *N* NaOH or 1ml of 0.1 *N* $H_2SO_4$. The former was used for the estimation of protein [18], total hexoses, hexosamine and fucose, while the latter was used for the estimation of sialic acid [19]. The results are expressed in µg/ml. The ratio of total carbohydrate (TC) (sum of total hexoses, hexosamine, fucose and sialic acid) to protein (P) has been taken as the index of mucin activity [19]. DNA content was estimated and expressed as µg/ml gastric juice [9].

*2.6 Statistical analysis*

It was done either by unpaired Student's *t* test or by One Way Analysis of Variance (ANOVA) followed by Dunnett's test. The value of P less than 5 ($P<0.05$) was considered statistically significant.

Table1. Effect of methanolic extract of dried pulp of plantain banana (Musa sapientum Var. paradisiaca, MSE), sucralfate (SFT) and glibenclamide (GLC) on blood glucose level in normal (NR) and NIDDM rats

| **Oral treatment (mg/kg, od for 6 days)** | | **NR rats** | **NIDDM rats** |
|---|---|---|---|
| Control | (DW) | 98.7 ± 6.1 | 186.1 ± 5.5[a] |
| MSE | 100 | 88.1 ± 3.1 | 165.8 ± 7.8 |
| | | (-11.7%) | (-10.9%) |
| SFT | 500 | 95.1 ± 3.1 | 178.2 ± 9.6 |
| | | (-3.7%) | (-4.2%) |
| GLC | 0.6 | 72.1 ± 3.2* | 110.3 ± 10.9* |
| | | (-27.0%) | (-40.7%) |

Values in parentheses indicate % decrease from respective control group
Values are mean ± SE of 8 rats in each group, P values: [a]<0.001 compared with NR control group (Student's *t* test) and *<0.05 compared to respective NR and NIDDM control groups (One-way ANOVA followed by Dunnett's test)

## 3. Results

MSE (100 mg/kg), GLC (0.6 mg/kg) and SFT (500 mg/kg) were given orally, once daily for six days prior to PL both in normal (NR) and NIDDM rats. MSE showed a tendency to reduce blood glucose level while GLC showed a significant decrease in blood glucose level both in NR and NIDDM rats. SFT did not show any effect on blood glucose level both in NR and NIDDM rats (Table 1).

NIDDM rats showed an increased propensity to ulceration against acute gastric ulcers induced by PL as indicated by significant increase in percent ulcer index (increase by 93%) compared with the NR control group (Table 2). Both MSE and SFT showed significant ulcer protection in the above acute gastric ulcer model in NR as well as in NIDDM rats. GLC did not show any ulcer protection in NR rats but reversed the increase in ulcer index of NIDDM rats near to the NR control value in PL ulcer model (Tables 2,3).

NIDDM rats showed a tendency to increase gastric mucosal offensive acid-pepsin secretion while both MSE and GLC did not produce any effect on acid-pepsin secretion in NR rats (Table 2). MSE and GLC treatment in NIDDM rats also showed a variable but insignificant effect on acid and pepsin secretion in NIDDM rats (Table 3). However, SFT decreased pepsin secretion only both in NR and NIDDM rats (Tables 2,3).

In NR rats, both MSE and SFT either increased or tended to increase the individual carbohydrate fractions of mucoprotein and total carbohydrates and decreased the protein content of gastric juice leading to a significant increase in TC:P ratio while GLC was ineffective (Table 2). NIDDM rats showed a significant decrease in sialic acid

Table 2. Effect of orally administered MSE (100 mg/kg), SFT (500 mg/kg) and GLC (0.6 mg/kg) on gastric juice offensive acid-pepsin and defensive mucin secretion and cell shedding and ulcer index in normal and NIDDM rats.

| **Gastric Juice** | **NR** | **MSE** | **SFT** | **GLC** | **NIDDM** |
|---|---|---|---|---|---|
| Volume | 2.04 ± 0.22 | 1.94 ±0.13 | 1.92 ± 0.19 | 2.00 ± 0.20 | 2.11 ± 0.20 |
| (ml/100g BW) | | | | | |
| Acid | | | | | |
| Conc. (µEq/ml) | 88.3 ± 7.2 | 91.5 ± 8.1 | 86.9 ± 5.1 | 93.1 ± 7.3 | 103.1 ± 9.1 |
| Output (µEq/4h) | 187.9 ± 17.2 | 177.5 ± 25.8 | 166.8 ± 15.8 | 186.2 ± 26.9 | 217.5 ± 22.6 |
| Pepsin | | | | | |
| Conc. (µmol/ml) | | | | | |
| Output (µmol/4h) | 269.4 ± 33.9 | 267.7 ± 15.1 | 216.8 ± 13.3* | 309.1 ± 11.0 | 306.6 ± 24.6 |
| | 555.6 ± 47.7 | 519.3 ± 36.3 | 416.3 ± 26.6* | 558.2 ± 35.9 | 646.9 ± 66.2 |
| Mucoprotein (µg/ml) | | | | | |
| Total hexoses | 287.2 ± 25.7 | 301.3 ± 17.1 | 292.8 ± 15.2 | 250.3 ± 10.8 | 274.5 ± 17.6 |
| Hexosamine | 187.1 ± 17.9 | 189.2 ± 15.3 | 173.1 ± 12.8 | 164.1 ± 14.6 | 154.4 ± 13.1 |
| Fucose | 53.1 ± 4.1 | 67.3 ± 5.7 | 65.7 ± 3.0 | 57.8 ± 2.3 | 46.2 ± 4.6 |
| Sialic acid | 33.9 ± 4.1 | 40.2 ± 3.9 | 39.8 ± 2.1 | 26.8 ± 1.5 | 19.3 ± 2.8[a] |
| Total carbohydrates (TC) | 561.3 ± 29.3 | 598.0 ± 31.1 | 571.4 ± 17.5 | 499.0 ± 19.6 | 494.4 ± 27.3 |
| Protein (P) | 530.1 ± 39.9 | 486.3 ± 27.3 | 439.5 ± 17.0* | 526.2 ± 19.0 | 698.2 ± 43.1[a] |
| TC:P | 1.06 ± 0.06 | 1.23 ± 0.05* | 1.30 ± 0.08* | 0.95 ± 0.07 | 0.72 ± 0.08[b] |
| Cell shedding | | | | | |
| (DNA µg/ml) | 265.3 ± 19.7 | 201.7 ± 17.1* | 162.7 ± 12.0* | 245.4 ± 16.3 | 341.7 ± 25.1[a] |
| Ulcer index | 15.1 ± 2.3 | 5.9 ± 2.5* | 4.0 ± 1.6* | 14.2 ± 1.3 | 29.2 ± 3.5[a] |

Values are mean ± SE of 8 rats in each group, P values: [a]<0.05, [b]<0.01 compared with respective NR group (Student's *t* test) and *<0.05 compared to respective NR groups (One-way ANOVA followed by Dunnett's test)

and total carbohydrates but increase in protein content leading to a significant decrease in mucin secretion (Table 2). However, all the three test drugs showed a reversal in the TC:P ratio decreased in NIDDM rats near to the NR control level (Table 3).

Both MSE and SFT showed decrease in cell shedding in terms of decrease in DNA (µg/ml) content of gastric juice while GLC was ineffective in PL normal rats. NIDDM rats showed an increase in mucosal shedding (Table 2). Again, the enhanced cell shedding induced by NIDDM tended to reverse or reversed by all the above test agents (Table 3).

## 4. Discussion

Recently we reported aggravation of acute gastric ulcers induced by CRS, ASP, EtOH and PL and delayed ulcer healing of chronic gastric ulcers induced by acetic acid and HCl respectively in NIDDM rats and this aggravation of ulcer/delayed healing in NIDDM rats were reversed by agents correcting blood sugar level [3]. Ulcerations by pyloric ligation-induced ulcers are thought to be due to autodigestion of mucosa by gastric juice leading to breakdown of mucosal barrier [16]. In the present study STZ-induced NIDDM rats showed increased propensity to acute gastric ulceration induced

Table 3. Effect of orally administered MSE (100 mg/kg), SFT (500 mg/kg) and GLC (0.6 mg/kg) on gastric juice offensive acid-pepsin and defensive mucin secretion and cell shedding in NIDDM rats

| **Gastric Juice** | **NIDDM** | **NIDDM + MSE** | **NIDDM + SFT** | **NIDDM + GLC** |
|---|---|---|---|---|
| Volume (ml/100g BW) | 2.11 ± 0.20 | 1.91 ± 0.22 | 1.88 ± 0.13 | 2.07 ± 0.25 |
| Acid | | | | |
| Conc. (µEq/ml) | 103.1 ± 9.1 | 92.2 ± 12.9 | 96.3 ± 9.2 | 88.8 ± 9.2 |
| Output (µEq/4h) | 217.5 ± 22.6 | 176.1 ± 29.3 | 181.0 ± 23.2 | 183.8 ± 28.0 |
| Pepsin | | | | |
| Conc. (µ mol/ml) | 306.6 ± 24.6 | 285.2 ± 24.4 | 230.2 ± 23.1* | 299.1 ± 19.7 |
| Output (µmol/4h) | 646.9 ± 66.2 | 544.7 ± 76.6 | 432.8 ± 33.2 | 619.1 ± 54.0 |
| Mucoprotein (µg/ml) | 274.5 ± 17.6 | 284.1 ± 17.9 | 320.0 ± 16.8 | 286.9 ± 13.0 |
| Total hexoses | 154.4 ± 13.1 | 183.3 ± 12.0 | 175.3 ± 11.7 | 169.9 ± 9.0 |
| Hexosamine | 46.2 ± 4.6 | 60.3 ± 7.2 | 67.7 ± 6.7 | 61.3 ± 5.0 |
| Fucose | 19.3 ± 2.8 | 34.1 ± 3.7* | 35.8 ± 2.5* | 29.1 ± 2.8 |
| Sialic acid | | | | |
| Total carbohydrates | 494.4 ± 27.3 | 561.8 ± 29.3 | 598.8 ± 27.1 | 547.2 ± 19.1 |
| (TC)1 | 698.2 ± 43.1 | 556.8 ± 35.5 | 596.8 ± 27.3 | 590.0 ± 45.0 |
| Protein (P) | 0.72 ± 0.05 | 1.01 ± 0.08* | 1.00 ± 0.09* | 0.93 ± 0.07* |
| TC:P | | | | |
| Cell shedding (DNA µg/ml) | 341.7 ± 25.1a | 230.0 ± 27.9* | 236.9 ± 21.3* | 275.0 ± 16.0 |
| Ulcer index | 29.2 ± 3.5 a | 8.1 ± 2.7* | 11.3 ± 2.8* | 17.8 ± 2.5* |

Values are mean ± SE of 8 rats in each group, P value: *<0.05 compared to respective NIDDM control group (One-way ANOVA followed by Dunnett's test)

by PL. STZ-induced diabetes has been reported to impair the gastric hyperemic response induced by back diffusion $H^+$ ion, following barrier disruption and leads to increase mucosal susceptibility to acid injury. In the diabetic rats, gastric acid secretion is stimulated at a basal level, presumably due to increase in the concentrations of histamine and gastrin in oxyntic mucosa [20]. Pentagastrin, carbachol and peptone-induced gastric acid output were increased in STZ diabetic rats [21]. The increased acid back diffusion has been reported to play an important role in the formation of acute hemorrhagic ulceration and impaired duodenal $HCO_3^-$ secretion in rats, possibly as a result of decreased sensitivity of the epithelial cell and dysfunction of neuronal pathway and was reported to increase the mucosal susceptibility to acid injury in the duodenum.

Both SFT and MSE showed significant ulcer protective effect against PL- induced acute gastric ulcers in normal as well as in NIDDM rats. The antiulcer effect of MSE and SFT may be due to their actions on gastric mucosal offensive and defensive factors though both tended to decrease acid secretion while, pepsin secretion was decreased significantly by SFT in NIDDM rats. On the other hand, GLC did not show any effect on acid-pepsin secretion in NR rats but tended to decrease them in NIDDM rats.

Both SFT and MSE significantly increased dissolved mucus as seen from the increase in TC: P ratio in both normal and NIDDM rats, which is taken as reliable marker for mucin secretion [22]. Mucus is endowed with an array of mucosal protective properties and also acts as a first line of defense. Thus significant increase in defensive factors may account for a major part in the activity of SFT and MSE, which have already been reported earlier [8]. Further, mucosal cell exfoliation was decreased by both SFT and MSE but not by GLC in NR rats though all were effective in decreasing an increased cell exfoliation induced by NIDDM and they thus, lead to strengthening of the mucosal barrier in NIDDM rats as observed from decrease in DNA content in gastric juice, which is taken as a reliable marker for cell shedding.

Plantain banana was taken in the present study because of presence of lecocyanidin flavonoid [12] reported to possess anti-ulcer property. Further its derivatives have been reported to possess anti-diabetic and anti-oxidant activity. GLC being an oral hypoglycemic agent only decreased the blood glucose level and decreased ulcer index only in NIDDM rats while, SFT showed predominant effect on mucosal defensive factors leading to protection both in normal as well as in NIDDM-PL rats. MSE was thus found to be more effective than standard anti-diabetic drug GLC or ulcer protective drug SFT in NIDDM rats. This may be due to the dual action of MSE both on blood sugar level (by virtue of presence of active ingredient leucocyanidin, [12-14]) and mucosal defensive factors [11].

## 5. Conclusion

Our results taken together suggest that the diabetic conditions increased the vulnerability of the gastric mucosa to ulceration by its various effects on mucosal offensive acid-pepsin secretion and defensive mucosal factors like mucin secretion and life span of mucosal cells. The ulcer protective effect of MSE was better than that of GLC both in normal and NIDDM rats which could be because of the presence of leucocyanidin flavonoid, reported to possess anti-ulcer activity while its derivatives were reported to possess both anti-diabetic and antioxidant properties reported to be useful in ulcer protection in diabetic rats.

However, further studies on the effects of MSE (leucocyanidin or other active principles present in it) on insulin signaling pathways and other mucosal defensive mechanisms may provide more insight on the therapy of NIDDM and NIDDM plus peptic ulceration.

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## References

1. Takeuchi K, Ueshima K, Ohuchi T, Okabe S. (1994) *Dig. Dis. Sci*. 39: 626-634.

2. Tashima K, Korolkiewiez RP, Kubomi M, Takeuchi K. (1998) *Br. J. Pharmacol*.124: 1395-1402.

3. Dorababu M, Prabha T, Priyambada S, Agrawal VK, Aryaa NC, Goel RK. (2004) *Indian J. Exp*. Biol. 42: 389-397.

4. Goel RK, Bhattacharya SK. (1991) *Indian J. Exp. Biol*. 29: 701-714.

5. Best R, Lewis DA, Nasser N. (1984) *Br. J. Pharmacol*. 82: 107-116.

6. Goel RK, Chakrabarti A, Sanyal AK. (1985) *Planta Medica*. 2: 85-88.

7. Goel RK, Das DG, Sanyal AK. (1985) *Indian J. Gastroenterol*. 4: 249-251.

8. Goel RK, Gupta S, Shankar R, Sanyal AK. (1986) *J. Ethnopharmacol*. 18: 33-44.

9. Mukhopadhyaya K, Bhattacharya D, Chakrabarti A, Goel RK, Sanyal AK. (1987) *J. Ethnopharmacol*. 21: 11-19.

10. Goel RK, Sairam K, Rao ChV, Raman A. (2001) *Indian J. Exp. Biol*. 39: 719-722.

11. Goel RK, Sairam K. (2001) *Indian J. Pharmacol*. 34 (2): 100-110.

12. Lewis DA, Fields WN, Shaw GP. (1999) *J. Ethnopharmacol*. 65: 283-288.

13. Vinod kumar R, Augusti KT. (1989) *Indian J. Biochem. Biophys*. 26: 400-404.

14. Daniel RS, Biju CM, Devi KS. (1998) *Indian J. Exp. Biol*. 36: 902-906.

15. Srinivas PS, Hakim JS, Santani DD, Goyal RK. (1997) *Pharmacol. Res*. 35: 423-428.

16. Sanyal AK, Pandey BL, Goel RK. (1982) *J. Ethnopharmacol*. 5: 79-89.

17. Debnath PK, Gode KD, Govinda Das D, Sanyal AK. (1974) *Br. J. Pharmacol*. 51: 213-216.

18. Lowry OH, Rosenborough NJ, Farr AL, Randall RJ. (1951) *J. Biol. Chem*. 193: 265-275.

19. Sanyal AK, Mitra PK, Goel RK. (1983) *Indian J. Exp. Biol*. 21: 78-80.

20. Inui H, Yasuno R, Takenoshita M, Ohnishi Y, Sakamoto M, Matsuzaki J, Yamaji R, Miyatake K, Yamatodani A, Nakano Y. (2000) *J. Nutr. Sci. Vitaminol*. (Tokyo) 46(3): 144-148.

21. Tashima K, Nishijima M, Fujita A, Kubomi M, Takeuchi K. (2000) *Dig. Dis. Sci*. 45(7): 1352-1358.

22. Menguy R, Desbaillets L. (1968) *Annals Surgery*. 168: 475-482.