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Modulatory influence of Green tea polyphenols on lipid peroxidation during L-Arginine induced acute pancreatitis in Wistar rats

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Abstract: *Green tea polyphenols have gained enormous attention as an effective natural herb that is used in most parts of the world and is a rich source of polyphenols, which are antioxidant in nature. Despite medical treatment, the lethality of severe acute pancreatitis is still high (20-30%). Experimental acute necrotizing pancreatitis was induced by intraperitoneal administration of a high dose of L-arginine in rats. This study was focused to study the effect of green tea polyphenols (GTP) on L-Arg induced AP in rats. GTP was pretreated at a dose of 20 mg/ kg b.wt. for 3 days then AP was induced by intra peritoneal (i.p.) injection of L-Arg (3.2 g/kg b. wt.) twice at 1 hour time interval. Markers such as serum amylase, pancreatic and lung myeloperoxidase (MPO) were significantly increased in AP induced animals, oxidants and antioxidants such as malendialdehyde (MDA) and glutathione (GSH), glutathione peroxidase (GPx), glutathione reductase (GR), superoxide dismutase (SOD) and catalase (CAT) were significantly decreased in AP induced animals except MDA, these parameters were brought back to near normal status on pretreatment with GTP. The result suggests that GTP pretreatment offered a significant protection against AP and might be a promising potential candidate.*

Key Words*: Green tea polyphenols, acute pancreatitis, L-arginine, antioxidants*

I. Introduction

Acute pancreatitis (AP) is a disease with high morbidity and mortality. AP is characterized by elevated serum amylase and lipase activities, decreased free radical scavenging activities [1]. Histologically, AP is characterized by edema, vacuolization, inflammation, and acinar cell necrosis [2, 3]. In the earlier stages, inflammation is limited only in the pancreas. Due to the systemic action of diverse inflammation mediators, this locally limited inflammation rapidly spreads and develops into systemic inflammatory response syndrome and eventually into multiple organ failure; the latter is responsible for most pancreatitis associated mortalities [4, 5]. Lung injury frequently associated with pancreatitis, but hepatic injury is minor during acute pancreatitis [6]. Severe lung injury [acute respiratory distress syndrome (ARDS)] has been reported to contribute to early death in patients with severe acute pancreatitis [7].

L-Arginine induced pancreatitis is an experimental model of severe necrotizing acute pancreatitis. Twenty-four hours after intraperitoneal (*i.p.*) injection of L-Arg, causes inflammation of the tissue that was confirmed by histology alterations and changes in biochemical parameters. The model is highly reproducible, noninvasive and produces dose-dependent acinar necrosis, and is therefore ideal for studying the pathogenesis of acute pancreatitis [8, 9]. Though there are number of reports addressing the role of Arginine in inducing AP, the mechanism by which Arg causes pancreatitis is not fully known. But accumulating evidence suggests that oxygen free radicals [8, 10] nitric oxide [11], inflammatory mediators [12] all have a key role in the development of this disease [13].

Green tea is widely consumed throughout the world and is also known to possess beneficial properties including antipyretic, diuretic, anti-oxidative, and anti-hepatotoxic activities [14, 15, 16, 17, 18]. The main components of green tea are catechins including (-)-epigallocatechin gallate (EGCG; 9–13%), (-)-epicatechin gallate (ECG; 3–6%), (-)-epigallocatechin (EGC; 3–6%), (-)-epicatechin (EC; 1–3%), (-)-catechin (C; less than 1%) [18]. Early reports suggest that, oxidative stress has been shown to be involved in the pathophysiology of AP. However, the effects of GTP on L- Arg induced AP and its effect on pancreas have not yet been investigated clearly. Therefore, in this study, we investigated whether GTP can ameliorate the severity of AP in L-Arg induced model.

II. Materials And Methods

2.1. Chemicals

L-Arginine and other fine chemicals were purchased from SISCO Research Laboratories Pvt. Ltd, Mumbai. All other chemicals and reagents used were of analytical grade.

2.2. Animals

Male albino rats of Wistar strains weighing approximately 220–240 g used in this study and were obtained from Tamilnadu Animal Science and Veterinary University, Madavaram, Chennai, India. The animals were acclimatized to the laboratory conditions for a period of 2 weeks. They were maintained at an ambient temperature of 25±2° C and 12/12 hours of light–dark cycle and given standard rat feed (Hindustan Lever Ltd., Bangalore) and tap water *ad libitum*. The experiments were conducted according to the ethical norms approved by Ministry of Social Justices and Empowerment, Government of India and Institutional Animal Ethics Committee Guidelines.

2.3. Preparation of GTP

Fresh green tea leaves were collected from The Nilgris, India. Extraction of green tea polyphenols (GP 80%) was done by adapting the procedure followed by Srinivasan et al. [17, 18]. Briefly, Green tea leaves were extracted with 6 times volume of 30% grain alcohol under 60 - 70^{o}C for 20 minutes, filtered, cooled and the solution was extracted. The residual green tea leaves were extracted with 4 times volume of 30% grain alcohol. The extract was filtered and cooled, and extracts were pooled, concentrated and the grain alcohol was recovered under low temperature (<50^{o}C) to obtain the concentrated solution (solid >25%) and subsequently purified using ethyl acetate. From the concentrated extract, ethyl acetate was recovered and Green tea polyphenols was spray dried into powder.

2.4. Experimental Protocol

The rats were divided into four groups (n = 6 per group). Rats in group I served as control animals and received intra peritoneal injections (*i.p.*) of physiological saline. In group II (AP induced), pancreatitis was induced by intra peritoneal (*i.p.*) injection of L-Arg (3.2 g/kg body weight) twice at 1 hour time interval. Rats in group III (AP + GTP) were treated with a single dose of (20 mg/kg body weight) GTP orally 3 days prior to L-Arg administration as mentioned as group II. Rats in group IV received the same dose of GTP alone for 3 days.

2.5 Biochemical parameters

The ratio pancreatic weight/body weight ratio (pancreatic weight g/body weight g ×1000) was done to evaluate the degree of pancreatic edema. The serum α-amylase levels were measured using a commercial kit (Sigma Chemical Company, St. Louis, USA). The cytosolic fractions were prepared by the method of Dignam et al [19]. Pancreatic and lung myeloperoxidase (MPO) activity, as a marker of leukocyte infiltration, was assayed by method of Kuebler et al. [20]. The level of malanoldehyde (MDA) in tissue homogenate was determined using the method of Ohkawa et al [21]. MDA levels were expressed as a nanomol per milligram of protein (nm/mg protein). Protein was assayed by Lowry method [22]. The activity of superoxide dismutase (SOD) was measured by the method Misra and Fridovich [23]. Catalase was assayed by measuring the rate of decomposition of H_2O_2 [24]. Glutathione peroxidase (GPx) activity was measured spectrophotometrically at 340 nm following Paglia and Valentine method [25]. Reduced glutathione (GSH) was estimated by Ellman method [26].

2.6 Histological analysis

A portion of the pancreas, liver and lung was fixed in 8% neutral formaldehyde solution and embedded in paraffin. Tissue slices were stained with hematoxylin and eosin and examined under light microscope. Photomicrographs were obtained using a Nikon Y-FL ECLIPSE 400 (Japan) microscope connected to a Nikon FDX-35 camera (Japan).

2.7. Statistical analysis

All data were analyzed with SPSS/10 Student Software. Hypothesis testing methods included one way analysis of variance (ANOVA) followed by Bonferroni's multiple comparisons test. The values are expressed as mean±S.D, *P* value of less than 0.001, 0.01 and 0.05 was considered to indicate statistical significance.

III. Results

GTP was found to have a protective effect on AP. All animals survived until the end of the experiment. Animals from control group did not show much histological alterations and were found to be normal. As shown in the (Fig. 1a) the pw/bw ratio of GTP treated group (4.78±0.23) was significantly (p<0.001) decreased compared to L-Arg induced group (5.42±0.31). The levels of serum amylase (Fig. 1b) which is frequently used as a marker of AP was found to be significantly (p <0.001) increased in AP induced group. Pretreatment with GTP significantly (p <0.01) decreased the serum amylase activity when compared with L-Arg induced rats.

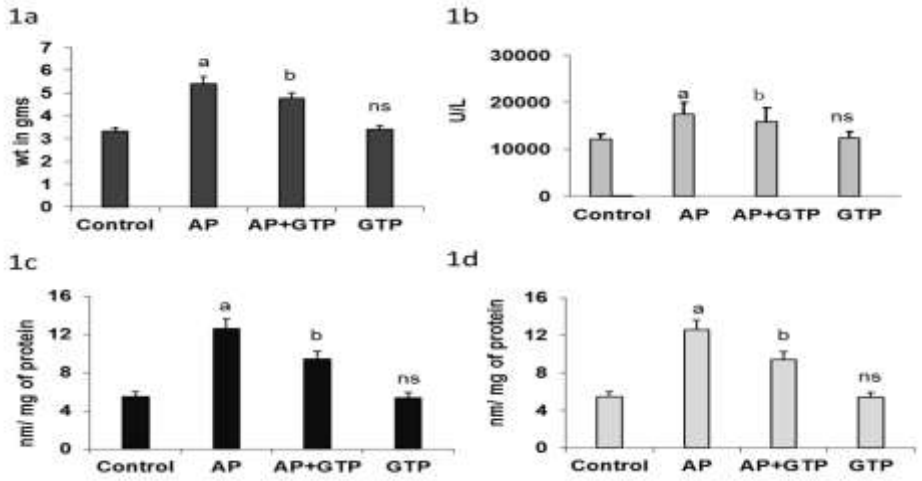
Pancreatic MPO activity was significantly (p <0.001) elevated in L-Arg induced group relative to that of control group. A significant (p <0.01) reduction in pancreatic MPO activity was observed in group III (GTP + AP),

when compared to L-Arg induced group (Fig. 1c). A significant (p <0.001) increase in MDA (Fig. 1d) was observed in pancreas of AP induced rats when compared to that of control rats, pretreatment with GTP showed a significant (p <0.001) fall in the level of pancreatic MDA when compared to that of AP induced rats.
The activities of enzymic antioxidants are shown in (Table 1). The activity of SOD, CAT, GR, GPx and GSH in the AP induced rats was found to be decreased significantly. GTP pretreatment to L-Arg induction in rats showed a notable increase in the activities of these antioxidant enzymes.

Administration of 3.2g of L-Arg/ kg body weight induced acute pancreatitis characterized by marked edema in pancreas, leukocyte infiltration, scattered foci, acinar necrosis and numerous inflammatory cells (Figure. 2b) when compared with the control (Figure. 2a). These histological changes and abnormalities were ameliorated on pretreatment with GTP (Figure. 2c).

IV. Figures And Tables

Figure 1. Effect of GTP on amylase, MPO and MDA on AP induced pancreas



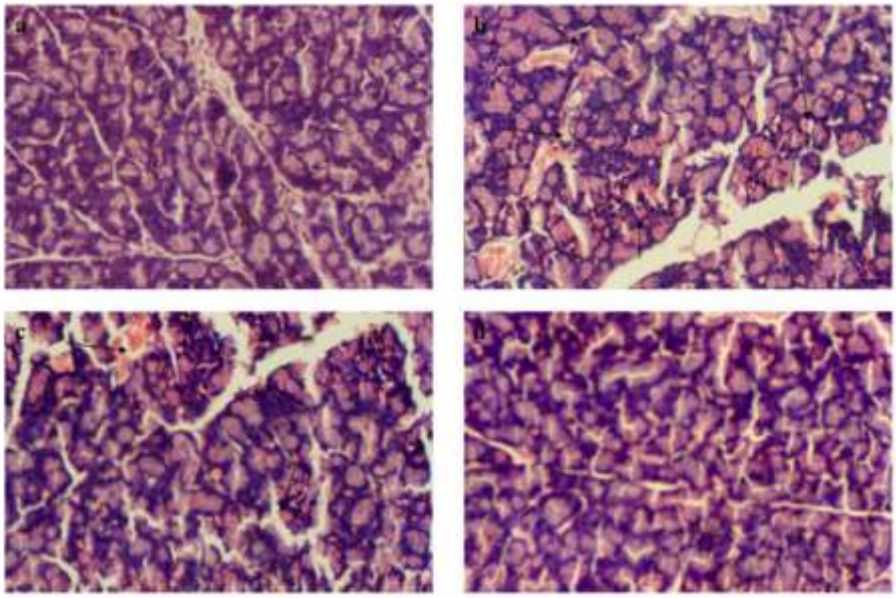
(1a): Pancreatic weight/Body weight, **(b):** serum amylase, **(c):** Pancreatic MPO, **(d):** Pancreatic MDA. Values are expressed as mean ± S.D. Control Vs AP,AP Vs AP+GTP, a-p < 0.001; b-p < 0.01; ns: non-significant. (Bonferroni's multiple comparision test).

Table. 1. Effect of GTP pretreatment on the antioxidant enzyme levels in L-Arg induced AP in rats.

Parameter	Control	AP	AP + GTP	GTP
SOD	7.06 ± 0.69	3.10 ± 0.30*	5.42 ± 0.52*‡	7.27 ± 0.66^{ns}
CAT	42.54 ± 4.50	25.4 6± 2.82*	36.69 ± 3.12*‡	42.92 ± 4.53^{ns}
GR	2.54 ± 0.20	0.70 ± 0.06*	2.14 ± 0.21*‡	2.57 ± 0.24^{ns}
GPx	11.10 ± 1.14	7.04 ± 0.77*	8.56 ± 0.83*‡	11.26 ± 1.14^{ns}
GSH	28.78 ± 1.92	17.42 ± 0.87*	21.49 ± 1.42*‡	28.92 ± 0.79 ^{ns}

Values are expressed as mean ± SD. Statistically significant variations are compared as follows: *-control Vs AP, *‡ -AP Vs AP+GTP, ^{ns} -control Vs GTP, p< 0.05.
Units: SOD-50% autooxidation of epinephrine/min, CAT-µg of H_2O_2 consumed/min, GPX-µg of glutathione consumed/min, GR-µg of NADP+ formed/min, GSH-mg/100 g of tissue.

Figure 2. Effect of GTP on AP induced pancreas.



2a: (Control)Normal histological appearance of pancreatic cells, 2b: (AP) Obvious acinar cell degeneration, edema, vacuolization and inflammation, 2c: (AP+GTP) Minimal infiltration neutrophils, 2d: (GTP) Normal histological appearance to that of control.

V. Discussion

The present study demonstrated the ameliorating and antioxidant effect of GTP during L-Arg induced AP. The dose of 20mg/kg GTP was chosen according to Srinivasan *et al*. L-Arg-induced pancreatitis is an experimental model of severe necrotizing acute pancreatitis. Twenty-four hours after intraperitoneal (*i.p.*) injection of L-Arg, inflammation of the tissue was confirmed by histology and characteristic changes of the laboratory parameters. The model is highly reproducible, noninvasive and produces dose-dependent acinar necrosis, and is therefore ideal for studying the pathogenesis of acute pancreatitis [8, 27, 12].

In the current investigation GTP pretreatment significantly decreased pancreatic weight/body weight ratio in GTP + AP induced rats as shown in the (Fig. 1a) when compared to AP induced animals. In the present investigation, the activity of MPO and the level of MDA were significantly increased in AP induced animals, which correlated with many previous reports that suggested ROS may play an important role in the initiation and development of pancreatitis [28, 29]. Lungs from rats treated with L-Arg had increased levels of MPO activity in comparison to control suggesting neutrophil infiltration as a result of the pancreatitis. Drugs with anti-inflammatory and antioxidant features are potential candidates for the treatment of AP [8]. EGCG a component of GTP is a potent antioxidant, [30] and posses anti-inflammatory property [31]. Pretreatment with GTP and on AP induction showed a significant decrease in the MPO activity in both pancreas and lung and MDA levels which is in accordance with the previous studies by Di Paola et al [32] and Xu et al. [33], in which green tea significantly decreased the activity of MPO and MDA levels. Green tea extracts inhibit lipid peroxidation in *in vitro* systems and experimental animals [34, 35, 18]. Katiyar and Mukhtar, [36] suggested that, EGCG a component of Green Tea Polyphenols can interact with peroxyradicals and inhibit lipid peroxidation.

In our present study, elevation of serum amylase activity (Fig. 1b) is one of the distinctive parameter of acute pancreatitis. The levels of serum amylase is most commonly obtained as biochemical marker of pancreatic disease, particularly AP. Amylase activity in serum has been used for many years for the evaluation of patients with acute abdominal pain and suspected pancreatic disorders [37]. Therefore, pretreatment with GTP decreased the activity of serum amylase in AP induced animals which correlates with the previous, that green tea was found to decrease the activity of serum amylase [38].

The administration of L-Arg to experimental animals resulted in development of an edematous pancreatitis. The free radical scavenging enzymes (such as SOD, CAT, GR, GPx and GSH) levels were significantly decreased. Czako et al, [8] suggested that, among the endogenous scavengers Mn-superoxide dismutase (SOD) and catalase activities decreased significantly throughout the entire study after L-Arg injection. Antioxidant enzymes such as SOD, CAT and GPx can directly counter attack the oxidant attack that

may protect cells against lipid peroxidation. The SOD catalyzes the dismutation reaction and the product of SOD is H_2O_2, which is toxic and must be rapidly removed [39]. In mammalian cells, two enzyme families accomplish this: the Glutathione peroxidases and the Catalases. Both Glutathione peroxidases and catalases detoxify H_2O_2 by reducing it to water and oxygen [40]. In the present study, there was a significant decrease in enzymic antioxidant status (Table. 1) such as SOD, CAT, GR, GPx and GSH in AP induced animals. This decrease in enzymic antioxidant status and increase in MDA level (Fig. 1d) might be the crucial factor for induction of AP by L-Arg.

Tea catechins can chelate iron and copper preventing the metal catalyzed free radical formation [40, 41]. Green tea catechins can act as scavengers of free radicals caused by reactive oxygen species and prevent radical damage. In addition to directly quenching reactive oxygen species, tea flavonoids can chelate iron and copper to prevent their contribution in Fenton and Haber–Weiss reactions [42]. It has been suggested by Sutherland et al. [43] that catechins like EGCG elicit antioxidant actions in a number of ways; they scavenge ROS [44] and reactive nitrogen species [45]. Green tea by scavenging superoxide may reduce the formation of H_2O_2 and in addition it possess a direct scavenging effect on H_2O_2 [46]. An increase in superoxide dismutase activity was observed when administered to rats [47], occurred suggesting that catechins have both direct antioxidant effects and indirect influences to increase the activity of other antioxidants or enzymes. In the present investigation a significant increase in the enzymic antioxidant status was observed in GTP pretreated AP induced animals, the possible reason might be due to inhibition of oxidant production thereby maintaining the antioxidant status and preventing the inflammation of pancreas.

In conclusion, GTP treatment ameliorates the oxidant production and improves the antioxidant status thereby decreases severity of L-Arg induced AP. GTP is a potential candidate to counter attack some of L-Arg-induced changes in laboratory parameters of acute pancreatitis. GTP significantly decreased markers of AP such as serum amylase, pancreatic and lung MPO activity. Oxidant level was significantly decreased and antioxidant status was significantly increased by pretreatment with GTP. Histological studies showed a clear vicinity of protection against the inflammation produced by L-Arg induced AP thereby preventing lung and liver damage. Multiple organ failure is the main reason for pancreatitis-associated mortality. Thus, GTP might be a promising candidate in preventing AP and associated multiple organ failure leading to mortality.

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