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SALUTARY ACTIVITY OF BERBERINE AGAINST DIBUTYLTIN DICHLORIDE INDUCED ACUTE PANCREATITIS IN ALBINO WISTAR RATS

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Keywords:

Dibutyltin dichloride, Berberine, Acute pancreatitis, Serum amylase, Serum lipase

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ABSTRACT: Background: Berberine is a natural alkaloid used as traditional Chinese medicine. **Objective:** The objective of the present study was to evaluate the effect of berberine on dibutyltin dichloride (DBTC) induced acute pancreatitis in albino Wistar rats. **Methods:** In this study 24 Wistar rats were randomly selected and divided into four groups. Each group consists of 6 animals. Group-1 (-ve control), Group 2 (+ve control or disease control), Group 3 (Berberine 50 mg/kg p.o), Group 4 (Berberine 100 mg/kg, p.o). Acute pancreatitis was induced with dibutyltin dichloride at a single dose of 6mg/kg body weight i.p to all the groups except negative control. At 3rd day, serum amylase and lipase levels were elevated in all groups except negative control meanwhile rats were treated with berberine up to 28 days. The estimation of blood parameters and tissue parameters was carried out by the auto-analyzer. **Results:** Berberine treated groups significantly reduce elevated serum parameters, and significantly increase the tissue antioxidant parameters to normal. Serum parameters like serum amylase, lipase, fasting blood glucose levels, liver function parameters, CRP and total protein content were calculated. Tissue antioxidant parameters, MDA, Protein carbonyl levels were significantly reduced to normal in berberine treated groups when compared with positive control or disease control. Histopathological study of the pancreas was also carried out. **Conclusion:** Berberine at a dose of 100 mg/kg p.o., could effectively normalize the serum biochemical parameters, antioxidant parameters when compared to the disease control and berberine at a dose of 50 mg/kg treated rats.

INTRODUCTION: Sudden inflammation of the pancreas is observed in acute pancreatitis. Due to the biliary tract obstruction, the pancreatic cells may release either trypsin instead of trypsinogen or insufficient amount of trypsin inhibitor, and the trypsin begins to digest the pancreatic cells [1-2].

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It is mainly caused by gall stones and alcohol abuse [3]. Excess body fat has accumulated in obese that promote gall stone formation. Alcohol damages the pancreas by inducing oxidative stress.

The annual incidence of acute pancreatitis was ranging from 13 to 45/100000 persons and chronic pancreatitis was ranging from 5 to 12/100000. Chronic pancreatitis prevalence rate is increasing than acute pancreatitis because of high alcohol consumption in developing countries like China and India, due to rapid Urbanization and increased affluence leads to the burden of alcohol-related pancreatitis *i.e.* the incidence of acute pancreatitis

in these countries would be expected to increase [4]. Stable or decreased incidence of acute pancreatitis is observed in many North American and European countries which were assumed to be due to decreased alcohol consumption [5]. At present antibiotics and analgesics are used to treat acute pancreatitis symptomatically, and a lot of side effects are associated with it. WHO recommends the international community to use the traditional Ayurvedic system of medicine in the management of acute pancreatitis [6-7].

Berberine is a chemical found in plant parts like root, rhizome, stem, and bark of Goldenseal (*Hydrastis canadensis*), Oregon grape (*Berberis aquifolium*), Barberry (*Berberis vulgaris*), Chinese goldthread (*Coptis chinesis*), Tree turmeric (*Berberis aristata*), and Yellow root (*Xanthorhiza simpicissima*) [8]. Berberine is yellow color isoquinoline alkaloid and it activates an enzyme called AMP-activated protein kinase (AMPK) [8-9]. This enzyme is also called a metabolic master switch or AMP-activated protein kinase (AMPK) activator which is mainly present in organs like brain, muscle, kidney, heart, and liver. It plays a major role in regulating metabolism and helps to regulate the biological activities that normalize lipid glucose and energy imbalance activities were understood by **Fig. 1**. eNOS is activated by AMP kinase activator. The central role of endothelial AMP kinase is maintaining physiological functions such as regulation of inflammations, modulating endothelial energy supply, protection from apoptosis, and angiogenesis and maintenance of perfusion. By acting on eNOS AMP kinase activators reduce the inflammation caused due to acute pancreatitis and also reduces the acinar cell damage by inhibiting the JNK signaling pathway resulting in the inhibition of the inflammatory mediator's activity. They also reduce the oxidative stress by its antioxidant properties [10].

In the present investigation, a scientific attempt was made to estimate biochemical parameters obtained from berberine treatment and it was tested for acute pancreatitis induced by dibutyltin dichloride.

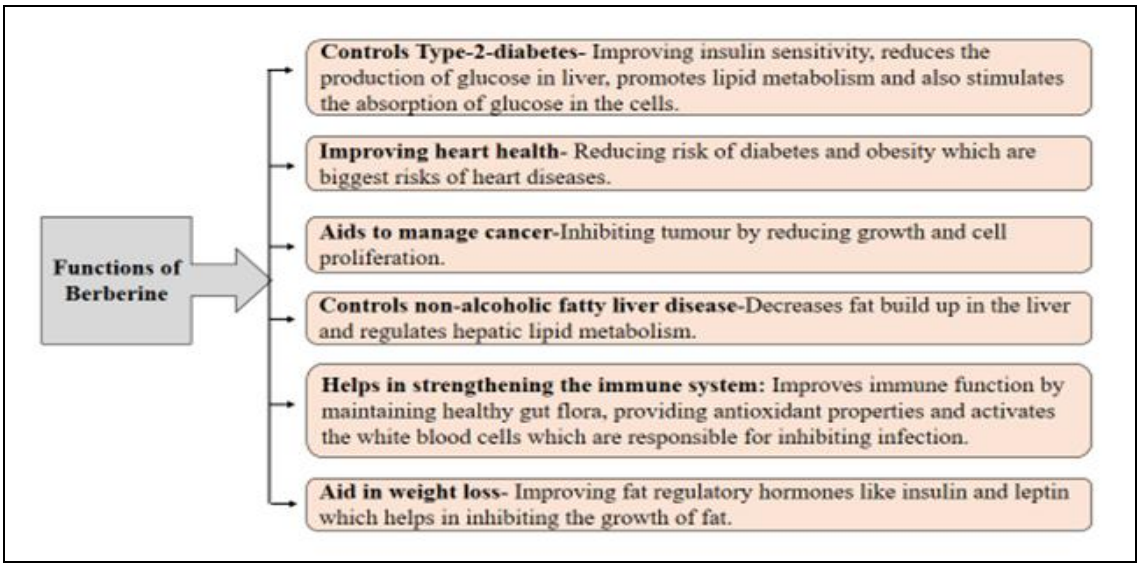


FIG. 1: FUNCTIONS OF BERBERINE

METHODOLOGY:

Herbal Isolate: Berberine was procured from Laila Nutraceuticals.

Chemicals: Dibutyltin dichloride was procured from Sigma Aldrich. Ethanol, glycerol is used for the preparation of Dibutyltin dichloride solution. All other chemicals are used for analytical grade.

Selection of Experimental Animals and Animal Care: Animals were procured from Mahaveer laboratories, Hyderabad. Wistar albino rats (180-200 g) of male were used in the present study. The animals were housed under standard environmental conditions (23 ± 1 ºC) with a relative humidity of 50 ± 10% and maintain 12:12 dark and light cycle, maintained with free access to water and *ad libitum* standard laboratory diet (70% carbohydrates, 25% proteins, 5% lipids (Hindustan liver Bangalore). After randomization before the experiment, the rats were acclimatized for a period of two weeks.

Animal Ethics: Animal housing and handling were in accordance with CPSCEA guidelines. Our college was approved by CPCSEA for conducting animal experiments with the registration no. 516/01/A/CPCSEA.

The prior permission for the study was obtained from our Institutional Animal Ethics Committee (IAEC).

Acute Toxicity Studies: The acute oral toxicity study was done according to OECD 423 guidelines. The study was conducted on albino mice of either sex weighing between 25-35 g and was divided into 4 groups each group containing 3 mice. They were fasted overnight and maintained with water *ad libitum*. The selected functional foods were administered at a dose level of 2000 mg/kg body weight. Standard diet and water were continued during the study as usual. The doses of the selected herbal isolate were fixed based on the acute toxicity study.

Preparation of Dibutyltin Dichloride (DBTC) and Induction of Acute Pancreatitis: DBTC was dissolved in 96% ethanol (2 parts) and then mixed with glycerol (3 parts). The dibutyltin dichloride solution was injected intraperitoneally with a record syringe at a dose of 6mg/kg body weight.

Preparation of Treatment Drug Solution: Berberine was dissolved in distilled water and administered at a dose of 50 mg and 100 mg/kg body weight as low dose and high dose respectively as peracute toxicity studies. 53.5 mg of drug dissolved in 3 ml of distilled water. From this solution, 0.5 ml was administered orally which delivers 50 mg/kg body weight. 103.8 mg of drug was dissolved in 3 ml of distilled water. From this solution, 0.5 ml was administered orally which delivers 100 mg/kg body weight.

Grouping of Animals:

Group 1: Normal control (Vehicle, -ve control)

Group 2: Disease control (+ve control, DBTC; 6 mg/kg body weight, i.p.)

Group 3: 6 mg/kg dibutyltin dichloride + test (Low dose)

Group 4: 6 mg/kg dibutyltin dichloride + test (High dose)

Dibutyltin dichloride (6mg/kg body weight i.p) will be given to Group 2 to Group 4 for the induction of acute pancreatitis observed till the 28th day. Control group was received distilled water (group-1), selected herbal isolate berberine was administered orally at 50 mg/kg and 100mg/kg body weight group-3 and group 4 respectively. Blood sampling was done from the retro-orbital plexus at 3rd and 28th day and serum biochemical parameters were analyzed using SCREEN MASTER 3000 auto analyzer. Groups that received selected herbal isolates were compared with the disease control group and normal control group.

Pancreatic Function Parameters:

Estimation of Serum Amylase: Serum amylase was carried out by a direct substrate method *i.e.* a kit method. To the 0.02ml of a sample, add the 1.0 ml of amylase reagent (L1) and mixed well absorbance was measured recorded at 405nm. The level of serum amylase was expressed as U/L.

Estimation of Serum Lipase: Serum lipase was carried out by the methyl resorufin method, *i.e.* a kit method. To the 20 µl of sample add the lipase reagent 1 mixed carefully incubate for 15 min at 37 ^{o}C. To this add the 250 µl reagent 2 mixed well and absorbance was measured. The level of serum lipase was expressed as µl. For calibrator add 20 µl reagent 3 (lipase calibrator) in the place of sample and then measured the absorbance was recorded at 580 nm.

Calculation:

LipaseU/L= (ΔOD min) sample-(ΔOD min) blank / (ΔOD min) calibrator - (ΔOD min) blank

Liver Function Parameters:

Estimation of SGOT: SGOT test was carried by kinetic technique (International federation for clinical chemistry method (IFCC) *i.e.* a kit method. One vial of enzyme reagent was reconstituted with 10 ml buffer solution to give working reagent. 1ml of this working reagent was added to 0.1ml of the sample, mixed and after a minute of incubation, changes in optical density were measured per minute (ΔOD/min) during 3 min. Levels of SGOT was expressed as U/L.

Calculation:

$$\text{Activity (U/L)} = \Delta OD/\text{min} \times 1768$$

Estimation of SGPT: SGPT test was carried by kinetic technique (International federation for clinical chemistry method (IFCC) *i.e.* a kit method. One vial of enzyme reagent was reconstituted with a 10 ml buffer solution to give working reagent. 1 ml of this working reagent was added to 0.1 ml of sample, mixed and after a minute of incubation, changes in optical density were measured per minute (ΔOD/min) for 3 min. Levels of SGPT was expressed as U/L.

Calculation:

Activity (U/L) = ΔOD/min. × 1768

Estimation of Alkaline Phosphatase: ALP test was carried using p-NPP (p-nitrophenylphosphate) kinetic method *i.e.* a kit method. ALP kit is based on the recommendations of the International Federation of Clinical Chemistry (I.F.C.C). One vial of the p-NPP substrate was reconstituted with 5 ml of buffer solution to produce a stable working reagent. To one 1ml of working reagent 0.02 ml of sample was added, mixed well and absorbance was recorded. Levels of ALP was expressed as U/L.

Calculation:

Serum ALP activity in U/L = ΔA/min. × 2742.

Estimation of Total Bilirubin: Total bilirubin was carried by Modified Jendrassik and Grof's method *i.e.* a kit method. For the preparation of $T_1$0.2ml of serum sample was added to the solution it contains 1.0 ml DIAZO A, 0.1ml of DIAZO B and then adds 1 ml of ACTIVATOR finally add 2.5 ml of distilled water.

Absorbance was recorded T2 was prepared by using 1ml of DIAZO A, 1 ml of ACTIVATOR and finally makeup with 2.6 ml of distilled water absorbance was recorded at 540 nm. The level of total bilirubin was expressed as mg%.

Estimation of Total Protein in Serum: Total protein content test was carried by Modified biuret, Endpoint assay method *i.e.* a kit method. To the 0.05 ml of a serum sample, 1 ml of biuret reagent and 2 ml of distilled water were mixed. For standard preparation, the standard was replaced with sample solution and absorbance was recorded at 555 nm. The level of total protein content was expressed as gm%.

Calculations:

Total proteins in gm% = A of (T)X std.conc / A of (S)

Estimation of C-reactive Protein: C-reactive protein (CRP) level was assayed using a solid-phase ELISA that uses affinity-purified anti-rat CRP antibodies for solid phase (microtiter wells) immobilization and horseradish peroxidize (HRP) conjugated anti-rat CRP antibodies for detection. Level of C-reactive protein was expressed as $ng.ml^{-1}$.

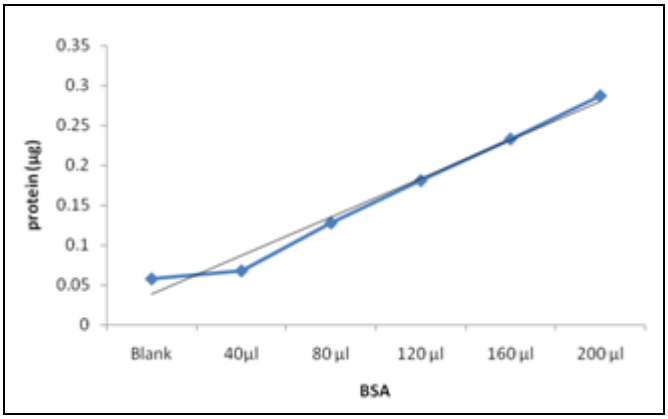
Determination of Antioxidant Activity:

Determination of Protein Estimation Methods: The dry pancreatic samples were used for the estimation of all parameters. The pancreatic samples were weighed and homogenate prepared by using the TWEEN buffer pH 8. According to the weight of the pancreatic sample prepare 10% homogenate. The dry pancreatic samples were used for the estimation of all parameters. The pancreas was weighed and homogenate prepared by using 0.1N NaOH.

Method: Protein was estimated by the method developed by Lowry *et al.*, 1955. The sample volumes are based on tissue and diluted up to 400µl with buffer and add 2 ml of alkaline copper solution incubate for 10 min, add 0.2 ml of folin ciocalteau reagent and incubate for 45 min. Finally, add 0.9 ml of distilled water.

Measure the absorbance at 540 nm by spectro-photometer. Protein levels were determined by comparing the known concentration of standard bovine serum albumin. The protein levels were expressed as mg protein/gm tissue.

Standard Graph of BSA:



STANDARD GRAPH OF BSA

BSA concentration	Protein estimation (µg)			
	Set1	Set 2	Mean	Mean after blanking
Blank	0.060	0.056	0.058	-----
40µl	0.125	0.128	0.068	0.068
80 µl	0.186	0.187	0.128	0.064
120 µl	0.245	0.234	0.181	0.060
160 µl	0.299	0.284	0.233	0.058
200 µl	0.342	0.349	0.287	0.057

Estimation of Superoxide Dismutase (SOD): Tissue was homogenized with Remi homogenizer with ice-cold tris buffer to produce a 10% w/v homogenate**.** The homogenate was centrifuged at 12000 rpm for 15 min at 4 ºC. A liquid of 0.1 ml of supernatant was added to 1.2 ml of 0.052 M sodium pyrophosphate buffer (pH 9.3) followed by the addition of 0.1 ml of 196 µM phenazine methosulphate, 0.3 ml of 300 µM nitroblue tetrazolium, 0.2 ml of 790 µM NADH. The reaction mixture was incubated for 90 sec at 37 ºC, and the reaction was stopped by the addition of 0.1 ml of glacial acetic acid. The reaction mixture was stirred vigorously and shaken with 4.0 ml of n-butanol and centrifuged at 4000 rpm for 10 min. The absorbance of the organic layer was measured at 560 nm. A control was prepared using 0.1 ml of distilled water devoid of 0.1ml of homogenate.

One unit of the enzyme activity, defined as enzyme concentration required for inhibiting the absorbance of chromogen production by 50% in control sample under assay conditions. The SOD levels were expressed as units per mg protein.

Estimation of Catalase (CAT): Tissues were homogenized with Remi homogenizer in ice-cold tris buffer to produce a 10% w/v homogenate. The homogenate was centrifuged at 12000 rpm at 4 ºC for 15 min. 0.1 ml of the supernatant was added to a cuvette containing 1.9 ml of 50 mM phosphate buffer. To this mixture, 1 ml of freshly prepared 30 mM H_2O_2 was added and changes of absorbance for 3 min at 240 nm at an interval of 30 sec were recorded. A control was prepared using 0.1 ml of distilled water devoid of 0.1 ml of homogenate. The activity of catalase was expressed as units of hydrogen peroxide were metabolized /mg protein/minute.

Estimation of Reduced Glutathione (GSH): The sulfhydryl group of GSH reacts with DTNB to produce a yellow colored 5-thio2-nitrobenzoic acid (TNB). The mixed disulfide, GSTNB that is concomitantly produced is reduced by glutathione reductase to recycle the GSH and produce more TNB. The rate of TNB production is directly proportional to this recycling reaction which is in turn, proportional to the concentration of GSH in the sample.

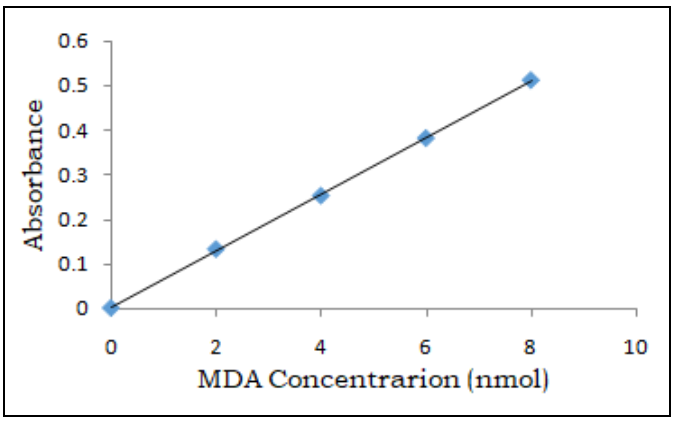
Procedure: Take 0.2 ml of tissue homogenate and 0.2 ml of 0.8 mM EDTA, 0.1 ml of Sodium azide, 0.1 ml of 4 mM GSH, 0.1 ml of H_2O_2 solution, and 0.4 ml of 0.4 M phosphate buffer (pH-7). Incubate at 37 ºC for 10min: keep the tubes at room temperature and to this add 0.5 ml of 10% TCA and centrifuged at 2000 rpm for 10 min, to this supernatant add 0.1ml of 0.04% DTNB solution. Read the optical density at 420nm against blank. Levels expressed as µmoles of glutathione oxidized /min/mg protein.

Estimation of Protein Carbonyl Content: 500 µl of the sample was diluted with 500 µl of distilled water. To this 500 µl of 2, 4 DNPH was added and incubated for 1 hour at room temperature. Then 700 µl of ice-cold TCA was added, Centrifuged at 10,000 rpm for 20 min. The supernatant was discarded and the precipitate was separated. The pellet was washed 3 times with 1 ml of 1:1 ratio of ethanol: ethyl acetate. The pellet was dissolved with 0.9 ml of urea, 0.1 ml of tris EDTA and absorbance was measured at 365nm.

Estimation of Malondialdehyde (MDA): The assay is based on the reaction of malondialdehyde (MDA) with thiobarbituric acid (TBA) at 95 ºC in the presence of acetic acid, which forms an MDA-TBA2 adduct which shows maximum absorption at 532nm.

Preparation of Standard Curve: Stock solution of 1,1,3,3-tetraethoxypropane (TEP) was prepared by taking 220 mg of TEP in a volumetric flask and the volume was made up to 10 ml with distilled water in a volumetric flask. From this stock solution, 1 ml was taken into a volumetric flask and

volume was made up to 100 ml with distilled water to obtain a 10 µM solution. From this working standard solution 0.2 ml, 0.4 ml, 0.6 ml, 0.8 ml, 1.0 ml quantities representing 20 nmol, 40 nmol, 60 nmol, 80 nmol, and 100 nmol of TEP, respectively were transferred into centrifuge tubes. Then 0.2 ml of 9.1% sodium dodecyl sulphate, 1.5ml of 20% acetic acid solution and 1.5 ml of 0.9% aqueous solution of thiobarbituric acid (TBA) were added and mixed thoroughly. The final volume in all tubes was made up to 5 ml with distilled water. The reactants were heated on an oil bath at 95 ºC for about 60 min. The tubes were cooled to room temperature and the resulting chromogen was extracted with 5 ml of 15:1 v/v n-butanol and pyridine mixture by vigorous shaking. Separation of the organic phases was facilitated by centrifuge at 4000 rpm for 10 min and its absorbance was measured at 532 nm. The standard graph was prepared by taking a concentration of MDA on X-axis and the corresponding absorbance on Y-axis.



STANDARD CURVE

Procedure for Preparation of Sample: MDA levels in tissue homogenate were measured by the method developed by Ohkawa *et al*., 1979. To the sample of 0.2 ml of tissue homogenate, 0.2 ml of 9.1% sodium dodecyl sulphate, 1.5 ml of 20% acetic acid solution and 1.5 ml of 0.9% aqueous solution of TBA were added. The mixture was made up to 5 ml with distilled water and then heated on an oil bath at 95 ºC for 60 min using a condenser. After cooling with tap water, 5ml of a mixture of n-butanol and pyridine (15:1 v/v) was added and shaken vigorously. After centrifugation at 4000 rpm for 10 min, the organic layer was taken and its absorbance was measured at 532 nm. The tissue MDA levels were measured from the standard curve and expressed nmol/mg of protein.

RESULTS AND DISCUSSION:

TABLE 1: EFFECT OF BERBERINE ON SERUM AMYLASE LEVELS OF DBTC INDUCED ACUTE PANCREATITIS IN ALBINO WISTAR RATS AT 3RD AND 28TH DAY

Groups	3rd day	28th day
	Mean ± SEM	Mean ± SEM
Control	674.76±5.59	671.23±5.13
Disease control	1220.16±60.98	388.58±21.59***
Berberine (50 mg/kg)	1082.39±36.14	502.35±23.27***
Berberine (100 mg/kg)	1032.00±24.64	622.70±12.85***

All the values represents MEAN±SEM, n=6, ***p<0.001, **p<0.01, *p<0.05 when compare to 3rd day. Two way ANOVA-Bonferroni post-test to compare replicate means by row.

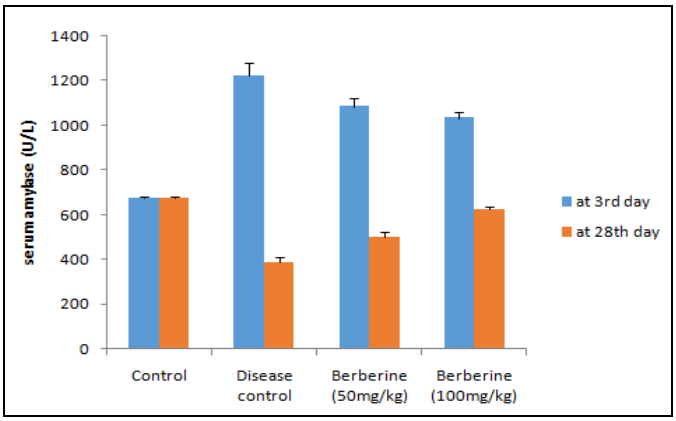


FIG. 2: EFFECT OF BERBERINE ON SERUM AMYLASE LEVELS OF DBTC INDUCED ACUTE PANCREATITIS IN ALBINO WISTAR RATS AT 3RD AND 28TH DAY

TABLE 2: EFFECT OF BERBERINE ON SERUM LIPASE LEVELS OF DBTC INDUCED ACUTE PANCREATITIS IN ALBINO WISTAR RATS AT 3RD AND 28TH DAY

Groups	3rd day	28th day
	Mean ± SEM	Mean ± SEM
Control	21.99±0.17	22.25±0.20
Disease control	30.27±0.70	30.81±0.88ns
Berberine (50mg/kg)	28.85±0.19	25.48±1.22**
Berberine (100mg/kg)	28.57±0.29	22.08±0.84***

All the values represents MEAN±SEM, n=6, ***p<0.001, **p<0.01, *p<0.05 when compare to 3rd day. Two way ANOVA-Bonferroni post-test to compare replicate means by row.

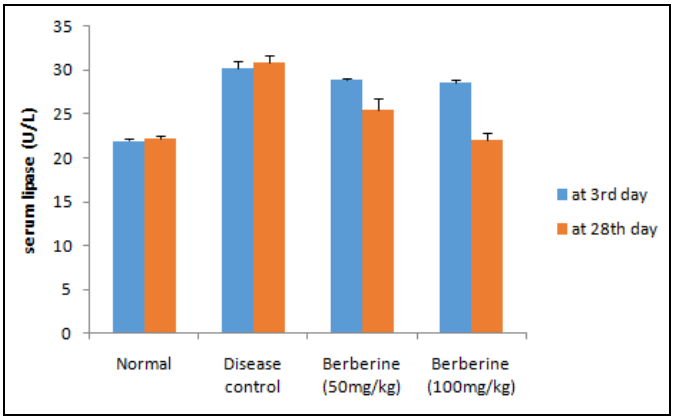


FIG. 3: EFFECT OF BERBERINE ON SERUM LIPASE LEVELS OF DBTC INDUCED ACUTE PANCREATITIS IN ALBINO WISTAR RATS AT 3RD AND 28TH DAY

DBTC administration showed a significant elevation in amylase and lipase levels in serum when compared to the normal control group which was observed on 3rd day after the single intraperitoneal injection. A significant reduction of amylase levels to normal was observed on 28th day in DBTC induced groups when compared with 3rd day are clearly observed by **Table 1, 2** and **Fig. 2, 3**. But there was no significant difference in lipase levels between on 3rd day and 28th day that means their levels were maintained in DBTC administered group. In 1997 Merkord J *et al*., reported that DBTC causes toxic necrosis of the biliopancreatic duct epithelium, which may cause obstruction of the pancreas that may lead to digestion of pancreatic tissue and barrier which prevents the enzymes getting into circulation is broken. Hence, the elevation of amylase and lipase levels in circulation [11].

TABLE 3: EFFECT OF BERBERINE ON FASTING GLUCOSE LEVELS OF DBTC INDUCED ACUTE PANCREATITIS IN ALBINO WISTAR RATS

Groups	Glucose (mg/dL) Mean ± SEM
Normal	74.1 ± 1.44
Disease control	110.0 ± 3.13$^{###}$
Berberine (50 mg/kg)	93.5 ± 2.95**
Berberine (100 mg/kg)	76.8 ± 1.44***

All the values represents MEAN±SEM, n=6, ***p<0.001, **p<0.01, *p<0.05 when compare to Disease Control, ###p<0.001 comparing the diseased control with control group One way ANOVA- Dunnett's multiple comparison test.

The berberine treated groups showed the significant elevation of serum amylase (after 3rd-day amylase levels were reduced in DBTC administered group) and significant reduction lipase levels to normal when compared to the diseased control group evidenced by **Table 1, 2** and **Fig. 2, 3**. This might be due to the protective action of berberine against pancreatic injury. Evidence, In 2017, Kris G *et al*., reported that berberine can activate AMP kinase inside the cell or berberine is also known as AMP kinase activator [12]. In 2017 Choi SB *et al*., reported the protective action of berberine against choline-deficient ethionine-supplemented (CDE) diet-induced severe acute pancreatitis [13].

DBTC administration showed a significant elevation of fasting glucose levels when compared to the normal control group was observed in **Table 3**. Elevation of fasting blood glucose levels may indicate the prediabetic stage. Evidence, In 2018 Chamorro-Garcia R *et al*., reported that perinatal exposure to Dibutyltin led to increased fat storage, glucose intolerance in humans [14]. In 1980 Solomon SS *et al*., reported that acute pancreatitis is associated with damage to both the endocrine and exocrine pancreas glucose intolerance seen with this disease appears to be the result of hyperglucagonemia and relative hypoinsulinemia [15], this might be due damage of beta cells of pancreas by the reactive oxygen species, they impair second phase insulin secretions. The berberine treated groups showed a significant reduction of elevated fasting blood glucose levels to normal was observed in **Table 3**. This might be due to the hypoglycemic activity of berberine.

TABLE 4: EFFECT OF BERBERINE ON LIVER FUNCTION PARAMETERS OF DBTC INDUCED ACUTE PANCREATITIS IN WISTAR ALBINO RATS

Groups	Liver function parameters		
	SGOT (U/L)	SGPT (U/L)	ALP (U/L)
Control	26.833±3.13	26.16±1.62	25.5±1.54
Disease control	68.66±1.99$^{###}$	56.16±2.76$^{###}$	80.33±3.38$^{###}$
Berberine (50 mg/kg)	44.00±2.62**	34.33±1.22**	41.83±1.47***
Berberine (100 mg/kg)	30.66±0.98***	26.33±1.40***	34.16±2.18***

TABLE 5: OTHER SERUM BIOCHEMICAL PARAMETERS

Groups	Serum biochemical parameters			
	Bilirubin (mg/dL)	Creatinine (mg/dL)	Total protein (g/dL)	CRP (ng.ml^{-1})
Control	5.01±0.40	1.15±0.02	6.28±0.08	2.73 ± 0.03
Disease control	8.15±0.34$^{###}$	1.75±0.05$^{###}$	9.47±0.32$^{###}$	12.47 ± 0.03$^{###}$
Berberine (50 mg/kg)	6.40±0.43*	1.35±0.02***	7.38±0.27***	3.54 ± 0.04***
Berberine (10 0mg/kg)	5.36±0.39***	1.25±0.02***	6.37±0.27***	2.90 ± 0.05***

All the values represents MEAN±SEM, n=6, ***p<0.001, **p<0.01, *p<0.05 when compare to Disease Control, ###p<0.001 comparing the diseased control with control group One way ANOVA- Dunnett's multiple comparison test.

Evidence, In 2007 Libin Z *et al*., reported that berberine acutely decreased glucose-stimulated insulin secretion (GSIS) and palmitate-potentiated insulin secretion in high-fat diet-induced diabetics in rats [16]. Berberine is a hypoglycemic agent, exerts a regulatory effect on beta cells *via* AMP kinase signaling pathway. In 2010 Wang Y *et al*., reported the Hypoglycemic and insulin-sensitizing effects of berberine in high-fat diet- and streptozotocin-induced diabetic rats [17].

DBTC administration a significant elevation of the SGOT, SGPT, ALP, CRP, total bilirubin and serum creatinine levels, when compared to the normal control, was clearly observed in **Table 4, 5** this may indicate the obstruction of biliopancreatic duct, leads to triggering of enzymes. Elevated enzyme levels may cause cellular damage and leakage of enzymes into the circulation leads to severe inflammation. Evidence, In 1999 Merkord J *et al*., reported that DBTC administration shows persistently elevated levels of alkaline phosphatase activity and it indicates the obstruction of the common biliopancreatic duct during the whole observation period [18]. Bile duct obstruction results in increased synthesis of ALP triggered by the bile duct epithelial cells and finally release of ALP into the serum (ClinLab navigator. 2018) [19]. In 2010 Tetangco EP *et al*., reported that markedly elevated AST levels have also been described in acute pancreatitis [20]. It may due to obstruction of the biliary duct, reflux of liver enzymes into hepatic sinusoids, increased production of transaminases, increased permeability of hepatocytes results to release of enzymes into the bloodstream. In 2009 Lankisch PG *et al*., reported that high serum creatinine is a well-known unfavorable prognostic parameter in acute pancreatitis [21]. Acute renal failure in acute pancreatitis is caused by the release of vasoactive compounds, enzymes and cytokines from the pancreatic tissue into the circulation.

The berberine treated groups showed a significant reduction of elevated SGOT, SGPT, ALP, CRP, total bilirubin and serum creatinine levels when compared to the diseased control group was clearly observed in **Table 4, 5**. This might be due to the protective action of berberine against bile duct lesions and liver and renal injury. Evidence, In 2010 Yibin F *et al*., reported that hepatoprotective effects of berberine on carbon tetrachloride-induced acute hepatotoxicity in rats [22]. Berberine protected liver injury evidenced by decreased ALT and AST activities and shows hepatoprotective activity. Berberine reduces the ALP levels by acting on the bile duct (Mohamed NA *et al*., 2018) [23]. In 2018 Zheng Z *et al*., reported the hepatoprotective effects of berberine on acetaminophen-induced hepatotoxicity in mice [24]. In the 2013 Wan X *et al*., reported the berberine ameliorates chronic kidney injury caused by atherosclerotic renovascular disease in rats[25]. This self-perpetuating cycle can lead to progressive renal disease. Experimental blockade of the oxidative stress pathway with antioxidant vitamins in several disease models has been shown to decrease renal injury. In the 2018 Zhu L *et al*., reported that berberine ameliorates diabetic nephropathy by reducing renal injury by reducing the serum creatinine levels [26].

TABLE 6: EFFECT OF BERBERINE ON OXIDATIVE STRESS ENZYMES OF DBTC INDUCED ACUTE PANCREATITIS IN ALBINO WISTAR RATS: (PANCREATIC HOMOGENATE)

Groups	SOD (IU/mg of protein) Mean ± SEM	Catalase (IU/mg of protein) Mean ± SEM	GSH(nmol/mg of protein) Mean ± SEM	Protein carbonyl (nmol/mg of protein) Mean ± SEM	MDA (nmol/mg of protein) Mean ± SEM
Normal	1.91±0.02	0.88±0.02	9.01±0.28	03.57±0.15	6.06±0.09
Disease control	0.82±0.03$^{###}$	0.29±0.01$^{###}$	3.92±0.15$^{###}$	10.72±0.38$^{###}$	9.96±0.30$^{###}$
Berberine (50 mg/kg)	1.57±0.03***	0.64±0.02***	6.28±0.19***	07.12±0.27***	8.43±0.12***
Berberine (100 mg/kg)	1.84±0.02***	0.86±0.02***	8.52±0.16***	04.32±0.11***	6.72±0.08***

Oxidative stress is the molecular and cellular damage resulting from excessive ROS (reactive oxygen species) production or reduced endogenous antioxidants. DBTC administration increased the production of ROS in the course of pancreatitis, activates pro-inflammatory cytokines leads to tissue damage [27].

In the present study DBTC administration, significantly reduced the SOD, catalase, and GSH levels in the pancreas homogenate when compared to the normal control group was clearly observed in **Table 6**. It might be due to the free radicals generated by the oxidative stress and tissue inflammation.

Evidence, In 2007 Lian X *et al*., reported that DBTC administration, showed a significant reduction in the antioxidant enzymes like SOD, Catalase, GSH levels [27]. It was mainly due to the increased production of reactive oxygen species (ROS) in the course of pancreatitis activates nuclear factor B (NF- B), which subsequently results in the transcription of various proinflammatory cytokines, leading to tissue damage and ultimately fibrosis. DBTC administration showed a significant elevation in the MDA, Protein carbonyl levels in pancreatic homogenate when compared to a normal control group. It might be due to the reactive oxygen and nitrogen generated by inflammation of the tissue. In 2017 Merkord J *et al*., reported that DBTC treated rats revealed increased serum concentrations of MDA after 1, 2 and 4 h and with the highest level after 1 h [28]. In DBTC induced toxicity, serum malondialdehyde as a marker of oxidative stress was evaluated. LPO is indirectly measured by measuring MDA levels.

The berberine treated groups showed a significant elevation of reduced SOD, catalase, and GSH levels in pancreatic homogenate when compared to the diseased control group was observed in **Table 6**. This might be due to the protective action of berberine against oxidative stress caused by tissue damage. Evidence, In 2014 Zheng L *et al*., reported that, glutathione (GSH), which often declines during oxidative stress [29]. GSH is an antioxidant itself and is a substrate of glutathione peroxidase (GSH-Px) in the clearance of peroxides. In addition to GSH-Px, another well-known antioxidant enzyme, superoxide dismutase (SOD), is also involved to evaluate the inhibitory effect of berberine on oxidative stress. An antioxidant enzyme is a part of the antioxidant defense mechanisms, which helps to maintain the balance of redox in organisms and could be damaged in the pathogenesis of the disease. The berberine treated groups significantly reduced the elevated MDA, and protein carbonyl levels were observed when compared to the diseased control group. This might be due to the protective action of berberine on tissue damage. Evidence, In 2017 Hassanein K *et al*., reported that alkaloid berberine shows protective action on lipid peroxidation, antioxidant defense system by inhibiting liver damage due to its antioxidant defense mechanism [30]. In 2014 Mojarad TB *et al*., reported that the anticonvulsant and antioxidant effects of berberine in kainate-induced temporal lobe epilepsy in rats [31].

Histopathological observation in the pancreas may reveal infiltration and fibrosis of pancreatic tissue in DBTC induced group *i.e.* **Fig. 6** and **Fig. 7** when compared with **Fig. 4** *i.e.* normal control and these changes were clearly understood in **Fig. 5**.

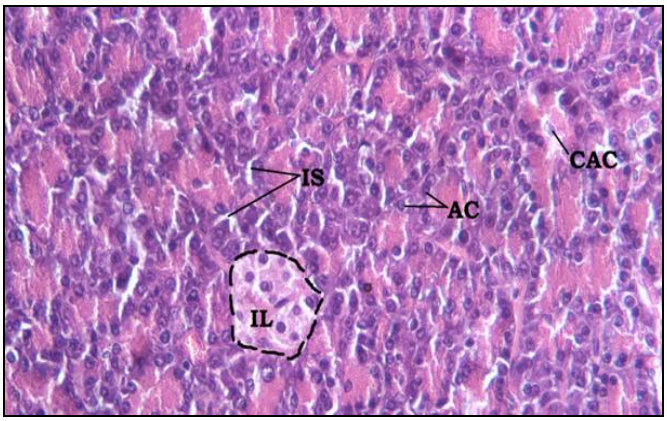


FIG. 4 GROUP-1 NORMAL CONTROL: ACINI, CENTRAL ACINI CELLS, ISLETS OF LANGERHANS, IS-INTERCALATED DUCTS IN EXOCRINE PANCREAS AND VASCULAR STROMA IN ENDOCRINE PANCREAS. AC-Acini, CAC-Central acini cells, IL-islets of Langerhans, IS-Intercalated space

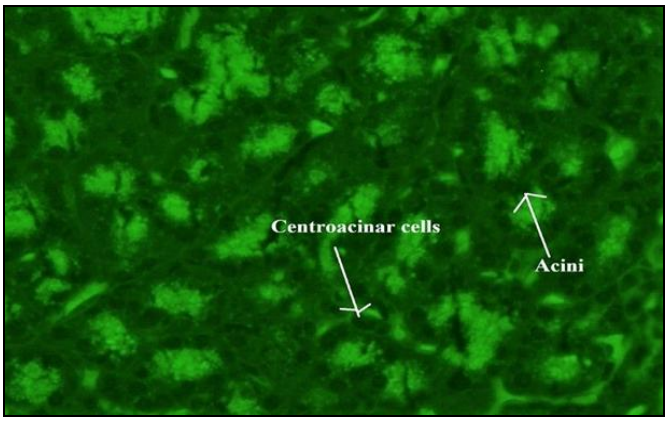


FIG. 5: CAPTURED USING FLUORESCENCE MICROSCOPE GROUP-1 NORMAL CONTROL: ACINI, CENTRAL ACINI CELLS, ISLETS OF LANGERHANS, IS-INTERCALATED DUCTS IN EXOCRINE PANCREAS AND VASCULAR STROMA IN ENDOCRINE PANCREAS

The moderate changes in acini, reduced the gap between intercalated ducts and Islets of Langerhans were observed in a low dose of berberine (50 mg/kg body weight) treated group in **Fig. 8** and these changes were clearly understood in **Fig. 9**. Normalizing of pancreatic tissue was observed in a high dose of berberine (100 mg/kg body weight) treated group when compared to a diseased and low

dose of berberine treated group was observed in **Fig. 10** and these changes were clearly understood in **Fig. 11**. There was evidence, damage of pancreatic tissue by oxidative stress induced by DBTC in albino Wistar rats proven by histo-pathological examination (Merkord J *et al.*, 2016). In 2014 Mojarad TB *et al.*, reported that antioxidant activity of berberine might show the protective action against tissue damage [31].

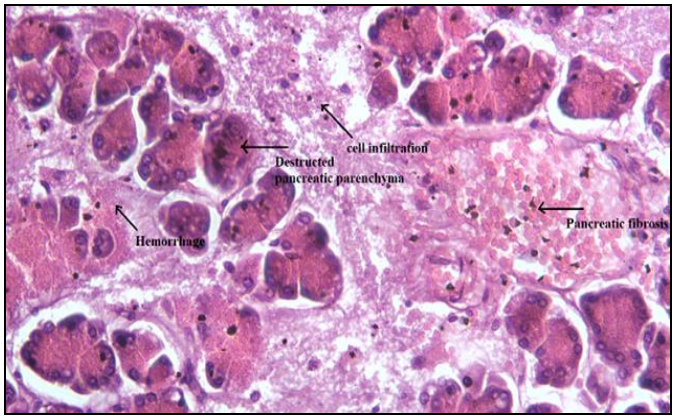


FIG. 6: GROUP-2 DISEASED CONTROL: DBTC (6mg/kg BW) INDUCED RATS- HEMORRHAGE, CELLULAR INFILTRATION, PANCREATIC FIBROSIS, AND DESTRUCTED PANCREATIC PARENCHYMA

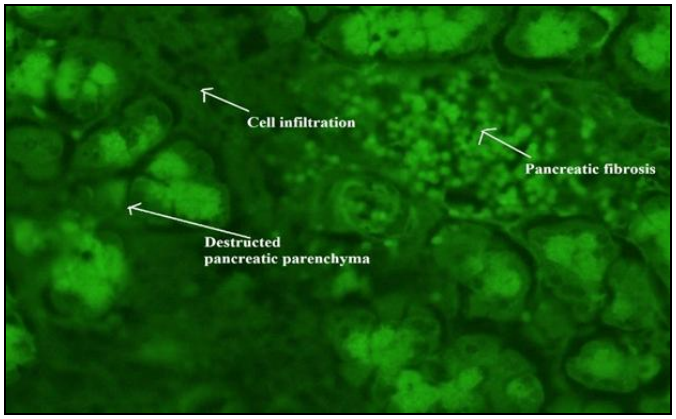


FIG. 7: CAPTURED USING FLUORESCENCE MICROSCOPE, GROUP-2 DISEASED CONTROL: DBTC (6mg/kg BW) INDUCED RATS- HEMORRHAGE, CELLULAR INFILTRATION, PANCREATIC FIBROSIS, AND DESTRUCTED PANCREATIC PARENCHYMA

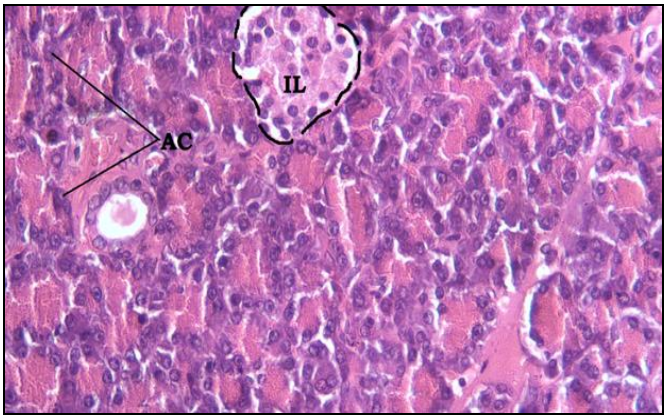


FIG. 8: GROUP 3- TREATMENT 1-LOW DOSE- OBSERVED THE MODERATE CHANGE IN ACINI, REDUCED THE GAP BETWEEN INTERCALATED DUCTS AND ISLETS OF LANGERHANS

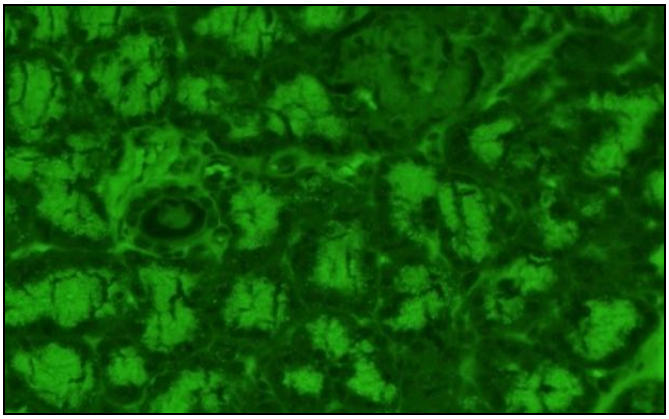


FIG. 9: CAPTURED USING FLUORESCENCE MICROSCOPE,GROUP 3- TREATMENT 1-LOW DOSE- OBSERVED THE MODERATE CHANGE IN ACINI, REDUCED THE GAP BETWEEN INTERCALATED DUCTS AND ISLETS OF LANGERHANS

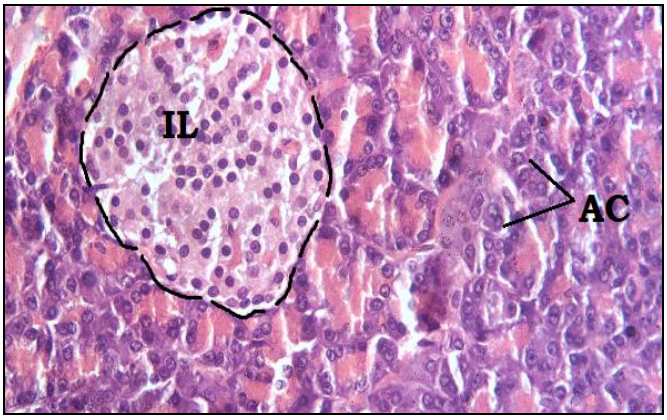


FIG. 10: GROUP 4-TREATMENT 2-HIGH DOSE- NORMALIZING OF EXOCRINE AND ENDOCRINE PANCREAS

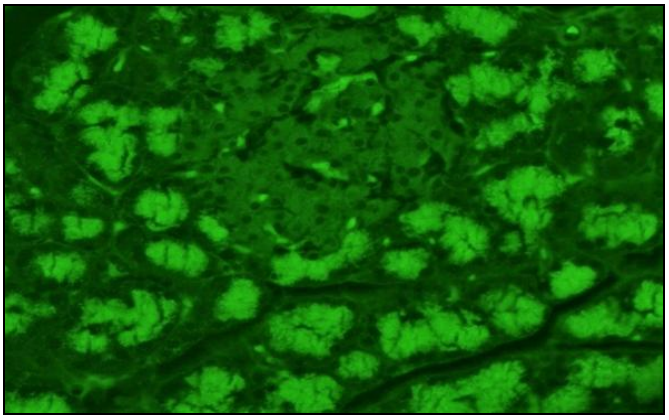


FIG. 11: CAPTURED USING FLUORESCENCE MICROSCOPE, GROUP 4-TREATMENT 2-HIGH DOSE- NORMALIZING OF EXOCRINE AND ENDOCRINE PANCREAS

From the histopathology examination data normalizing pancreatic tissue was observed in berberine treated groups when compared to DBTC treated group.

CONCLUSION: The study concluded that berberine found to improve the pancreatic functions by increasing the amylase and lipase levels to normal which might be due to inhibition of fibrosis caused by DBTC in acinar cells, and which was further supported by histopathological studies. The berberine dose of 100mg/kg bodyweight could effectively normalize the serum amylase and lipase levels. The serum biochemical parameters like SGOT, SGPT, ALP, bilirubin, creatinine, total protein levels were reduced to normal, which supports berberine found to improve the pancreatic functioning. The beneficial effect of berberine might be due to the improvement of antioxidant activity which is evident from increased levels of endogenous antioxidants SOD, CAT, GSH.

The other parameters like MDA, PCO and nitrate showed improvement in levels to normal which further supports the improvement in the functioning of the pancreas and other organs like the liver. The possible protective action against acute pancreatitis could be the action of berberine to counter act the oxidative damage caused by radicals or may attenuate the inflammatory infiltration induced by DBTC. Hence the berberine could be a potential source of the traditional drugs for acute pancreatitis.

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CONFLICTS OF INTEREST: None

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