

Effect of Wheatgrass Juice on Fertility Changes Induced by Cyclophosphamide in Male Wistar Albino Rats

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In the present study, reproductive toxicity caused by cyclophosphamide on sperm parameters, biochemical parameters, histopathology and protective effect of wheatgrass juice was examined. There were total 6 groups of adult Wistar albino rats. Each group had 6 rats. After 24hrs of the last treatment, rats were sacrificed by cervical dislocation and sperm parameters like sperm count and motility, plasma malonaldehyde (MDA) levels, catalase (CAT) levels, superoxide dismutase (SOD) and glutathione (GSH) activities in testicular tissue, and testicular histopathological changes in the testicular tissue were examined. Administration of cyclophosphamide showed an increase in plasma MDA level and decrease in SOD, GSH and Catalase activity. These values were statistically significant. However, wheatgrass juice treatment along with cyclophosphamide markedly showed improvement in reducing cyclophosphamide-induced oxidative stress, and normalized sperm characteristics and testicular histopathology. Thus, in conclusion, cyclophosphamide causes fertility changes which leads to male infertility, whereas wheatgrass juice has a protective effect on reversing the testicular damage and male infertility.

Keywords: Antifertility; Antioxidant; Sperm morphology; Testicular health.

Infertility is a major issue faced by couples worldwide. WHO defines infertility as an inability of a couple to conceive after one

or more years of unprotected intercourse. It affects approximately 15% of couple who have unprotected intercourse. Infertility affects 8 percent

of the world's population globally¹. About 20-30% of population is affected by male factor infertility. In India, around 3.9-16.8% of male suffer from male infertility with the incidence being high in Kashmir². There are many physiological and conventional causes for infertility, among these one of the potential factor is oxidative stress³. It is the condition where the homeostasis between ROS and antioxidants are disturbed which leads to decreased sperm count & motility, increased DNA damage, deformity and male infertility. This is due to increased levels of ROS or decreased antioxidant counter mechanism^{4,5}

Cyclophosphamide (CP) is an alkylating, anticancer agent, which is extensively used to treat several types of cancers. It also has a potent immunosuppressive effect because of which it is used in organ transplantation and autoimmune diseases⁶. Acrolein is one of the metabolites of cyclophosphamide, which causes lethal effects on normal cells by activating reactive oxygen species (ROS) and nitric oxide production. Cyclophosphamide is known to cause sperm damage to the DNA and alteration in the gene expression of male germ cells^{7,8}

Wheatgrass (WG) (*T. aestivum*) belongs to the family Graminae. It has high chlorophyll content and rich source of vitamins, minerals, and antioxidants (SOD & Cytochrome oxidase). Wheatgrass also has rich amount of flavonoids, phenolic compounds, and alkaloids which have antioxidant property. Literature studies showed that wheatgrass has antidiabetic, anticancer, antiulcer, antiarthritic and hepatoprotective properties⁹.

Antioxidant supplementation may be effective as treatment depending on the pathology of male infertility¹⁰. Thus, the aim of present study was to evaluate the protective role of WGJ on cyclophosphamide-induced male infertility.

MATERIALS AND METHOD

In current study, 150-250gm weighing adult male Wistar albino rats were used and kept under standard laboratory conditions. Rats were housed in polypropylene cages in a room at temperature 25°C with 12:12hr dark and light cycle. Water and food were given ad libitum.

Cyclophosphamide (Endoxan, 500mg) was purchased from Manipal pharmacy of

Manipal and the test drug Wheatgrass powder (Brand-Organic India) was obtained by online store. Wheatgrass juice was used to administer by dissolving the powder in distilled water.

A total of 36 animals were randomly divided into 6 groups. Each group consisted of 6 rats. The grouping and dosing of animals is illustrated in table 1. Cyclophosphamide was given as a single dose and Wheatgrass juice was given for about 30 days. After 24 hours of last treatment, rats were sacrificed by cervical dislocation and further studies were carried out.

Parameters measured

Body weight

Body weight of each rat was measured at the beginning and after the completion of treatment period.

Reproductive organ weight

Reproductive organs were removed and weighed after sacrifice of rats.

Sperm functions analysis

Sperm count

After weighing the epididymis, using 1ml of phosphate buffer solution the epididymis was minced with anatomical scissors to obtain a suspension. This was then filtered through an 80µm of nylon mesh. Using standard method Neubauer chamber, sperm count was conducted in the filtrate.

Sperm Mass motility

Percentage of motile sperms were calculated by using three different fields in each sample, motility estimations were performed and the mean of the three samples was taken as the final motility score¹¹.

Histopathology

Testis of each rat was taken for histopathological evaluation. Qualitative analysis was done to look for vacuoles, gaps & sloughing of epithelium, which indicate the extent of tissue damage.

Procedure

The testicular tissues were embedded in paraffin and washed with series of graded alcohol. The tissue sections were taken and mounted on slides later staining of the tissues were done by using hematoxylin and eosin stain followed by dehydration and lastly mounted by using DPX¹².

Biochemical analysis

Antioxidant assay

Testicular tissue was used for checking

MDA (malondialdehyde) levels, catalase, SOD and Glutathione levels.

Statistical analysis

One-way ANOVA followed by Tukey's post hoc analysis was used to compare the means between the groups. p -value < 0.05 accepted as statistically significant. Results of all groups were expressed in mean value \pm SD.

RESULTS

Body weight

At the baseline, there was no significant difference was seen in body weight among any group. Animals treated with cyclophosphamide showed significant reduction in final mean body weight as compared all the other groups. The bodyweight was preserved in groups treated with cyclophosphamide plus wheatgrass juice and there was no statistically significant difference was seen on intergroup comparison. (Table 2)

Testicular and epididymal weight

The mean weight of testicular tissue and epididymis was significantly reduced in cyclophosphamide administered group. The reproductive organ weights of groups 3 and 4 were significantly reduced when compared with control. However, on intergroup comparison between group 3 & 4, no statistically significant difference was seen. (Table 2)

Sperm parameters

Sperm count and Sperm motility

Significant reduction in number of sperms and percentage of motile sperms were seen in CP alone treated group when compared with the remaining groups. Significant increase in sperm quantity and quality was seen in groups treated with wheatgrass juice along with cyclophosphamide as compared to cyclophosphamide alone treated group. However, there was no significance was seen between the groups. (Table 3)

Biochemical analysis

In biochemical analysis, changes in the activities of redox and antioxidant enzymes were estimated. The MDA levels of cyclophosphamide alone treated group was significantly higher in comparison to other groups. The SOD and MDA levels of groups 3 and 4 were significantly increased as compared with control, whereas all other parameters such as SOD, GSH and Catalase levels were decreased significantly in cyclophosphamide alone treated group in comparison to other groups. (Table 4)

Histopathological examination:

The testicular tissues stained in haematoxylin and eosin and observed for, I- Gaps between the seminiferous tubules, L- Lumen of the tubule S- Seminiferous epithelium

Group 2 (figure 1 b) showed marked testicular damage which has increased gaps between the tubules and abnormal epithelium as compared to control (figure 1 a) group

Group 3 and 4 (figure 1c & 1d) showed better results compared to group 2 by retaining the comparatively normal epithelium and gaps between the tubules.

Group 5 and 6 (figure 1e & 1f) are comparatively similar to control in gaps, epithelium and lumen of the seminiferous tubules

DISCUSSION

Most of the chemotherapeutic drugs particularly alkylating agents are known to cause gonadotoxic effects. This study was done to evaluate the antioxidant effect of wheatgrass juice on male infertility, which was caused by cyclophosphamide.

The results from present study showed that use of cyclophosphamide (150mg/kg)

Table 1. Outline of different groups and doses of drugs

Groups	Drugs	Rats
1 (positive control)	Normal saline	6
2 (negative control)	Cyclophosphamide (150mg/kg) i.p	6
3 (test)	Cyclophosphamide (150mg/kg) i.p+ Wheatgrass juice (100mg/kg) orally	6
4 (test)	Cyclophosphamide (150mg/kg) i.p+ Wheatgrass juice (400mg/kg) orally	6
5 (test)	Wheatgrass juice (100mg/kg)(10)orally	6
6 (test)	Wheatgrass juice (400mg/kg)(11)orally	6

produced fertility changes in the rats and the test dose of drug wheatgrass juice (100mg & 400mg) showed a positive effect by preventing the reproductive toxicity/oxidative damage produced by cyclophosphamide.

Cyclophosphamide is an anticancer drug, which has been used in various studies. In our study, the results clearly shown that administration of cyclophosphamide-produced decrease in sperm parameters and reproductive organ weights as

A) **Table 2.** Results showing initial and final body weight, testicular weight and epididymis weight of rats.

Group	Body weight Day 1(gm)	Body weight Day 30 (gm)	Testicular weight (gm)	Epididymis weight (gm)
1. Control	191.66 ± 26.39	236.67 ± 32.66 ^{a,c}	1.49 ± 0.05 ^a	0.31 ± 0.01 ^a
2. Cyclophosphamide	193.33 ± 30.11	135.00 ± 28.81	0.87 ± 0.08	0.12 ± 0.01
3. Cyclophosphamide+ wheatgrass juice 100mg/kg	193.5 ± 24.44	196.67 ± 10.33 ^a	1.30 ± 0.01 ^{a,d}	0.21 ± 0.01 ^{a,d,f}
4. Cyclophosphamide + wheatgrass juice 400mg/kg	211.66 ± 26.96	193.33 ± 31.57 ^a	1.34 ± 0.03 ^{a,d}	0.22 ± 0.02 ^{a,d,f}
5. Wheatgrass juice 100mg/kg	200.83 ± 18.55	243.50 ± 7.58 ^{a,b,c}	1.46 ± 0.04 ^{a,c}	0.31 ± 0.01 ^a
6. Wheatgrass juice 400mg/kg	194.66 ± 17.11	221.67 ± 12.11 ^a	1.40 ± 0.04 ^{a,d,e}	0.24 ± 0.03 ^{a,d}

Level of significance $p < 0.05$

a-compared to Group 2, b- compared to Group 3, c- compared to Group 4, d- compared to control, e- compared to Group 3 and f- compared to Group 5

B) **Table 3.** Sperm function analysis.

Group	Sperm count(106/mL)	Sperm motility(%)
1. Control	105.33 ± 2.80 ^a	26.92 ± 2.13 ^a
2. Cyclophosphamide	34.40 ± 2.47	6.20 ± 0.84
3. Cyclophosphamide+ wheatgrass juice 100mg/kg	69.86 ± 4.94 ^{a,b,c}	23.69 ± 4.91 ^{a,d}
4. Cyclophosphamide + wheatgrass juice 400mg/kg	78.13 ± 2.97 ^{a,b,c}	27.86 ± 3.73 ^{a,d}
5. Wheatgrass juice 100mg/kg	108.26 ± 11.87 ^a	20.70 ± 1.79 ^{a,d,e}
6. Wheatgrass juice 400mg/kg	111.46 ± 9.01 ^a	26.65 ± 3.06 ^a

Level of significance $p < 0.05$

a-compared to Group 2, b- compared to control, c- compared to Group 5&6, d- compared to control and e- compared to Group 4

C) **Table 4.** Enzymatic and non-enzymatic anti-oxidants levels in the rats testes of various groups

Group	SOD Units/mg	Catalase Units/mg	GSH μ M/mg	MDA μ M/mg
1. Control	0.077 ± 0.005 ^a	2.055 ± 0.019 ^a	0.201±0.023 ^{a,c}	0.818 ± 0.026 ^a
2. Cyclophosphamide	0.057 ± 0.004	1.645 ± 0.021	0.090 ± 0.008	1.319 ± 0.072
3. Cyclophosphamide e+ wheatgrass juice 100mg/kg	0.093 ± 0.008 ^{a,b}	1.995 ± 0.071 ^a	0.152 ± 0.011 ^a	1.005±0.023 ^{a,b}
4. Cyclophosphamide e + wheatgrass juice 400mg/kg	0.092 ± 0.004 ^{a,b}	2.066 ± 0.023 ^{a,d}	0.183±0.014 ^{a,c}	0.995±0.039 ^{a,b}
5. Wheatgrass juice 100mg/kg	0.085 ± 0.003 ^a	2.019 ± 0.008 ^a	0.195±0.008 ^{a,c}	0.836±0.139 ^{a,f}
6. Wheatgrass juice 400mg/kg	0.095 ± 0.003 ^{a,b,c}	2.104 ± 0.017 ^{a,d,c}	0.218±0.011 ^{a,f}	0.837±0.139 ^{a,f}

Level of significance $p < 0.05$

a-Compared to Group 2, b- compared to control, c- compared to Group 5, d- compared to Group 3, e- compared to group 3, and f- compared to Group 3&4

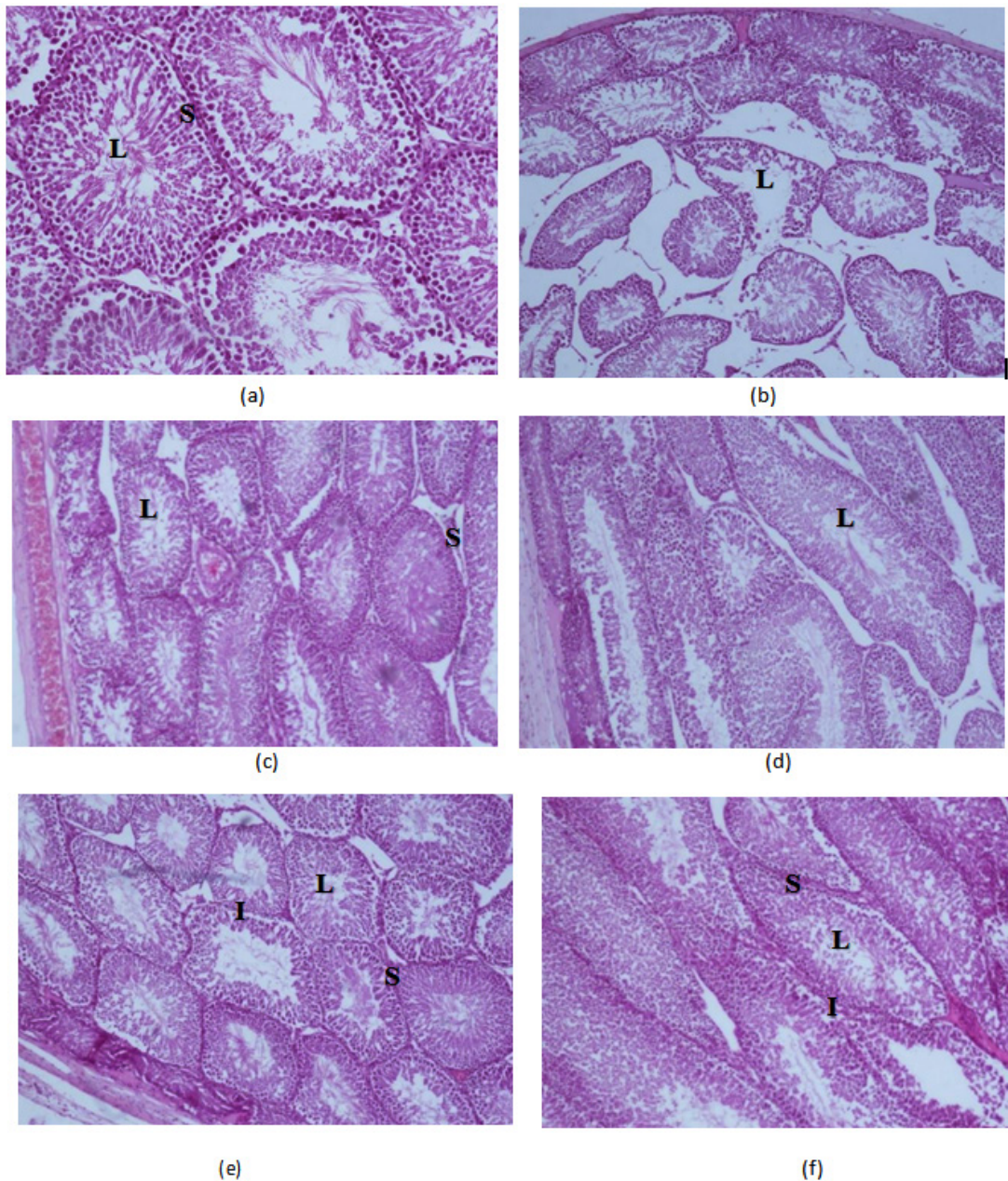


Fig. 1. Histopathological examination of testicular tissues.

a) Control group; (b) CP 150mg/kg; (c) CP 150mg/kg + WGJ 100mg/kg; (d) CP 150mg/kg + WGJ 400mg/kg; (e) WGJ 100mg/kg (f) WGJ 400mg/kg I- Gaps between the seminiferous tubules, L- Lumen of the tubule S- Seminiferous epithelium [Magnification: 40x10 X]

compared with other groups.

The possible mechanism behind this is, cyclophosphamide exposure causes oxidative stress by increasing the production of ROS and generation of free radicals¹³. Acrolein is one of

the metabolite of cyclophosphamide, which is known to cause reproductive toxicity by activating ROS and other free radicals, which are likely to cause DNA- protein or DNA-DNA crosslink and fragmentation of single strand, by alkylation.

In our study from the results, we have noted that both 100mg and 400mg of wheatgrass juice was able to reverse the fertility changes caused by cyclophosphamide by increasing the reproductive organ weights and sperm parameters as compared to cyclophosphamide alone treated group, the possible mechanism behind this is antioxidant effect of wheatgrass juice.

Jangle SN *et al.* and Rana S *et al.* said that wheatgrass is rich in antioxidants and they are used to treat various diseases like cancer, asthma, ulcerative colitis, IBD, thalassemia, diabetes, rheumatoid arthritis *etc*¹⁴. Antioxidants protect other molecules from free radical damage by preventing their oxidation (*in vivo*). Free radicals and reactive oxygen species have been held responsible for many diseases.¹⁵

It is well established that reactive oxygen species (ROS) is the culprit behind many cardiovascular diseases like hypertension, atherosclerosis, cardiac hypertrophy, heart failure and restenosis. NAD (P)H oxidase is said to be the major source of ROS¹⁶. Because of oxidative stress, there will be excess generation of ROS and free radicals such as O₂, OH and other radicals which results in increased MDA levels which is a by-product of lipid peroxidation.¹⁷ The biochemical parameters of present study showed that administration of 150mg/kg of cyclophosphamide resulted in increased MDA levels and decreased GSH, SOD and Catalase levels as compared to all the other groups whereas co-administration of wheatgrass juice along with cyclophosphamide reversed the effect of cyclophosphamide by decreasing the MDA levels and increasing GHS, Catalase and SOD levels in rats.

Histopathological findings from current study are clear that cyclophosphamide causes damage to the testicular tissue and the test dose of wheatgrass juice has protective effect against it by reversing the effect of cyclophosphamide. Protective effect of wheatgrass juice on testicular tissue is also suggested in the studies done by Eissa and Skoracka.^{18,19}

In present study, 2 doses (100mg/kg and 400mg/kg) of wheatgrass juice were used to evaluate the antioxidant property in which both the doses were effective in preventing male infertility.

Even a low dose of wheatgrass juice (100mg/kg) with cyclophosphamide was effective in reversing the fertility changes induced by cyclophosphamide by increasing the sperm counts and motility.

On comparison between wheatgrass 100mg and wheatgrass 400mg doses, which were given along with cyclophosphamide, wheatgrass 400mg was showed better effects than wheatgrass 100mg but there was no statistical significance.

CONCLUSION

The present study suggests that wheatgrass juice has a very good antioxidant effect, co- administration of wheatgrass juice with cyclophosphamide preserved the male fertility by overcoming the adverse effects of cyclophosphamide.

Conflict of Interest

There is conflict of interest.

Funding Sources

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