Research Paper

Effects of *Pterocarpus marsupium* on NIDDM-induced rat gastric ulceration and mucosal offensive and defensive factors

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Received: 12.9.2003
Revised: 23.2.2004
Accepted: 29.2.2004

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ABSTRACT

Objective: To evaluate the vulnerability of gastric mucosa to ulceration in non-insulin-dependent diabetes mellitus (NIDDM) rats *vis*-à-*vis* the protective effects of the methanolic extract of *Pterocarpus marsupium* heartwood (PMS, an antidiabetic herbal plant).

Material and Methods: NIDDM was produced in 5-day-old rat pups by administering streptozotocin (70 mg/kg, i.p). The animals showing blood glucose level > 140 mg/dl after 12 weeks of STZ administration were considered as NIDDM positive rats. The effective hypoglycemic dose of PMS (750 mg/kg/day, p.o.) for 6 days was studied for its gastric ulcer (GU) protective effects against cold restraint stress (CRS), aspirin (ASP), ethanol (EtOH) and pylorus ligation (PL)-induced GU both in normal (NR) and NIDDM rats. To ascertain the mechanism of action, the effects of NIDDM and that of PMS treatment in NIDDM rats on mucosal offensive acid-pepsin, free-radicals (LPO,NO) and defensive mucin secretion, cell shedding, cell proliferation, glycoproteins and antioxidant enzymes (SOD and CAT) were studied.

Results: PMS (750 mg/kg) decreased the blood sugar level both in NR and NIDDM rats. NIDDM rats exhibited an increased propensity to GU, induced by CRS, ASP, EtOH and PL. Though, PMS did not protect the NR rats against GU induced by the above methods it reversed their increased propensity in NIDDM rats. NIDDM PL-rats showed an increase in acid-pepsin secretion, cell shedding and decrease in mucin secretion and mucosal glycoproteins with little effect on cell proliferation. PMS treatment in NIDDM rats reversed the acid-pepsin secretion, enhanced mucin and mucosal glycoproteins and decreased cell shedding without any effect on cell proliferation. NIDDM-CRS rats showed a significant increase in LPO and NO and a decrease in SOD and CAT levels, which were, reversed by PMS treatment.

Conclusion: NIDDM increased the propensity to GU by affecting both offensive (increased) and defensive (decreased) mucosal factors. Though PMS, a hypoglycemic agent, did not show any protection against ulceration induced by CRS, ASP, EtOH and PL in normal rats, it protected the mucosa against the same in NIDDM rats by affecting the above mucosal offensive and defensive factors.

KEY WORDS: Diabetes mellitus, peptic ulcer, gastric mucosa, vijaysar

Introduction

Recent experimental studies indicate that a prolonged diabetic state produces deleterious influences on various functions of the gastrointestinal tract with reports of an aggravation of gastric mucosal ulcerogenic responses to starvation or stress in diabetic rats.[1,2] Peptic ulcer occurs due to an imbalance between offensive acid-pepsin secretion and free radical generation and defensive mucosal factors which include mucin-bicarbonate secretions, lifespan of mucosal cells, cell proliferation, glycoproteins, and antioxidant enzymes status.[3,4]

Diabetes being a chronic disease may lead to a decrease in the mucosal defensive factors with a concomitant increase in propensity to ulceration in response to various physical (cold restraint stress and pylorus ligation) and chemical (aspirin, ethanol) agents. The changes induced by diabetes (DM) could

be reversed by using drugs which can either promote mucosal defensive factors or correct the blood glucose levels or both.[5] Hence, we selected *Pterocarpus marsupium* (PMS, Leguminnaceae family), commonly known as vijaysar that has been recommended as early as 1000 BC by Sushruta for the treatment of diabetes. Various reports indicate the hypoglycemic activity of PMS both in experimental and clinical studies.[6-8] The objectives of the present study were to evaluate the effects of PMS on the susceptibility of the gastric mucosa of NIDDM rats to various ulcerogenic stimuli. The results were also compared with the standard oral hypoglycemic drug, glibenclamide and ulcer protective drug, sucralfate, on the ulcer models in NR/NIDDM rats.

Material and Methods

Animals: Adult Inbred Charles-Foster (CF) albino mice (20-22 g) and rats (130-180 g), of either sex, obtained from the central animal house of the Institute of Medical Sciences, Banaras Hindu University, Varanasi, were kept in the departmental animal house at 26±2° C and relative humidity 44-56%, light and dark cycles of 10 and 14 h respectively. The mice were used for acute toxicity study to find the LD_{50} dose of the test drug, PMS, while the rats were kept for breeding to obtain pups. The 5-day-old pups were divided into two groups, one group received streptozotocin to induce non-insulin dependent diabetes mellitus (NIDDM), while the other group served as normal (NR) control. Animals were provided with standard rodent pellet diet (Hind liver) and the food was withdrawn 18 h before the experiment though water was allowed *ad libitum*. 'Principles of laboratory animal care' guidelines were followed and prior permission was sought from the Institute Animal Ethics Committee for conducting the study.

Drug collection and extraction: The heartwood of PMS (Ayurvedic Gardens, Banaras Hindu University) was collected and identified with the standard sample preserved in the department of Dravyaguna, Institute of Medical Sciences, Varanasi. The heartwood was cut into very small pieces and macerated with methanol for 7 days. The extract (PMS) was vacuum dried and stored in a refrigerator until further use. The yield was 9.2 %.

Drug treatment

Acute toxicity study (Mice): To find the LD_{50} of PMS, six groups of mice, containing six in each group, were given PMS in the doses of 500, 1000, 2000, 4000 and 8000 mg/kg orally, while the control group received 1% carboxymethyl cellulose (CMC) in distilled water. The concentration of test drug/vehicle was so prepared that each animal received 1 ml/100 g body weight of the test solution/suspension. The animals were observed for 5 min every 30 min till 2 h and then at 4, 8 and 24 h after treatment for any behavioral changes/mortality. They were further observed daily for 7 days for mortality.

Rat study: PMS, glibenclamide (GLC, 0.6 mg/kg)[9] and sucralfate (SFT, 500 mg/kg)[4] were suspended in 1% CMC in distilled water (1 ml/100 g, bw). The test drugs were administered orally, once daily for five days and a last dose on Day 6 in 18 hr-fasted rats, one hour before the experiments on NR or NIDDM rats. The control group of animals received suspension of 1% CMC in distilled water. A dose-dependent hypoglycemic effect of PMS (500, 750 and 1000 mg/kg) was seen on the blood glucose level, both in NR and NIDDM rats and an optimal dose of 750 mg/kg was selected for antiulcer studies.

Experimental methods

Induction of Type II Diabetes mellitus (NIDDM) in rats: NIDDM was induced by injecting streptozotocin (STZ, 70 mg/kg, i.p.) to 5-day-old rat pups.[10] The control pups received saline alone. The pups were weaned after one month. Twelve weeks after injection of STZ, the rats were checked for fasting glucose level and those with glucose level greater than 140 mg/dl were considered as NIDDM rats and used for further studies.

Estimation of blood glucose: Blood samples were collected from the retro-orbital plexus of the rat and the blood glucose level was estimated by the GOD-POD method (Ranbaxy diagnostic kits, New Delhi).

Antiulcer study

The following experimental rat gastric ulcer models were used.[4]

Cold restraint stress (CRS)-induced ulcers: On Day 6, 18 h-fasted normal (NR) and NIDDM rats were subjected to cold restraint stress by strapping on a wooden plank and keeping them for 2 h at 4-6°C. The animals were then sacrificed by cervical dislocation and ulcers were scored on the dissected stomachs. The ulcer index was calculated by adding the total number of ulcers per stomach and the total severity of ulcers per stomach.[11]

Pylorus-ligation (PL)-induced ulcers: Drugs were administered for a period of 5 days as described above. On Day 6, 1 h after administration of the test drugs, the animals (fasted for 18 h) were anaesthetized using pentobarbitone (35 mg/kg, i.p.), the abdomen was opened and pylorus ligation was done without causing any damage to its blood supply. The stomach was replaced carefully and the abdomen closed in layers. The animals were deprived of water during the postoperative period and were sacrificed with an overdose of ether after 4 h. Stomachs were dissected out and the contents collected for estimation of biochemical parameters. Ulcer index was calculated as described in the CRS group.

Aspirin (ASP)-induced ulcers: ASP at a dose of 200 mg/kg (20 mg/ml suspension in 1% CMC) was administered orally to 18 h-fasted animals, 1 h after administration of the test drug on Day 6. The ulcers were scored after 4 h. The stomach was taken out and cut open along the greater curvature and ulcers were scored by a person unaware of the experimental protocol in the glandular portion of the stomach. Ulcer index was calculated as described in the CRS group.

Ethanol (EtOH)-induced ulcers: The test drugs were administered in the same dose and duration as mentioned above. One hour after the administration of the last dose of test drugs to 18 h-fasted rats on Day 6, gastric ulcers were induced by administering EtOH (95%, 1 ml/200 g). The animals, 1 h after administration of EtOH, were sacrificed by cer-

vical dislocation and the stomach was taken out and incised along the greater curvature and examined for ulcers. The ulcer index was scored based upon the product of the length and width of the ulcers present in the glandular portion of the stomach (mm^2/ rat).

Statistical analysis for the above models was done by using one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparisons test. The difference were considered to be significant when $P<0.05$.

Gastric secretion study

The gastric juice was collected 4 h 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/4h. Peptic activity was determined using hemoglobin as substrate and was expressed as μmol of tyrosine/4 h.[12] 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 1 ml of 0.1 N H_2SO_4. The former was used for the estimation of protein,[12] total hexoses, hexosamine and fucose, while the latter was used for the estimation of sialic acid.[13] 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.[14] DNA content was estimated and expressed as mg/ml gastric juice.[15]

Estimation of mucosal glycoproteins

Samples of gastric mucosal scraping were homogenized in distilled water and treated with 90% ethanol. The carbohydrates and the proteins in the samples were estimated using the methods described above for gastric juice contents.[16]

Cell proliferation

Mucosal scraping was homogenized in 2.5 ml of ice-cold 0.6 N perchloric acid (PCA). DNA[17] and protein,[13] were then estimated. Cell proliferation was expressed as mg DNA/ mg protein.

Estimation of free radical generation

The fundus of the stomach was homogenized (5%) in ice-cold 0.9% saline with a Potter - Elvehjem glass homogeniser for 30 sec. The homogenate was used for the following estimations.

Lipid peroxidase (LPO) activity: LPO product malondialdehyde (MDA) was estimated using 1,1,3,3-tetraethoxypropane as the standard and was expressed as nmoles/g wet tissue.[18]

Superoxide dismutase (SOD) activity: SOD was estimated by following the procedure of Kakkar and associates, 1984.[19] The inhibition of the reduction of nitro blue tetrazolium (NBT) to blue-colored formozan in the presence of phenazine metha sulphate (PMS) and NADH was measured at 560 nm using n-butanol as blank. One unit of enzyme activity was defined as the amount of enzyme that inhibits the rate of reaction by 50% in 1 min under the defined assay conditions and the result has been expressed as units (U) of SOD activity/g wet tissue.

Catalase (CAT) activity: Decomposition of H_2O_2 in presence of catalase was followed at 240 nm.[20] One unit (U) of CAT was defined as the amount of enzyme required to decompose 1 m mol of H_2O_2 per min, at 25° C and pH 7.0. Result was expressed as U of CAT activity/g wet tissue.

Estimation of nitric oxide: Nitric oxide levels were estimated by the Griess reaction method[21] spectrophotometrically. Result was expressed as μmol/g wet tissue.

Statistical analysis for the above estimations was done by unpaired 't' test when control and diabetes groups were compared. One-way analysis of variance (ANOVA) followed by Dunnett's test was used for multiple comparisons. The differences were considered to be significant when $P<0.05$.

Results

Acute toxicity study: The result of the acute oral administration of PMS in various doses of 500, 1000, 2000, 4000 and 8000 mg/kg indicated no mortality up to 7 days after treatment.

Effect on blood glucose: PMS (500, 750 and 1000 mg/kg) dose-dependently decreased the blood glucose levels in both NR and NIDDM rats. The effect was comparable to the oral hypoglycemic drug, glibenclamide (NR 26.6% decrease and NIDDM 42.9% decrease). However, SFT did not show any effect on blood glucose level both *per se* as well as in NIDDM rats (Table 1).

Ulcer-protective effects: NIDDM rats showed increased propensity to ulceration in all models of rat gastric ulcers (156.1 to 193.4% increase, $P<0.05$). PMS showed more ulcer protective activity in NIDDM rats in various ulcer models (25.8 to 44.5% protection) as compared to NR rats (3.1 to 16.5%), while SFT showed significant ulcer-protective effect both in NR and NIDDM rats. Glibenclamide (GLC), like PMS, did not show any ulcer-protective activity in NR rats but it reversed the increased propensity to ulceration in NIDDM rats (Tables 2 and 3).

Effect on gastric juice and mucosal parameters: NIDDM rats showed a tendency to increase gastric mucosal offensive acid-pepsin secretion (acid output- 11.8% increase; peptic output-11.5% increase) but defensive mucin secretion was decreased significantly as indicated by decrease in TC: P ratio (35.7% decrease, $P<0.05$). NIDDM rats also showed an increase in gastric mucosal cell shedding (as indicated by an increase in DNA content in gastric juice, 29 % increase, $P<0.05$) (Table 4), a decrease in mucosal glycoproteins (28.2% decrease, $P<0.05$) with little or no effect on cell proliferation (Table 5). PMS treatment in NIDDM rats reversed the increased output of acid (16.1% reversal) and pepsin (15% reversal), decreased cell shedding (27.5 % reversal), enhanced mucin (27.8 % reversal) and mucosal glycoproteins (28.6 % reversal) without any effect on cell proliferation. Sucralfate (SFT), an ulcer-protective drug, did not show any effect on acid output but it significantly decreased the peptic output. It also increased the mucin secretion and mucosal glycoproteins and decreased cell shedding in NIDDM rats (Tables 4 and 5). Standard oral anti-diabetic drug, GLC did not show any effect on acid-pepsin secretion but it pre-

Table 1

Effect of graded doses of methanolic extract of *Pterocarpus marsupium* heartwood (PMS), glibenclamide (GLC) and sucralfate (SFT) on blood glucose level in normal and NIDDM rats

Oral treatment (mg/kg, p.o., od x 6 days)		*Normal rats*		*NIDDM rats*	
		Before treatment	*After treatment*	*Before treatment*	*After treatment*
Control	(DW)	100.3 ± 5.9	98.7 ± 6.1 (-1.60%)	170.9 ± 8.9	168.1 ± 8.3 (-1.64%)
PMS	500	105.1 ± 5.9	90.3 ± 4.7 (-14.1%)	170.8 ± 8.1	138.8 ± 9.8 (-18.7%)
	750	107.3 ± 6.0	77.2 ± 6.4* (-28.1%)	180.8 ±12.3	123.0 ± 7.2* (-32.0%)
	1000	101.3 ± 5.7	61.3 ± 5.3* (-39.5%)	169.6 ± 10.3	107.3 ± 6.7* (-36.7%)
GLC	0.6	98.2 ± 4.3	72.1 ± 3.2* (-26.6%)	193.2 ± 7.9	110.3 ±10.9* (-42.9%)
SFT	500	97.6 ± 3.8	95.1 ± 3.1 (-2.60%)	180.6 ± 7.8	178.2 ± 9.8 (-1.33%)
One-way ANOVA		F	8.911		11.288
		df	42,5		42,5
		P	<0.001		<0.001

Each value represents mean±SEM of 8 rats in each group. Values in parentheses indicate % change.
One-way analysis of variance (ANOVA) followed by Dunnett's multiple comparisons test was applied both in NR and NIDDM rats groups. The difference were considered to be significant when **P<0.05* compared to respective NR- and NIDDM-control groups.

Table 2

Effect of PMS, GLC and SFT on 2 h cold restraint stress (CRS)-, aspirin (ASP, 200 mg/kg, p.o., 4 h)-induced gastric ulcers in normal and NIDDM rats

Oral treatment (mg/kg, od x 6 days)			*Normal rats*		*NIDDM rats*	
			Ulcer index	*% protection*	*Ulcer index*	*% protection*
CRS–induced ulcers						
Control	(DW)		29.6 ± 2.9	-	46.2 ± 5.8**	-
PMS	750		24.7 ± 5.3	16.5	29.0 ± 5.4	37.2
GLC	0.6		24.3 ± 2.7	17.9	30.3 ± 2.3*	34.4
SFT	500		9.7 ± 1.3*	67.2	27.3 ± 3.8*	40.9
One-way ANOVA		F	6.634		3.713	
		df	28,3		28,3	
		P	<0.002		<0.023	
ASP-induced ulcers						
Control	(DW)		18.7 ± 2.3	-	32.4 ± 3.9**	-
PMS	750		16.3 ± 2.6	12.8	20.0 ± 2.4*	38.3
GLC	0.6		17.9 ± 2.5	4.3	21.0 ± 2.1*	35.2
SFT	500		3.7 ± 0.7*	80.2	16.7 ± 2.1*	48.5
One-way ANOVA		F	10.133		5.126	
		df	28,3		28,3	
		P	<0.001		<0.002	

Each value represents mean ± SEM of 8 rats in each group.
One-way ANOVA followed by Dunnett's multiple comparisons test was applied both in NR and NIDDM rats groups. The difference was considered to be significant when **P<0.05*. The different NIDDM groups were also compared with the NR control group. The difference was considered to be significant when ***P<0.05* compared to respective NR-control groups.

vented the decrease in mucin secretion and mucosal glycoproteins and increase in DNA content in gastric juice in NIDDM rats without any effect on cell proliferation (Tables 4 and 5).

Effect on free radicals: Oxidative free radicals LPO and NO levels were increased significantly both in CRS and NIDDM rats. Both LPO and NO were further increased in NIDDM-CRS rats when compared to NIDDM rats. Both PMS and GLC tended to reverse or reversed these enhanced free radicals in NIDDM-CRS rats (Table 6).

Table 3

Effect of PMS, GLC and SFT on ethanol (EtOH, 100%, 1 ml/200 g, p.o., 1h)- and 4 h pylorus ligation (PL)-induced gastric ulcers in normal and NIDDM rats

Oral treatment (mg/kg, od X 6 days)			*Normal rats*		*NIDDM rats*	
			Ulcer index	*% protection*	*Ulcer index*	*% protection*
EtOH-induced ulcers						
Control	(DW)		19.5 ± 3.8	-	36.5 ± 3.1**	-
PMS	750		18.9 ± 2.9	3.1	27.1 ± 3.9	25.8
GLC	0.6		18.5 ± 2.8	5.1	28.8 ± 3.3	21.1
SFT	500		6.9 ± 1.6*	64.6	21.2 ± 3.4*	41.9
One-way ANOVA		F	4.416		3.368	
		df	28,3		28,3	
		P	<0.012		<0.032	
PL-induced ulcers						
Control	(DW)		15.1 ± 2.3	-	29.2 ± 3.5**	-
PMS	750		13.4 ± 2.6	11.3	16.2 ± 4.3*	44.5
GLC	0.6		14.2 ± 1.3	6.0	17.8 ± 2.5	39.0
SFT	500		4.0 ± 1.6*	73.5	11.3 ± 2.8*	61.3
One-way ANOVA		F	4.382		5.085	
		df	28,3		28,3	
		P	<0.012		<0.006	

Each value represents mean ± SEM of 8 rats in each group.
One-way ANOVA followed by Dunnett's multiple comparisons test was applied both in NR and NIDDM rats groups. The difference was considered to be significant when **P<0.05*. The different NIDDM groups were also compared with the NR control group. The difference was considered to be significant when ***P<0.05* compared to respective NR-control groups.

Table 4

Effect of PMS, GLC and SFT on NIDDM-induced changes in gastric juice parameters

Parameters	*Normal control*	*NIDDM control*	*NIDDM +PMS*	*NIDDM +GLC*	*NIDDM +SFT*
Volume (ml/100 gm/bw)	2.0 ± 0.2	2.0 ± 0.2	1.7 ± 0.2	2.1 ± 0.3	2.1 ± 0.2
Acid output (µEq/ 4 h)	188 ± 17	210 ± 23	180 ± 20	188 ± 28	221 ± 23
Pepsin output (µmol/4 h)	556 ± 48	620 ± 66	527 ± 39	621 ± 54	477 ± 33
Mucoproteins (µg/ml)					
Total hexoses	317 ± 26	275 ± 18	267 ± 18	287 ± 13	320 ± 17
Hexosamine	187 ± 18	154 ± 13	173 ± 14	170 ± 9	175 ± 12
Fucose	53 ± 4	46.2 ± 4.6	59 ± 5	61 ± 5	68 ± 7
Sialic acid	34 ± 4	19.3 ± 2.8**	30 ± 3*	29 ± 3	36 ± 3*
Total carbohydrate (TC)	591 ± 29	494 ± 27**	528 ± 21	547 ± 19	599 ± 27
Protein (P)	530 ± 40	698 ± 43**	574 ± 45	590 ± 45	597 ± 27
TC: P	1.12 ± 0.09	0.72 ± 0.05**	0.92 ± 0.06	0.93 ± 0.07*	1.00 ± 0.09*
Cell shedding					
DNA (µg/ml)	265 ± 20	342 ± 25**	248 ± 15*	275 ± 16	237 ± 21*

Each value represents mean ± SEM of 8 rats in each group.
One-way ANOVA followed by Dunnett's multiple comparisons test was applied for comparing the NIDDM groups with the NR control group. The difference was considered to be significant when ***P<0.05* compared to NR-control group. The Test was also applied separately in NIDDM groups. The difference was considered to be significant when **P<0.05* compared to NIDDM-control group.

Effect on antioxidant enzymes: Antioxidant enzymes SOD and CAT were significantly decreased in NIDDM rats. SOD level was increased but CAT level was decreased in NR-CRS rats (Table 4). However, both SOD and CAT were not affected further in NIDDM-CRS rats compared to the NIDDM group. Both PMS and GLC treatments significantly reversed SOD and CAT levels in NIDDM-CRS rats (Table 6).

Discussion

Our present study showed that gastric ulcers induced by various (physical and chemical) agents were aggravated in STZ-induced NIDDM rats. This confirmed the earlier observations where increased propensity to ulceration in both experimental and clinical diabetes was reported.[2] The increased suscep-

Table 5

Effect of PMS, GLC and SFT on NIDDM-induced changes in gastric mucosal parameters

Parameter	*Normal Control*	*NIDDM Control*	*NIDDM +PMS*	*NIDDM +GLC*	*NIDDM +SFT*
Glycoproteins (µg/ml)					
Total hexoses	2810 ± 181	2276 ± 129**	2820 ± 189*	2729 ± 123	2714 ± 97*
Hexosamine	1648 ± 88	1546 ± 53	636 ± 47	1604 ± 33	1778 ± 81
Fucose	288 ± 29	208 ± 17**	278 ± 15*	290 ± 17*	311 ± 21*
Sialic acid	106 ± 9	68 ± 11**	92 ± 7	88 ± 7	99 ± 9
Totalcarbohydrate (TC)	4852 ± 270	4098 ± 219**	4826 ± 181	4711 ± 129	4902 ± 201*
Protein (P)	6240 ± 476	7280 ± 489	6674 ± 329	6518 ± 412	6728 ± 413
TC : P	0.78 ± 0.06	0.56 ± 0.06**	0.72 ± 0.05	0.72 ± 0.05	0.73 ± 0.05*
Cell proliferation					
Proteins (µg)	6996 ± 562	7173 ± 671	7066 ± 701	7143 ± 593	6658 ± 497
DNA (µg)	757 ± 32	726 ± 21	747 ± 24	761 ± 34	703 ± 32
µg DNA/mg protein	108 ± 9	101 ± 5	106 ± 6	107 ± 4	106 ± 6

Each value represents mean ± SEM of 8 rats in each group.
One-way ANOVA followed by Dunnett's multiple comparisons test was applied for comparing the NIDDM groups with the NR control group. The difference was considered to be significant when ***P<0.05* compared to NR-control group. The Test was also applied separately in NIDDM groups. The difference was considered to be significant when **P<0.05* compared to NIDDM-control group.

Table 6

Effect of PMS and GLC on gastric mucosal LPO, NO, SOD and CAT levels in NIDDM-CRS rats

Treatment (mg/kg, od, p.o. *X 6 days)*		*LPO (nmol/g wet tissue)*	*NO (µmol/g wet tissue)*	*SOD (Units/g wet tissue)*	*CAT (Units/g wet tissue)*
NR		242.8 ± 17.0	241.3 ± 6.3	93.1 ± 9.1	25.3 ± 2.5
NR-CRS		333.7 ± 15.7	283.2 ± 8.2*	126.4 ± 10.4*	19.0 ± 3.0
NIDDM		315.0 ± 14.3	305.0 ± 8.9*	56.4 ± 4.2*, @	14.8 ± 1.8*
NIDDM + CRS		452.1 ± 36.3*, @, #	330.1 ± 12.2*, @	52.1 ± 4.8*, @	13.4 ± 1.9*
NIDDM + CRS+ PMS 750		318.3 ± 26.1$	299.0 ± 8.8*	87.1 ± 5.8@, #, $	18.7 ± 1.9
NIDDM + CRS+ GLC 0.6		391.5 ± 40.3*	285.7 ± 9.4*, $	91.0 ± 4.2@, #, $	22.4 ± 2.0#, $
One-way ANOVA	F	7.074	9.599	15.879	4.033
	df	42,5	42,5	42,5	42,5
	P	<0.01	<0.001	<0.004	<0.004

Each value represents mean ± SEM of 8 rats in each group.
One-way ANOVA followed by Dunnett's multiple comparisons test showed as **P<0.05* compared to respective NR-control, *@P<0.05* compared to respective NR-CRS, *#P<0.05* compared to respective NIDDM group, *$P<0.05* compared to respective NIDDM-CRS group.

tibility to ulceration could be due to back diffusion of hydrogen ions in the stomach of diabetic rats, which played an important role in the formation of acute hemorrhagic ulcers.[2] Gastric ulcers have multiple etiopathogenesis. Ulcers caused by ethanol are due to superficial damage to mucosal cells[22] and damage by NSAIDs are due to a decrease in PG synthesis, and ulcers due to stress are due to both physiological and psychological factors and those by pyloric ligation are due to increased accumulation of gastric acid and pepsin leading to auto digestion of the gastric mucosa.[3]

Mucus is secreted by the mucus neck cells and coats the gastric mucosa, thereby preventing physical damage and back diffusion of hydrogen ions. Even though the causative factors of ulcerogenesis may be different, the net imbalance in offensive and defensive factors brought about by these changes, is thought to be the detrimental factor in ulcerogenesis.[3] The decreased mucin secretion in NIDDM rats indicates the decreased ability of the mucosal membrane to protect the mucosa from physical damage and back diffusion of hydrogen ions. The decrease in the glycoprotein content of the gastric mucosa[18] further proved the decreased ability of the gastric mucosa to withstand the offensive onslaught.

The decrease in the DNA content of the gastric mucosa indicates decreased cell shedding and increased lifespan of cells and increase in mucosal resistance.[15] NIDDM rats showed a tendency to increase acid-pepsin secretion and increased cell shedding (in terms of DNA content of gastric juice) and decreased mucosal defensive mucin secretion and mucosal glycoproteins (in terms of TC:P ratio, a reliable index of both mucin secretion and mucosal glycoproteins).[3] Treatment with both PMS and GLC reversed the NIDDM-induced acute adverse effect on the above parameters thus indicating the deleterious effects of diabetes and its reversal by hypoglycemic agents.

Reactive oxygen species (ROS) are generated through nu-

merous normal metabolic processes and are needed for normal functioning of the organism. Various antioxidant enzymes like SOD, CAT and glutathione peroxidase (GPX) control their accumulation.[23] Any imbalance in the activity of these enzymes normally leads to faulty disposal of free radicals and its accumulation. These ROS are responsible for the oxidation of tissues leading to lipid peroxidation and tissue damage. Oxidative damage is considered to be an important factor in the pathogenesis of ulcers as evidenced in different experimental and clinical models. Gastric mucosal LPO has been reported to increase in CRS-induced gastric ulcers[4] and there are reports which indicated an increase in the erythrocytic LPO level in STZ-induced diabetes.[4,24,25] In our present work, we also observed an increase in the oxidative free radicals, lipid peroxides and nitric oxide in CRS and NIDDM rats, with a further increase when CRS was given to NIDDM rats. This could be due to an increase in the generation of ROS leading to oxidative damage. Normally, the increase in damage due to O_2^- is contained by dismutation with SOD.[23] SOD converts the reactive O_2 to H_2O_2, which if not scavenged by the CAT causes lipid peroxidation by an increase in the generation of hydroxyl radicals.[26] Hence, a decrease in SOD and CAT levels in NIDDM rats may lead to an increase in the accumulation of these reactive products and LPO resulting in tissue damage. The above effect could be further aggravated by the decreased activity of CAT during stress.[27] Treatment with PMS and GLC both reversed these oxidative changes induced by NIDDM and stress. The decrease in ulcer index could be due to concomitant decrease in LPO, NO and an increase in SOD and CAT as seen in NIDDM-CRS rats.

Thus, the increased propensity to ulceration as found in NIDDM rats could be due to an increase in offensive acid-pepsin secretion and free radicals along with a decrease in defensive mucosal factors like mucin secretion, mucosal glycoproteins, lifespan of mucosal cells and antioxidant enzymes. Both PMS and GLC (a known oral hypoglycemic agent) significantly decreased the blood glucose levels in NR and NIDDM rats and showed significant ulcer-protective effect in NIDDM rats but not in NR rats. This could be due to the correction of blood glucose level by PMS and GLC, which in turn could have caused the reversal of the diabetes-associated changes on mucosal offensive and defensive factors. The standard anti-ulcer drug, SFT decreased the ulceration in all ulcers models in both NR and NIDDM rats by virtue of its various effects on mucosal offensive and defensive factors. The maintenance of blood glucose level at a nearly normal level could thus possibly protect the NIDDM patient from peptic ulceration.

Acknowledgement

RKG is thankful to ICMR for grant-in-aid.

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