Wheatgrass (Triticum Aestivum): An Effective Anti-Oxidant in L-Arginine Induced Chronic Pancreatitis Model of Rat: A Dose Dependent Study

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Abstract: Wheatgrass (Triticum aestivum) has been found effective in preventing various diseases in human as well as animals. However its role in chronic pancreatitis has not yet been studied.

Methods: 42 Wistar rats of either sex were divided into 7 groups. Group 1(Control): Rats were given IP injections of normal saline on days 1,4,7,10,13,16&19 and water intragastrically daily 2 days before starting IP injections. Group 2: L-arginine hydrochloride (250mg/100g bw/day) IP in 2 repeated doses of 1 hr interval on day 1 and single dose on day 4,7,10,13,16&19. Group 3: Animals in this group received wheatgrass alone at highest dose of 1.2 gm/kg b.wt. Group 4-7: Rats received wheatgrass dissolved in water orally at different doses i.e.0.6,0.8,1.0,1.2 gm/kg b.wt daily 2 days before starting of L-arginine as per group 2. Levels of serum amylase, lipase and pancreatic lipid peroxidation (LPO) & Glutathione (GSH) were studied on day 21. Histopathological changes were studied by H&E stain.

Results: Levels of serum amylase & lipase were significantly higher in arginine group as compared to controls. Interestingly, supplementation of wheatgrass at all doses brought down the already increased activity of amylase, lipase but there was significant decrease with 0.8, 1.0 & 1.2gm/kg b wt. dose of wheatgrass. Also, it was found that levels of LPO in pancreatic tissue were significantly higher where as GSH levels were significantly lower in arginine group as compared to controls. Interestingly, supplementation of wheatgrass at all doses brought down the already increased activity of amylase, lipase but there was significant decrease with 0.8, 1.0 & 1.2gm/kg b wt. dose of wheatgrass. Also, it was found that levels of LPO in pancreatic tissue were significantly higher where as GSH levels were significantly lower in arginine group. In wheatgrass treated groups, GSH levels were increased while LPO decreased as compared to L-arginine group (group 2). Histologically, inflammation, fibrosis, fat infiltration and edema scores were significantly higher in arginine group as compared to Wheatgrass + L-arginine groups (groups 4-7).

Conclusion: Wheatgrass supplementation at dose of 0.8gm/kg bw is effective in controlling chronic pancreatitis in rats.

1. Introduction

Chronic pancreatitis (CP) is an inflammatory condition affecting the pancreas. CP induces irreversible fibrotic destruction of pancreas [1]. Chronic pancreatitis is characterized by diffuse or focal parenchymal destruction with damage to the acini, islet cell destruction, chronic infiltration of inflammatory cells, loss of normal architecture, and progressive fibrosis [2]. Fibrosis ultimately leads to irreversible morphological and structural changes resulting in impairment of both exocrine and endocrine functions of the pancreas.

Oxidative stress is involved in the pathogenesis of pancreatitis, and has a substantial role in the development of pancreatic fibrosis also [3]. Antioxidant deficiencies were reported in patients with both temperate and tropical pancreatitis [4-5] and it was anticipated that CP is related to oxidative stress. Oxidative stress defined as a state of potential injury due to imbalance between injurious free radicals and protective antioxidants [6]. Morris-Stiff et al (1999) reported that CP is related to antioxidant deficiency [7]. There is evidence that antioxidant supplementation in such cases reduces the demand for analgesics and the frequency of hospital admission due to painful exacerbations [8].

Diet and nutrition plays a major role in prevention of pancreatitis. The cereal grasses- wheatgrass, Alfa alfa have also been reported to improve health in animals as well as humans [9]. Wheatgrass has antioxidant properties. It has been reported that wheatgrass extract significantly inhibits ascorbate-Fe2+ induced lipid peroxidation in rat liver mitochondria [10]. Wheatgrass is rich in chlorophyll and various enzymes. Wheatgrass contains more than 60% chlorophyll and prevents the growth of pathogens. It is reported that chlorophyll is the main constituent in wheatgrass, which reduce oxidative stress [11]. Wheatgrass contains at least 13 vitamins, in which several are antioxidants like vitamin B12,
cytochrome oxidase, superoxide dismutase (SOD), mucopolysaccharide [12]. Wheatgrass also contain glycoprotein P4D1, which acts like an antioxidant and stimulates revival of DNA and RNA [13]. Wheatgrass reported to have good antioxidant properties. Therefore, present study was planned to investigate that wheatgrass if taken as a part of dietary supplement is beneficial in prevention of chronic pancreatitis. Because of its active ingredients, like chlorophyll, vitamins, minerals etc, wheatgrass may be powerful body healer. Till date there is no report available in which effect to wheatgrass has been tested in pancreatitis. Our study emphasizes on prevention of pancreatitis using wheatgrass in the daily diet.

2. Material and methods

2.1 Ethical consideration

The study was conducted after obtaining approval from Institute’s Animal Ethics Committee (IAEC) of PGIMER, Chandigarh. All the experiments and procedures including handling of the rats were carried out involving the highest standard of animal care, as per the norms laid down by the Institute’s Ethics Committee (I.E.C) of PGIMER, Chandigarh, to minimize pain and discomfort of the rats. Animals were sacrificed and carcasses were disposed off as per the principles of good laboratory practice (GLP).

2.2 Animals

The animals used in the study were Wistar rats of either sex in the weight range of 200 ± 50g and were acclimatized for one week prior of subjecting them to different treatments. They were kept in polypropylene cages under standard laboratory conditions of temperature and 12 hour light/dark cycle. Rats were fed standard laboratory diet and water ad libitum.

2.3 Chemicals

The chemicals used in present study were of analytical grade (AR) and laboratory grade (LR) received from various companies like BDH Chemicals, Merck, Ranbaxy, Sigma chemicals. L-arginine monohydrochloride (Cat. No. A5131) was procured from Sigma-Aldrich chemicals (India).

2.4 Production of plant material (Wheatgrass)

The seeds of *Triticum aestivum* L. are soaked for twelve hours and sown in place containing red soil. After 7 days when the wheatgrass is about 7-8 inches that is when it is at its nutritional peak. Wheatgrass trimmed above the soil surface and fresh plant material collected. These wheatgrass plants washed under running tap water to remove adhering dust, dried under shade for 2 days and pulverized in a mechanical grinder. The coarse powder is used for further studies and is available commercially in the form of tablet also. Wheatgrass tablets were procured from Sarvaayush Ayurveda and Herbals, Pune, India.

2.5 Experimental Protocol

42 Wistar rats of either sex were obtained from Central animal house, PGIMER, Chandigarh and divided equally into following seven experimental groups. All the animals were fed on standard laboratory diet and water ad libitum throughout the period of experimentation (Fig. 1).

Figure 1: Experimental design to standardize the dose of wheatgrass.

Group 1- Rats in this group were given water intragastrically daily and it was started 2 days before starting I.P injections of normal saline. Rats in this group were also given I.P injections of normal saline on day 1, 4, 7, 10, 13, 16 and 19. This group was used as the vehicle for giving arginine and served as control group.

Group 2- For arginine induced chronic pancreatitis, rats were given two I.P injections of arginine, 250 mg/100g body wt./day at 1 hr. interval on day 1. I.P injections were repeated with a single and same dose of arginine i.e 250 mg/100g b wt./day on day 4, 7, 10, 13, 16 and 19 as per our published criteria [14].

Group 3- Rats in this group were given wheatgrass tablets intragastrically 2 days before starting arginine injections. Wheatgrass tablets at a dose of 1.2 g/kg b wt./day were given daily as 16% solution in drinking water. Rats in this group served as wheatgrass control for groups 4, 5, 6 and 7. Maximum dose of wheatgrass was taken in this group to check its adverse effect.

Group 4- Rats in this group were given I.P injections of arginine in a similar way as in group 2 animals. Wheatgrass tablets dissolved in water were
given intragastrically 2 days before starting arginine injections. Wheatgrass tablets at a dose of 0.6 g/kg b wt./day were given daily as 16% solution in drinking water.

Group 5- Rats in this group were given I.P injections of arginine in a similar way as in group 2 animals. Wheatgrass tablets at a dose of 0.8 g/kg b wt./day were given intragastrically 2 days before starting arginine injections.

Group 6- Rats in this group were given I.P injections of arginine in a similar way as in group 2 animals. Wheatgrass tablets were intragastrically given 2 days before starting arginine injections. Wheatgrass tablets at a dose of 1.0 g/kg b wt./day were given daily as 16% solution in drinking water.

Group 7- Rats in this group were given I.P injections of arginine in a similar way as in group 2 animals. Wheatgrass tablets were intragastrically given 2 days before starting arginine injections. Wheatgrass tablets at a dose of 1.2 g/kg b wt./day were given daily as 16% solution in drinking water.

Food consumption by rats and their physical activities were recorded daily. All animals were weighed at the beginning of the experiment and at weekly intervals till end of experiments. All animals were sacrificed by giving I.P injection of thiopentone sodium (50 mg/kg) on day 21. Their blood was collected and stored at -80°C and homogenate was prepared in tris buffer (pH 8). Their blood was collected and stored at -80°C and homogenate was prepared in tris buffer (pH 8). Other portion of pancreatic tissues was dissected out from its attachment to the stomach, duodenum and spleen. Fat and peri-pancreatic tissues were removed. One portion of pancreas was stored at -80°C and homogenate was prepared in tris buffer (pH 8) for biochemical estimations. Other portion of pancreatic tissue was fixed in 10% buffered formaldehyde for H & E stain. Light microscopic studies were done to analyze histoarchitecture of pancreas in all groups.

2.6 Biochemical estimations
2.6.1 Serum Amylase
Estimation of serum amylase level was done by Amyloclastic method [15]. In this method, iodine gives blue colored complex when it comes in contact with starch. Thus, amylase activity of samples was evaluated by recording time in which known amount of starch was hydrolysed by amylase. Thus the end point of the reaction is absence of any substrate capable of forming the starch-iodine blue colored complex. The serum amylase activity was expressed as S.U/100 ml (Somogyi unit/100 ml).

2.6.2 Serum Lipase
Lipase levels were measured using method of MacDonald and LeFave (1962) [16]. This test involves incubation of sample containing lipase with olive oil emulsion. The fatty acids released during this reaction is then neutralized or titrated with 0.05 N sodium hydroxide. Amount of lipase present in sample is expressed as ml of 0.05 N sodium hydroxide required to neutralize the fatty acids produced by hydrolysis under the conditions of the test.

2.6.3 Preparation of pancreatic tissue homogenate
Pancreatic tissue homogenates (10%) were prepared in ice cold tris buffer (pH 8.0), using mechanically driven fitted Potter-Elvejhem type homogenizer for a few minutes till total disruption of cells. Homogenates were centrifuged at 10,000g for 10 minutes at 4°C. Aliquots of the post mitochondrial supernatants (PMS) were prepared for estimation of pancreatic LPO and pancreatic GSH levels.

2.6.4 Lipid peroxidation (LPO)
Lipid peroxidation is a marker to assess oxidative damage caused by reactive oxygen species. Malondialdehyde (MDA) is taken as an index to measure the extent of lipid peroxidation. Iron ascorbate catalyzed lipid peroxidation which was analysed by method of Ohkawa et al (1979) [17]. Lipids, mainly the polynsaturated fatty acids (PUFAS) are highly susceptible to peroxidation by various oxidizing free radicals, which are formed by ionizing, non-enzymatic oxidation reactions promoted by Fe2+ species. Cycloperoxides are formed as a result of these peroxidation reactions, which give MDA by cleavage reactions. MDA thus formed reacts with thiobarbituric acid (TBA) to form pink coloured chromophore which absorbs maximally at 532 nm. Units for LPO are nmoles MDA formed/min/100 mg protein for pancreatic tissue.

2.6.5 Glutathione (GSH)
Estimation of reduced glutathione was carried out in the pancreatic tissue according to the method of Ellman (1979) [18]. In this method 5, 5’ dialthio (2-nitrobenzoic acid) (DTNB) is reduced by sulphhydryl groups to form one mole of 2-nitro 5-mercaptobenzoic acid per mole of SH group. The nitromercaptobenzoic acid anion has an intense yellow colour, which absorbed maximum at 412 nm. Units for GSH are µmols non protein –SH/g for tissue homogeneate.

2.7 Histopathological examination
2.7.1 Haematoxylin and Eosin stain
Light microscopic studies were done to analyse histoarchitecture of the pancreas in all the groups. Samples of pancreatic tissues were fixed in buffered 10% formalin for at least 24 hours before processed through serial changes of alcohol, xylene and then embedded in paraffin. Paraffin sections were cut at 3 µm thick. The slides were then de-paraffinized in xylene, rehydrated through decreased concentration
of alcohol. The slides were then placed in haematoxylin stain for 15 minutes followed by water wash and dip in acid alcohol and then kept under running water for 15 minutes. This was followed by eosin treatment for 5 minutes, washed with water and dehydrated in ascending concentrations of alcohol and cleared in xylene. The slides were then mounted in DPX.

Histologic scoring of necrosis, inflammation, edema, acinar atrophy, fibrosis, and fat infiltration were performed as follows: 0, 0%; 1, 0-25%; 2, 25-50% and 3, >50%, in the total area of the specimen [19].

2.8 Statistical analysis

All values were presented as mean ± standard deviation (SD). Statistical analysis of the data was accomplished using either student’s t-test for comparison of two groups or analysis of variance (ANOVA) with Newman-Keuls post hoc test for comparison of more than two groups. Chi square test was applied for comparison of the categorical data. Histopathological differences of scores were tested using Man-Whitney U test. Value of p<0.05 was considered statistically significant. Statistical analyses were performed by using SPSS version 10.0 for Windows (SPSS, Inc., Chicago, IL).

3. Results

3.1 Animals

It has been observed that rats were sluggish and they were lethargic with administration of second dose of L-arginine. This phase remain same for 1-3 hr after that they gradually become active again. As far as condition of rats, we observed that after 72 hr of L-arginine injection they were less active compared with control rats. These changes were not observed in the control group of rats, which were injected with comparable doses of saline alone.

3.2 Weight of animals in different groups

Baseline weight (mean±SD) of control animals group 1, arginine treated animals group 2, wheatgrass alone treated group 3, wheatgrass along with arginine treated group 4- till group 7 were 194±10.5 g, 198±12.5 g, 195±8.7 g, 210±11.8 g, 200±7.4 g, 198±6.4 g and 201±9.4 g respectively. There were reduction in the body weight of the animals following arginine treatment however significant reduction were observed only in group 2 (185.5±15.0 g) and in group 4 (170.5±8.0 g). There was no reduction in the body weight of the animals in control group (215±13.6 g) as well as in wheatgrass alone group 3 (197±7.7 g) although there was slight increase in their body weight which was not statistically significant.

3.3 Serum amylase and serum lipase

Level of serum amylase was significantly higher in arginine treated group (1960.5±108.12 S.U/dl) when compared to control group (1620.6±80.1 S.U/dl). Highest dose of wheatgrass (1.2 g/kg) did not show any elevation in serum amylase levels. Serum amylase levels in wheatgrass 0.8 g/kg to 1.2 g/kg dose groups confirmed significant reduction (Table 1). Level of serum lipase was significantly higher in arginine treated group (4.9±0.35) when compared to control group (0.95±0.23). Highest dose of wheatgrass (1.2 g/kg) did not show any elevation in serum lipase levels. Serum lipase levels in wheatgrass 0.8 g/kg to 1.2 g/kg dose groups confirmed significant reduction (Table 1). Serum lipase levels in wheatgrass dose 0.8g/kg treated group (1.98±0.32) confirmed lowest level.

Table 1: Effect of different doses of wheatgrass on serum amylase and lipase.

<table>
<thead>
<tr>
<th>Groups (n=6)</th>
<th>Serum Amylase (S.U/dl)</th>
<th>Serum Lipase U (ml NaOH consumed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1620.6±108.1</td>
<td>0.95±0.23</td>
</tr>
<tr>
<td>Arginine</td>
<td>1960.5±108.12</td>
<td>4.9±0.35</td>
</tr>
<tr>
<td>Wheatgrass</td>
<td>1597.4±87.5</td>
<td>1.0±0.22</td>
</tr>
<tr>
<td>Arginine +</td>
<td>1910.6±99.5</td>
<td>3.6±0.5</td>
</tr>
<tr>
<td>Wheatgrass</td>
<td>1806.8±117.5</td>
<td>1.98±0.32</td>
</tr>
<tr>
<td>Arginine +</td>
<td>1790.5±120.1</td>
<td>2.02±0.24</td>
</tr>
<tr>
<td>Wheatgrass</td>
<td>1801.5±117.1</td>
<td>2.05±0.27</td>
</tr>
</tbody>
</table>

Data expressed as mean ± S.D.

a1p<0.05, a2p<0.01, a3 p<0.001, when values are compared to controls.
b1p<0.05, b2p<0.001; when values are compared to arginine treated animals.

3.4 Oxidative stress and antioxidant status in pancreatic tissue of rats.

LPO levels in pancreatic tissue of rats were increased significantly (p<0.001) following administration of arginine (8.10±0.68) as compared to control rats, 3.49±0.34 (Fig. 2). However the activity of pancreatic GSH, were decreased significantly (p<0.001) in arginine treated group (0.57±0.15) as compare to control group, 2.46±0.36 (Fig. 2). 0.8 g/kg w t./day, 1.0 g/kg b w./day and 1.2 g/kg b wt./day, dosages of wheatgrass showed significant (p<0.001) reduction in LPO (4.62±0.58, 4.35±0.59 and 4.53±0.91 respectively) when
compared with arginine treated animals (Fig. 2). On the other hand, pancreatic GSH levels show an increase activity following supplementation of wheatgrass.

But arginine treated rats supplemented with 0.6 g/kg of wheatgrass did not show significant changes in the activity of LPO and GSH, when compared with activity of arginine treated rats. However, enzymatic activity tended to restore with wheatgrass treatment and showed significant (p<0.05) recovery in 0.8 g/kg b wt./day to 1.2 g/kg b wt./day doses. It was also observed that recovery was almost similar in 0.8 g/kg b wt./day to 1.2 g/kg b wt./day dosed groups. Thus, not much difference was observed in biochemical indices in supplementation of 0.8 g/kg b wt./day as well as 1.0 g/kg b wt./day doses of wheatgrass.

3.5 Histoarchitecture of the pancreas

In arginine treated group exocrine pancreas of all animals showed extensive fat infiltration (30-50%) and fibro inflammatory changes. Acinar atrophy observed in 5 out of 6 animals (Fig. 3a). When these animals were treated with different doses of wheatgrass along with arginine it has been observed that wheatgrass dose 0.6 g/kg b wt./day showed no protection as majority of the animals i.e 5/6 showed extensive fibrosis, inflammation, acinar atrophy (Fig. 3b). Wheatgrass doses i.e 0.8, 1.0, 1.2 g/kg b wt./day showed significant protection as in all three groups, 3/6 animals showed only mild to moderate inflammation of the pancreas (Fig. 3 c-e) whereas rest 3/6 animals showed chronic pancreatic changes. One group of the animals was treated only with maximum dose of the wheatgrass i.e 1.2g/kg b wt./day. It has been observed in this group, that all animals i.e 6/6 showed normal pancreatic acinar cells (Fig. 3 f) which confirmed that wheatgrass alone did not created toxic effect to animals even if given at highest dose. However, normal histoarchitecture of acinar cells, observed in control rats (Fig. 3 g).

On the basis of these biochemical and histological finding wheatgrass dose 0.8g/kg b wt./day was considered minimum dose which showed maximum protection in experimental model of chronic pancreatitis.

4. Discussion

Chronic pancreatitis is a progressive, inflammatory disease of the pancreas, which ultimately lead to the development of pancreatic fibrosis and fat infiltration. Chronic pancreatitis is generally diagnosed at advanced stage which, limitize the treatment of the disease. Considering this problem, present study was planned with aim to explore the anti-oxidative, anti-fibrotic effect of wheatgrass on arginine induced chronic pancreatic changes in experimental model of rats.

In the present study wheatgrass were given to animals 30 minutes before induction of pancreatitis. Similarly a previously reported study by Batirel et al (1996) has administered aqueous garlic extract 30 minutes before induction of ischaemia and has reported protection against oxidative stress in rat brain [20]. No study till date explored the effect of wheatgrass in chronic pancreatitis neither in human beings nor in experimental models.

Serum enzymes i.e. amylase and lipase are the diagnostic biochemical tests for pancreatitis [21]. These investigations are technically simple and readily available that’s why they are continued to be used routinely in clinical settings [22]. As far as experimental studies are concerned, estimation of these two enzymes are important parameters in establishing the efficacy of investigational agents and markers for development of pancreatitis.

In the present study level of serum amylase in wheatgrass treated groups along with arginine showed significant reduction except in wheatgrass dose 0.6 g/kg b wt./day group when compared with arginine treated animals. Different doses of wheatgrass treatment along with arginine (group 4-7) also had significantly higher serum amylase levels compared to control group. Their rise in enzyme levels could be due to the injury caused by arginine to the pancreatic tissue thus causing the release of amylase in serum. Although the serum amylase levels were significantly high in wheatgrass treated groups as compared to controls but histopathological examination showed that severity of pancreatitis was significantly less in wheatgrass treatment along with arginine in group 5 till group 7. Thus based upon the results of serum amylase, it can be concluded that treatment with wheatgrass were beneficial in prevention of recurrent acute pancreatitis injury to the animals, which may lead to the development of chronic pancreatitis.

Levels of serum lipase were significantly elevated after arginine treatment compared to control group in present study. It has also been observed that wheatgrass treatment showed significant reduction in the level of serum lipase when compared with arginine treated group. However, there was significant elevation in the levels of serum lipase when compared with control group. This could be again due to the fact that arginine treatment injured pancreatic acinar cells that lead to excessive excretion of lipase in the circulation. The level of serum lipase was found to be significantly reduced in different doses of wheatgrass treated groups. This indicates the beneficial effect of these natural products. Similarly in a study, it has been reported that caerulein induced acute pancreatitis in rats lead to significant elevation in serum amylase and lipase.
When these animals were treated with a Shen-Fu injection (Chinese medicine), all biochemical indices became normal [23]. The beneficial effects seen in the present work with wheatgrass may be because these natural products controlled severity of arginine induced pancreatitis and prevented damage of acinar cells in pancreatic tissue.

At high concentrations free radicals and ROS preferentially react with the phospholipids of cell membranes inducing lipid peroxidation chain reactions, disintegration of membrane structure and irreversible cell damage [24]. They can also damage other important cellular molecules including DNA and proteins, provoking changes in physiochemical characteristics and subsequently, impairment in functional properties of intracellular proteins [25]. Oxidative stress and impairment of anti-oxidative mechanism are involved in pathogenesis of acute and chronic pancreatitis. It has been reported in literature that in patients of acute and chronic pancreatitis, levels of LPO increased while levels of GSH decreased when compared to healthy controls [26].

In the present study, increased levels of LPO and reduced level of GSH were observed in the pancreas of rats subjected to arginine treatment. This finding is consistent with the finding of Schoenberg et al (1995a), who reported significant elevation in MDA concentration in pancreatic tissue of acute and chronic pancreatitis individuals [24]. It was also observed in their study that level of reduced glutathione was significantly lower suggesting glutathione depletion due to oxidative stress. Enhanced expressions of MDA as a result of membrane LPO may indirectly reflect oxygen free radicals (OFR) activity [27]. Arginine is responsible for production of OS, which may be the reason for elevation of pancreatic LPO in our study.

Supplementation of different doses of wheatgrass along with arginine showed significant reduction in LPO whereas significant elevation in GSH levels except in wheatgrass dose 0.6 g/kg b.wt./day group. GSH has been reported as the main nonprotein thiol within the cell and part of a major intracellular defense system against OFR [28]. Wheatgrass supplementation to healthy volunteers during physical training for one month has been shown to reduce LPO levels in their blood [29]. It has been reported that wheatgrass supplementation significantly reduced MDA levels and increased GSH and vitamin C levels [30]. Antioxidant therapies have been shown to improve pancreatitis induced by caerulein administration [23]. Various antioxidant compounds have been already reported to reduce the oxidative stress in arginine induced pancreatitis by melatonin [31].

Histopathological examination is the confirmatory test for establishing the beneficial effect of different doses of wheatgrass. In the present study histopathological changes in the pancreatic tissue of rats were studied in control, arginine and different doses of wheatgrass along with arginine groups. It has been observed that arginine treated group exhibited acinar cell atrophy with altered ductal morphology, inflammatory cell infiltration, increased fibrosis and acinar replacement with fatty infiltration which is typical feature of chronic pancreatitis. However individual histological scores in control group was zero, indicating normal pancreatic architecture without any damage to pancreatic parenchyma. In animals treated with different doses of wheatgrass along with arginine it has been observed that severity of histopathological changes was markedly less compared to arginine group except in wheatgrass dose 0.6g/kg b wt./day group. Till date not a single study has reported the protective effect of wheatgrass on experimental model of pancreatitis in rats. Improvement in histopathological changes may be due to additive effect of wheatgrass to prevent free radical injury to the pancreas. Similarly, Zhou et al (2013) reported the effects of edaravone, a potent free radical scavenger, on Dibutyltin Dichloride induced CP [32]. They reported that edaravone improved histological scores and alleviated the fibrosis of pancreas.

In conclusion, 0.8g/kg b wt./day, dose of wheatgrass is the minimum dose showing maximum protection in CP model of rats. It were experimentally established on the basis of histological and biochemical parameters.

5. Acknowledgements

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6. References