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Original Research Paper

Pharmacology

CARDIO PROTECTIVE EFFECT OF WHEAT GRASS (*TRITIUM AESTIVUM LINN*) IN ALBINO RATS

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ABSTRACT

Context: Cardiovascular disease (CVD) is the principal cause of death worldwide. Whole grain like wheat (Tritium aestivum Linn.) may have cardioprotective effect.

Aims: This study was carried out to find out the cardio-protective potential of Triticum aestivum Linn by observing its effect on Isoproterenol induced myocardial infarction in rats.

Settings and Design: This was a longitudinal study done on thirty healthy albino rats

Methods and Material: Thirty albino rats were grouped into five groups of six rats in each, and housed in separate cages group-wise. All groups except control received Isoproterenol. In addition to Isoproterenol, two groups were given wheat grass at different dosage and one group received Carvediliol. Cardiac parameters and histopathological study was done.

Statistical analysis used: ANOVA test and post hoc Dunnett test using SPSS version 16, unpaired t test

Results: Significant reduction in cardiac parameters was seen.

Conclusions: *Triticum aestivum* prevented the Isoproterenol induced cardiotoxicity. Wheatgrass extract can be a good alternative or primary therapy in treating cardiovascular diseases myocardial infarction in future.

KEYWORDS : Tritium Aestivum Linn, Myocardial Infaraction

INTRODUCTION:

Cardiovascular disease (CVD) is the principal cause of death worldwide. According to World Health Organization (WHO) cardiovascular diseases contribute to 20% of mortality and is estimated to have caused over 15 million deaths comprising more than one third of all deaths in the year 2001.(1) There are various risk factors which promote the development of Cardiovascular disease. Hypertension is a major indepe ndent risk factor for coronary artery disease & its attendant complications like Infarction, cardiac failure and sudden cardiac death & also responsible for increased incidence of stroke and blindness.

The Joint National Committee (7th JNC2003) on Hypertension advocates non pharmacological therapy to be an important component of treatment of all patients with hypertension and prehypertension. Lifestyle changes play important role in control of hypertension and these are to be continued for lifetime. (3)

Whole grain like wheat (*Tritium aestivum Linn.)* finds a place in the recommended DASH diet and is an important component of human diet, particularly in developing countries. Epidemiological studies reveal that consumption of whole grain and its products are protective against chronic diseases such as cardiovascular diseases, diabetes and cancer. Wheat when harvested as young green shoots germinated over a period of 6-10 days is generally called 'wheat grass' and it is known as 'functional food' during which vitamins, minerals and phenolic compounds such as flavonoids are synthesized in wheat sprouts reaching maximum antioxidant potential.(4) Herbal products are highly acceptable and used 70 to 80% of the world population for their primary health care, especially in developing countries, due to their easy access, lesser side effects and low cost. It is also known to possess antioxidant as proven in several studies. (5,6) This property might protect the heart and preventing the most important cardiovascular complication. This study will definitely help and add knowledge in scientific exploration of cardio-protective potential of this promising herb in animal models. With this study, we had tried to find out the cardio-protective potential of *Triticum aestivum Linn.* by observing its effect on Isoproterenol induced myocardial infarction in rats.

SUBJECTS AND METHODS:

This was a longitudinal study done on thirty healthy albino rats weighing between 100-200gms. Prior to the dietary manipulation, all rats were fed standard rat chow, containing 60% vegetable starch, 11% fat and 29% protein, water ad libitum and maintained on 12 hours light/dark cycle. Simultaneously rats were acclimatized to the procedure of blood pressure measurement daily for one week.

DRUGS & CHEMICALS:

The drugs selected for the study were obtained in pure powder form from the following sources.

1- *Triticum aestivum* powder –Girmes wheat grass, Pune
2- Carvediliol – Alkem Company, Mumbai, Maharastra, 400013
3- Isoproterenol (ISO) – Get well Pharmaceuticals, New Delhi

Myocardial Infarction was induced in rats by intraperitoneal injection of Isoproterenol hydrochloride at a dose of 20mg/100g body weight, dissolved in normal saline for 2 consecutive days.

Thirty albino rats were grouped into five groups of six rats in each, and housed in separate cages group-wise, as follows.

Group 1 (Control) : Rats were administered Normal saline 0.5ml orally for 15 day followed by Normal saline 5ml s.c. on 14th& 15th day, at an interval of 24 hr.

Group 2 (ISO) : Rats were administered Normal saline 0.5 ml orally once daily for 15 days followed by ISO 20mg/100g body weight subcutaneously on the 14th& 15th day, at an interval of 24 hr.

Group 3 (TR low dose + ISO) : Rats were pretreated with *Triticum aestivum* low dose 200mg/kg orally for a period of 15 days followed by subcutaneous injection of Isoproterenol 20mg/100g body weight on 14th& 15th day at an interval of 24 hr.
Group 4 (TR high dose + ISO): Rats were pretreated with *Triticum aestivum* high dose 250mg/kg orally for a period of 15

days followed by subcutaneous injection of Isoproterenol 20mg/100g body weight on 14th& 15th day at an interval of 24 hr.

Group 5 (carvedilol + ISO): Rats were pretreated with carvedilol 5mg/kg orally for a period of 15 days followed by subcutaneous injection of Isoproterenol 20mg/100g body weight on 14th and 15th day at an interval of 24hr.

Group	Drug	Dose	Duration	Induction of MI
1	Distilled Water	0.5 ml	For 15 days	No treatment
2	Distilled Water	0.5 ml		Isoproterenol 20mg/100gbwt. SC Inj on 14th& 15th day.
3	Triticum aestivum Extract	200mg/kg		
4	Triticum aestivum Extract	250mg/kg		
5	Carvedilol	5mg/kg		

BIOCHEMICAL STUDIES :

At the end of the experimental period (after 24hr of the last dose of Isoproterenol), orbital blood samples were collected from all the rats and the serum was used for estimation of biochemical parameters (cardiac biomarkers) like creatine phosphokinase (CK) and lactate dehydrogenase (LDH), aspartate aminotransferase (AST), alanine transaminase (ALT) and alkaline phosphatise (ALP).

HISTOPATHOLOGICALSTUDIES :

At the end of the experiment, three rats of each group were sacrificed and the heart was isolated to measure its weight. The myocardial tissues from all groups were subjected to histopathological studies. The tissues were fixed in 10% formalin, routinely processed, embedded in paraffin wax and sectioned. Paraffin sections were then dewaxed,cut in glass slides, stained in hematoxylin and eosin and examined under light microscope.

STATISTICAL ANALYSIS–

The comparison were done using ANOVA test and post hoc Dunnett test using SPSS version 16, unpaired t test and $p<0.05$ was considered as statistically significant.

RESULTS:

Effect of *Triticum aestivum linn*. on various cardiac biomarkers is shown in in Isoproterenol induced myocardial infarction in albino rats. As evident in the table, it is having comparable effect to that of Carvedilol. (Table 1) Effect of *Triticum aestivum linn*. on histomorphology is shown.(Table 2) Carvedilol has a more protective effect compared to what grass. Group I revealed cardiac fibers with normal architecture and regular morphology and showed muscle fibers with striations in cytoplasm and elongated nuclei. Group II showed large areas of infarcted cardiac muscle tissue with extensive loss of muscle fibers, scattered inflammatory cells and isolated residual muscle fibers. Group III showed very small infracted cardiac muscle tissue, although it showed loss of muscle fibers, occasional inflammatory cells and isolated residual muscle fibers. Group IV revealed very small infarcted cardiac muscle tissue, although it showed loss of muscle fibers, scattered inflammatory cells in the infracted area. Group V revealed almost normal cardiac fibers and regular morphology and showed muscle fibers with striations in cytoplasm and elongated nuclei.

Table 1: Effect of *Triticum aestivum Linn*. on various cardiac biomarkers.

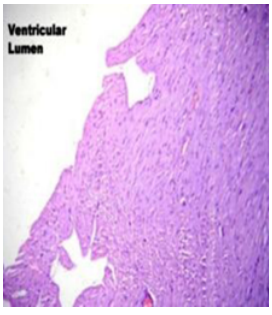
Parameters (mmHg)	Group1 CONTROL (Distilled Water)	Group2 CONTROL ISO 20mg/100gbwt	Group3 WG200mg/kg +ISO 20mg/100gbwt	Group4 WG250mg/kg +ISO 20mg/100gbwt	Group5 Carvedilol 5mg/kg+ ISO 20mg/100gbwt
CK-MB ± SE	75.34 ± 2.08	400.5 ±6.87	203.16 ±2.91	142.16 ±2.27	115.83 ±1.69
SGOT ± SE	31.567 ± 1.35	48.375*** ±0.93	39.49* ±0.81	34.82*** ± 0.54	31.40*** ± 0.63
SGPT ± SE	19.253 ± 0.64	67.22*** ± 1.59	52.117* ±1.69	38.133*** ± 2.86	22.96*** ± 1.25
ALP ± SE	92.59 ± 2.7	129.45** ±2.85	184.102* ±2.51	155.25** ±1.97	98.84 ±2.09
LDH ± SE	588.34 ±0.645	940.83*** ±3.64	740.16* ±3.42	691.5*** ±4.45	630.67*** ± 4.98

* – P<0.05, ** – P<0.01, *** – P<0.001

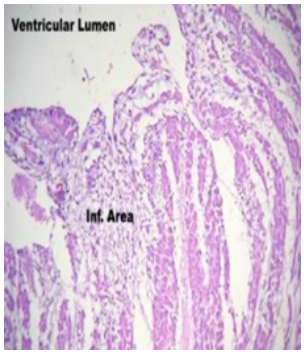
Table 2:Effect of *Triticum aestivum linn*. on histomorphology Of frontal section of heart in Isoproterenol induced myocardial infarction in albino rats

Group1 CONTROL (Distilled Water)	Group2 CONTROL ISO 20mg/100gbwt	Group3 WG200mg/kg +ISO 20mg/100gbwt	Group4 WG250mg/kg +ISO 20mg/100gbwt	Group5 Carvedilol 5mg/kg+ ISO 20mg/100gbwt
Grade-0	Grade-4	Grade-3	Grade-2	Grade-2
Grade-0	Grade-4	Grade-3	Grade-2	Grade-1
Grade-0	Grade-4	Grade-3	Grade-2	Grade-1

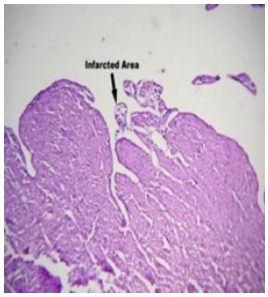
Figure 1:Photomicrograph in various group of rats



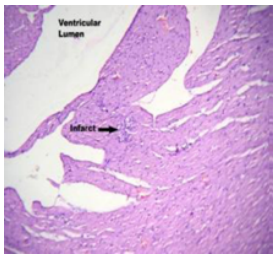
Group1 CONTROL (Distilled Water)



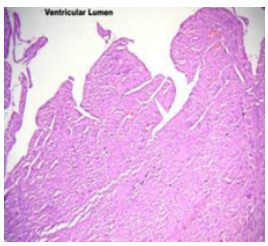
Group2 CONTROL ISO 20mg/100gbwt



Group 3 WG200mg/kg +ISO 20mg/100gbwt



Group4 WG250mg/kg +ISO 20mg/100gbwt



Group5 Carvedilol 5mg/kg+ ISO 20mg/100gbwt

DISCUSSION:

This study was carried out to find out the cardio-protective potential of *Triticum aestivum Linn.* by observing its effect on Isoproterenol induced myocardial infarction in rats. Wheat grass refers to the young grass of wheat (*Triticum aestivum*) germinated for a period of 6 to 10 days. It contains vitamin C and E, ß carotene, ferulic acid, vanilic acid and phenols, especially flavonoids. Wheat grass juice is found to have healing properties in various degenerative diseases and is known to benefit blood cells, bones, glands, kidney and other parts of the body. (7,8,9) Since little or no work has been done on cardio-protective effects of wheat grass, the present study was designed to analyse its role on Isoproteronol induced myocardial insufficiency.

Histopathological observations were in correlation with our biochemical results. Wheat grass treatment effectively reduced the pathological abnormalities and showed inflammatory cell infiltration, which is a positive immune response. This proves that Wheat grass is very effective in lowering the lipid levels induced by Isoproteronol.

In the cardioprotective study, first myocardial-infarction was induced with a -adrenergic agonist Isoproterenol, which caused severe stress in the myocardium causing coagulative necrosis (i.e. infarct like) of heart muscle. Cardiac markers are biomarkers measured to evaluate the heart function. They are creatine phosphokinase, Lactate dehydrogenase, aspartate aminotransferase etc.

The present study showed development of oxidative cardiac injury induced by ISO by the myocardial cell damage, the alteration in oxidative stress markers and the significant decrease in SOD as well as the levels of reduced GSH in the heart tissue. Cardioprotection was confirmed by the decrease level of serum markers of heart damage and elevated levels of GSH, SOD. (10)

Pre-treatment of *Triticum aestivum* was able to reduce the ISO induced cardiotoxic manifestations in multiple ways. Increase in the level of plasma triglycerides, total cholesterol, LDLs in the ISO treated group indicate that ISO may be interfering with metabolism or biosynthesis of lipids. Heart tissue injury induced by ISO in rats was indicated by elevated level of the marker enzymes such as serum. The increase of LDH level in serum suggests an increased leakage of this enzyme from mitochondria as a result of toxicity induced by treatment with ISO. (11)

Treatment with ISO increased serum marker of heart damage, AST, ALT, ALP. The pre-treatment with *Triticum aestivum* attenuated this increase in AST, ALT and ALP level. Serum level of AST, ALT and ALP were significantly reversed by treatment with *Triticum aestivum* extract was found to be more effective.

Light micrograph of ISO injected rats showed necrosis of muscle fibers, inflammatory cell infiltration and oedema with fragmentation of muscle fibers as compared with the control group. Treatment with *Triticum aestivum linn* in ISO treated rats (*Triticum aestivum linn*.+ ISO) showed moderate degree of edema, necrosis and inflammatory cells compared to IRI injected rats.

To conclude, the present result suggests that *Triticum aestivum* prevented the ISO induced cardiotoxicity. Wheatgrass extract can be a good alternative or primary therapy in treating cardiovascular diseases myocardial infarction in future.

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