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Modulatory Effects of *Achyranthes aspera* Extracts Pre-Treatment on Cyclophosphamide Induced Alterations in Lipid Profile Parameters in Albino Rats

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Author's contribution

The sole author designed, analyzed, interpreted and prepared the manuscript.

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ABSTRACT

Cyclophosphamide is one of the widely used chemotherapeutic drugs to treat various sorts of cancer and immune- related diseases. However, its use is restricted due to pro- oxidant activity that can damage healthy cells leading to altered metabolism. The liver becomes a susceptible target for xenobiotics toxicity due to its involvement in metabolic reactions. In the present study, an effort has been made to deduce the effects of pre- treatment with extracts of *Achyranthes aspera* on lipid metabolism in cyclophosphamide treated rats. 30 male albino rats were randomly divided into 6 groups, namely, group I (VC), group II (CYP), group III (AAML), group IV (AAMS), group V (AAML + CYP) and group VI (AAMS + CYP). Methanolic extracts of leaves and seeds of *A. aspera* were

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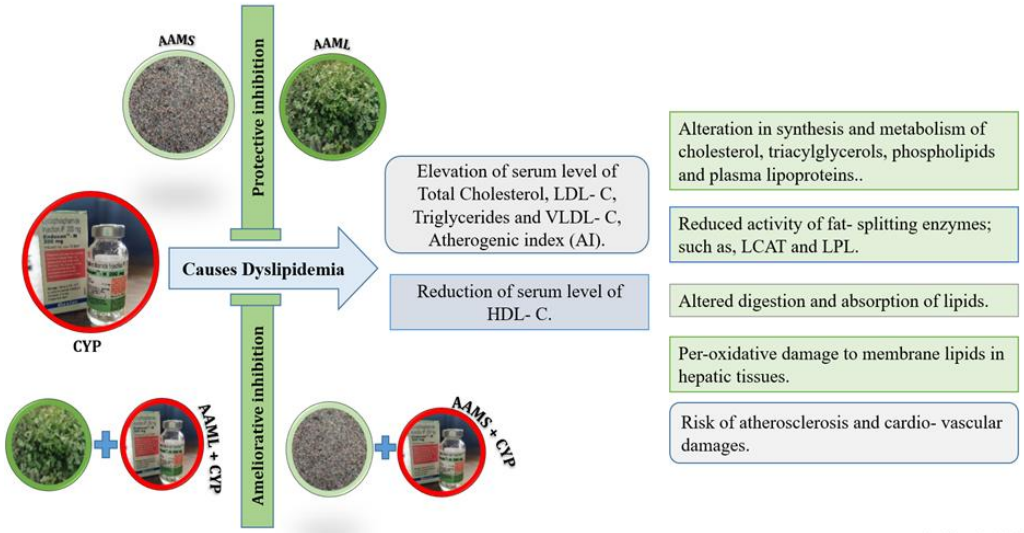
administered orally at the dose of 400 mg/kg body weight. A single dose of cyclophosphamide was injected intraperitoneally at the dose of 50 mg/kg body weight in rats of group II, V and VI on the 14th day of the experiment. The experiment was conducted for a duration of 16 days. Cyclophosphamide caused a significant elevation in serum level of TC, TG, LDL- C, VLDL- C and the atherogenic index, along with a decline in HDL- C. However, this effect was effectively countered by *Achyranthes aspera* extracts in pre- treatment groups (V & VI), showing its ameliorative effect against cyclophosphamide induced dyslipidemia and cardio- toxicity. % protection against cardiac risk was more in case of leaf extract pre- treatment. Treatment with extracts only did not show toxic alterations. *Achyranthes aspera* extracts potentially alleviated the cyclophosphamide- induced disturbances caused in lipid profile markers in albino rats. The anti-dyslipidemic property exhibited by the extracts can be possibly attributed to their phytochemical constituents responsible for anti- oxidative actions. Further pharmacological utilization of the plant warrants more scientific explorations.

GRAPHICAL ABSTRACT



Keywords: Achyranthes aspera; cyclophosphamide; lipid profile; dyslipidemia; cardio- toxicity, atherogenic index; anti- oxidant.

ABBREVIATIONS

AAML : Achyranthes Aspera Methanolic Leaf Extract
AAMS : Achyranthes Aspera Methanolic Seed Extract
AI : Atherogenic Index
CYP : Cyclophosphamide
HDL Cholesterol : High- Density Lipoprotein Cholesterol
LCAT : Lecithin Cholesterol Acyl Transferase
LDL Cholesterol : Low-Density Lipoprotein Cholesterol
LPL : Lipoprotein Lipase
TC : Total Cholesterol
TG :Triglycerides
VLDL Cholesterol: Very Low Density Lipoprotein Cholesterol

1. INTRODUCTION

Chemotherapy is a commonly used treatment modality for cancer (Mbong et al., 2014). During chemotherapy, single or combination of synthetic drugs are used to kill and restrict the growth of cancerous cells (Alam et al., 2018). The drugs used in chemotherapy can be alkylating agents, antimetabolites, mitotic inhibitors, antibiotics or topoisomerase inhibitors. Some of the commonly used chemotherapeutic drugs include cyclophosphamide, cisplatin, ifosfamide,

doxorubicin, bleomycin, topotecan, methotrexate, etc (Espinosa et al., 2003) But, many of them are non- selective in nature and can affect normal, non- cancerous cells also, especially the rapidly dividing cells (Alam et al., 2018). In this study, cyclophosphamide has been taken as experimental drug to analyse the toxic side effects of chemotherapeutic drugs on lipid profile of the experimental rats. Cyclophosphamide is an alkylating agent belonging to nitrogen mustard family and imparts its anti- neoplastic activity following its conversion into active metabolites, phosphoramide mustard and acrolein in the liver (Das et al., 2017). On account of its efficacy, cyclophosphamide is widely used not only for the treatment of different types of malignancies, but also in treating conditions with altered immune function, such as, rheumatoid arthritis, multiple sclerosis, systemic lupus erythematosus and organ transplantation (Moirangthem et al., 2016). However, it imposes severe side- effects on normal, healthy cells via acrolein, the metabolite with no anti- tumor activity (Ogino and Tadi 2023). Administration of cyclophosphamide even at recommended therapeutic dose has been reported to induce alopecia, immunotoxicity, hepatotoxicity, cardiotoxicity, nephrotoxicity, neurotoxicity, pulmonary toxicity and reproductive toxicity (Fraiser et al., 1991, Zhang et al., 2021, DeChiara et al., 2023, Kamel et al., 2022, Jali et al., 2023, Olowe et al., 2024). Cyclophosphamide induced liver toxicity and its impact on lipid profile markers and cardiac functions have been documented by many researchers. An intraperitoneal injection of cyclophosphamide at the dose of 200 mg/kg body weight caused hypercholesterolemia, hypertriglyceridemia and an increase in LDL-cholesterol along with a decrease in HDL-cholesterol level in adult Wistar rats (Das et al., 2017). It also lead to an increase in atherogenic index and damaged heart tissues (Das et al., 2017). Oral administration of cyclophosphamide at the dose of 2 mg/kg body weight for 14 days caused an increase in serum level of TC, LDL-c, TG in addition to an decrease in HDL-c level in albino rats (Mir et al., 2018). They also reported a depletion in enzymes involved in detoxification and oxidative defense in cyclophosphamide treated rats (Mir et al., 2018). It has been reported that cyclophosphamide mediates its toxic effects via the generation of free radicals, thereby causing an imbalance in oxidative status of the tissues (Moirangthem et al., 2016). So, a naturally occurring agent with anti- oxidative and free- radical scavenging property can be used as an adjuvant of a synthetic chemotherapeutic drugs to prevent or mitigate their side- effects without interfering with their anti- neoplastic activities (Yadav et al., 2023, Ayza et al., 2020, Ayza et al., 2022, Ahmed et al., 2023, Skrovankova et al, (2012) have summarized various medicinal plants belonging to different families. Some of them were, *Rosmarinus officinalis* (Rosemary), *Ocimum basilicum* (Basil), *Foeniculum vulgare* (Fennel), *Curcuma longa* (Turmeric), *Ginkgo biloba* (Ginkgo), *Eucalyptus globulus* (Eucalyptus), etc. These plants were reported to be rich in secondary metabolites responsible for their anti- oxidative property. These herbs and spices with antioxidative capacity were reported to have many health benefits (Skrovankova et al., 2012).

In the present study, *Achyranthes aspera* has been used as an experimental plant. *Achyranthes aspera* (family- amaranthaceae) is a weed that grows alongside roads and wastelands. It has been documented to have anti- oxidative properties in addition to other pharmacological effects (Samdershi et al., 2023). *Achyranthes aspera* has also been reported to have anti- hyperlipidemic activities (Sarvesh and Fernandes, 2017). The ethanolic and aqueous extracts of leaves of *Achyranthes aspera* were documented with efficient anti- hyperlipidemic effect in triton- induced hyperlipidemic rats, when administered at the dosage of 250 and 500 mg/kg body weight for an experimental period of 48 hours (Sarvesh and Fernandes, 2017). Cholesterol- induced hyperlipidemia was potentially inhibited by treatment with ethanolic and aqueous extract of leaves of *Achyranthes aspera* for 4 weeks at the dosage of 250 and 500 mg/kg body weight (Sarvesh et al., 2017). They quoted that some of the phytoconstituents found in extracts of *Achyranthes aspera*, viz. glycosides, triterpenoids, alkaloids, saponins and flavonoids were known to have antihyperlipidemic property (Sarvesh et al., 2017). Further, administration of aqueous extract of seeds of *Achyranthes aspera* (1.33g/kg body weight) for 45 days showed hypolipidemic effect in sesame oil fed rats (Krishnakumari and Priya, 2006). An anti- cholesteremic and anti- lipidemic activity of methanolic extract of *Achyranthes aspera* (150, 300 and 600 mg/kg body weight for 2 weeks) was observed in diet induced hyperlipidemic rats (Vijayabhaskar et al., 2014). In an another study, oral administration of saponins extracted from seeds of *Achyranthes aspera* for four weeks showed antihyperlipidemic properties in high cholesterol diet fed rats (Khan et al., 2015). So, in the current investigation, an

effort has been made to explore the toxicity of cyclophosphamide on the activity of liver in context of lipid metabolism and its possible prevention by pre- treatment with leaf and seed extracts of *Achyranthes aspera*.

2. MATERIALS AND METHODS

2.1 Preparation of Plant Extracts

Fresh leaves and ripe seeds were collected from healthy plants of *Achyranthes aspera* growing in local areas of Bihar and Jharkhand. Identification and authentication of the plant species was done by Dr. Malti Kerketta, Associate Professor (Taxonomist), University Department of Botany, Dr. S.P.M. University, Ranchi, Jharkhand. The methodology adopted for the preparation of methanolic extract of leaves and seeds was as per our previously published report (Samdershi, 2023). The obtained semi- solid extract was kept at 4 ℃ for further use.

2.2 Dosage of the Extracts

The dosage of the leaf and seed extracts that were administered were determined on the basis of previous study made by Zambare et al, (2011). They reported the lethal dose of *Achyranthes aspera* to be more than 2000 mg/kg body weight. So, in the present study, an oral dose of 400 mg/kg body weight was taken into consideration. The stored extracts were dissolved into distilled water for final administration.

2.3 Experimental Chemical

Cyclophosphamide, a widely used chemotherapeutic drug, was used as experimental chemical. It was obtained from local market of Ranchi, Jharkhand, India. It was available in the form of white, crystalline powder under the trade name of Endoxan- N; 200 mg. The cyclophosphamide powder was also dissolved in distilled water for preparation of dose and final administration to the experimental animals. It was administered intraperitoneally at the dose of 50 mg/kg body weight (Bin-Hafeez et al., 2001).

2.4 Experimental Animal

Male albino Wistar rats were used as experimental animal. 30 healthy albino rats with average body weight of 100-120 g were purchased from Jazz scientific, Ranchi, Jharkhand, India. The animals were maintained in well- ventilated cages in the animal house at the University Department of Zoology, Ranchi University, ranchi, Jharkhand, India. Prior to the start of experimentation, they were kept for acclimatization to laboratory conditions (Temperature- 20-25℃; Photoperiod- 12/12 Light/Dark) for two weeks. The rats were fed with standard rodent pellet diet and drinking water was made available *ad libitum*.

2.5 Experimental Design

The rats were randomly divided into 6 groups with 5 individuals in each. The treatment given to animals in different groups can be summarized as follows List 1.

The experiment was conducted for a period of 16 days. Rats in group I and II were given distilled water. In groups III and V, rats were orally administered with methanolic leaf extract of *A. aspera*, whereas rats of group IV and VI were administered with methanolic seed extract of *A. aspera* at the dose of 400 mg/kg body weight for 14 consecutive days. Rats in each group, except group I, were injected with cyclophosphamide (50 mg/kg body weight) on 14^{th} day of the experiment. After 48 hours of cyclophosphamide administration, blood was collected from rats of each group via cardiac puncture. Serum was separated and analysed for parameters of lipid profile using automated analyser. The parameters studied were; Total cholesterol (TC), triglycerides (TG) and lipoproteins (Low Density Lipoprotein: LDL, High Density Lipoprotein: HDL and Very Low Density Lipoprotein: VLDL). Further, the atherogenic index and protection percentage was calculated using following formula (Das et al., 2017). *Atherogenic index= (Total cholesterol)/ (HDL-Cholesterol).*

*Protection percentage= [(AI of CYP control group) - (AI of Extract (Leaf or seed) + CYP treated group)/ AI of CYP control group]*100.*

2.6 Statistical Analysis

The data obtained were presented as mean ± Standard Deviation (SD). The variation in serum level of lipid profile parameters in different groups were statistically analysed using Student's *t*- test. The value of $p \leq 0.05/0.01/0.001$ was considered significant.

List 1. Naturae of treatment

GROUPS	Distilled water	Cyclophosphamide (50 mg/kg b.wt.; i.p.)	*A. aspera* leaf extract (400 mg/kg b.wt.; i.g.)	*A. aspera* seed extract (400 mg/kg b.wt.; i.g.)
I (VC)	✓	×	×	×
II (CYP)	✓	✓	×	×
III (AAML)	×	×	✓	×
IV (AAMS)	×	×	×	✓
V (AAML + CYP)	×	✓	✓	×
VI (AAMS + CYP)	×	✓	×	✓

3. RESULTS AND DISCUSSION

The liver plays a central role in the biosynthesis and metabolism of lipids and controls the serum level of cholesterol and lipoproteins (Ogbe et al., 2020). Alteration in the serum level of lipid profile parameters can be an indicative of liver and heart ailments in individuals undergoing chemotherapy (Moirangthem et al., 2016). Several medicinal herbs and spices have been reported to have lipid managing properties. Some of these plants are, *Allium sativum* (garlic), *Curcuma longa* (turmeric), *Linum usitatissimum* (flaxseeds), *Capsicum annuum*, etc. (Singh et al., 2022). Many medicinal plants with blood lipid lowering effects have also been documented by Al- Snafi A E, (2022). In the present study, lipid managing properties of *Achyranthes aspera* has been investigated in context of its effects on serum level of lipids and lipoprotein fractions in cyclophosphamide treated rats. Further, its probable effects on cardiac activity has also been deduced.

3.1 Effects on Serum Lipid and Lipoprotein Fraction

The effects of cyclophosphamide and extracts of *Achyranthes aspera* on the serum level of lipid profile parameters have been represented in Table 1 and Fig. 1 (a-e). The alterations in parameters of lipid profile can be termed as dyslipidemia. The pathological condition can be reviewed in terms of hyperlipidemia; i.e., increase in serum level of total cholesterol, triglycerides, LDL- cholesterol and VLDL- cholesterol; and decrease in the level of good cholesterol, i.e., HDL- cholesterol. A fluctuation in lipid profile parameters can be used as a marker to predict the impact of xenobiotics on liver and cardiac functioning as well as extent of lipid peroxidation (Moirangthem et al., 2016).

In the present study, treatment with cyclophosphamide at the dose of 50 mg/kg body weight resulted in a significant increase in the serum level of total cholesterol (TC), triglycerides (TG), LDL- cholesterol and VLDL- cholesterol and a decrease in HDL- cholesterol, when compared with control group rats (Table 1; Fig. 1). In corroboration with the present finding, chemotherapy induced dyslipidemia has been reported in many previous studies (Afsar et al., 2017, Afsar et al., 2019, Ogbe et al., 2020, Gungor et al., 2023). Cyclophosphamide induced hyperlipidemia can be due to an imbalance in the biosynthesis, utilization and excretion of cholesterol. It causes an increase in biosynthesis of cholesterol along with a decrease in the hydrolysis of cholesteryl ester, utilization and efflux (Das et al., 2017). Cyclophosphamide induced hypercholesterolemia can also be explained in terms of reduced activity of fat-splitting enzymes, namely, Lecithin cholesterol acyltransferse (LCAT) and Lipoprotein lipase (LPL). LCAT helps in esterification of free cholesterols and also helps in formation of the large- sized HDL particles, which help in the elimination of cholesterol from the circulation. Low levels of LCAT have been reported in animals treated with cyclophosphamide (Sudharsan et al., 2005). Cyclophosphamide also causes hypertriglyceridemia, which can be attributed to its inhibitory effect on the activity of lipoprotein lipase (LPL), an enzyme which facilitates hydrolysis of triglycerides into fatty acids and glycerol in plasma (Verma et al., 2018, Moirangthem et al., 2016). Accumulation of VLDL can also increase the serum level of cholesterol (Verma et al., 2018). Elevated levels of LDL-cholesterol in cyclophosphamide- treated rats can be due to the suppression of the activity of LDL- receptors present on the liver as well as extra- hepatic tissues (Sudharsan et al., 2005). Increase in LDL- cholesterol also increases the serum level of cholesterol, as it is the major transporter of cholesterol. Further, a decrease in HDL- cholesterol along with an increase in LDL-cholesterol facilitates the accumulation of

cholesterol in the circulation (Sudharsan et al., 2005) Cyclophosphamide also induces peroxidation of unsaturated membrane lipids, leading to their leakage in the circulation (Gungor et al., 2003).

However, pre- treatment with methanolic leaf and seed extracts of *Achyranthes aspera* (400 mg/kg body weight, p.o.) for 14 days prior to administration of cyclophosphamide prevented the dyslipidemic condition induced by cyclophosphamide. A comparatively lower value of serum level of cholesterol, triglycerides, LDL-cholesterol and VLDL- cholesterol along with an increase in HDL- cholesterol can be observed in rats of group V & VI, when compared with cyclophosphamide only treated group (Table 1; Fig. 1). However, the value did not reach the vehicle control level, showing only a partial inhibition of cyclophosphamide- induced lipid imbalance by *A. aspera* extracts. Supporting the present study, many herbal extracts have been reported to possess anti- dyslipidemic potential (Srivastava and Srivastava, 2018, Ebrahimi et al., 2019). Supplementation with leaves of *Solanum scabrum* and fruits of *Cola verticillata* has been documented to modulate lipid profile parameters in female Wistar rats treated with cyclophosphamide (Mbong et al., 2014). Extracts of *Phyllanthus fraternus* (Moirangthem et al., 2016) *Ipomoea aquatica* (Das et al., 2017) *Moringa oleifera* (Habeeb et al., 2018), *Chenopodium album* (Verma et al., 2018) *Lophira lanceolata* (Singh et al., 2022) and *Acacia hydaspica* (Afsar et al., 2017) have been reported to inhibit the cyclophosphamide-

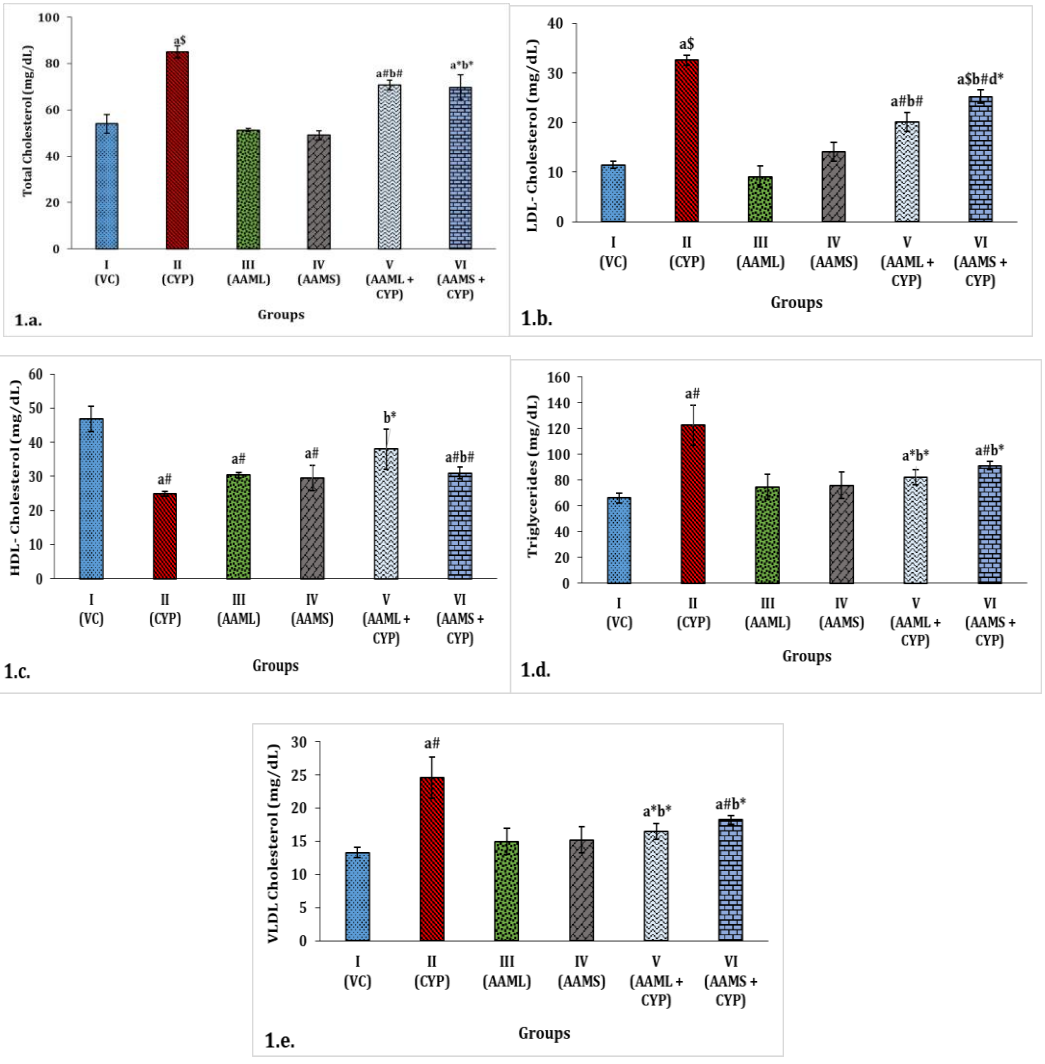


Fig. 1. Graph showing alterations in serum level of lipids and lipoprotein fractions in different experimental group rats 1.a.- Total cholesterol, 1.b.- LDL- cholesterol, 1.c.- HDL- Cholesterol, 1.d.- Triglycerides and 1.e.- VLDL- Cholesterol

Table 1. Effects of treatment with cyclophosphamide and *Achyranthes aspera* extracts on lipid profile parameters of albino rats of various experimental groups

Groups	Treatment and Dose (mg/kg body weight)	Total Cholesterol (mg/dL)	LDL-Cholesterol (mg/dL)	HDL-Cholesterol (mg/dL)	Triglycerides (mg/dL)	VLDL-Cholesterol (mg/dL)
I	Vehicle Control (Distilled Water)	54.00 ± 4.00	11.48 ± 0.76	46.84 ± 3.72	66.33 ± 3.84	13.27 ± 0.77
II	Cyclophosphamide (CYP) (i.p., 50)	85.00 ± 2.64$^{a\$}$	32.59 ± 1.04$^{a\$}$	24.87 ± 0.65$^{a\#}$	122.77 ± 15.56$^{a\#}$	24.55 ± 3.11$^{a\#}$
III	AAML (i,g., 400)	51.33 ± 0.58	9.12 ± 2.08	30.37 ± 0.84$^{a\#}$	74.90 ± 9.60	14.98 ± 1.92
IV	AAMS (i,g., 400)	49.00 ± 2.00	14.15 ± 1.89	29.43 ± 3.72$^{a\#}$	76.08 ± 10.07	15.22 ± 2.01
V	AAML (i,g., 400) + CYP (i.p., 50)	70.67 ± 2.08$^{a\#b\#}$	20.16 ± 1.89$^{a\#b\#}$	37.95 ± 5.85^{b*}	82.25 ± 5.85^{a*b*}	16.45 ± 1.17^{a*b*}
VI	AAMS (i,g., 400) + CYP (i.p., 50)	69.78 ± 5.30^{a*b*}	25.22 ± 1.36$^{a\$b\#d*}$	30.97 ± 1.66$^{a\#b\#}$	91.17 ± 3.25$^{a\#b*}$	18.23 ± 0.65$^{a\#b*}$

*Values were Mean ± S.D (n=5); a showed significant difference from Group I; b showed significant difference from Group II; d showed significant difference from Group V; *****p=.05, #p=.01, **$**p<.001*

induced hyperlipidemia and reduction in the level of HDL- cholesterol. Treatment with flaxseed oil showed a dose- dependent inhibition of cyclophosphamide induced dyslipidemia in mice (Singh, 2020). The protective as well as ameliorative potential of these extracts against cyclophosphamide induced toxicity can be attributed to their phyto- constituents, especially flavonoids and other polyphenolic compounds. Many plant- based antioxidants have been listed to prevent cisplatin- induced hepatotoxicity (Rashid et al., 2021). In this study, no potential damaging effects were observed in serum level of lipid profile markers in rats of group III and IV, which were administered with leaf and seed extracts of *Achyranthes aspera* only, for 14 consecutive days (Table 1). It advocated for the non- toxic nature of the extracts used.

Additionally, bio- active components isolated from plant- based extracts have also been documented to exhibit remarkable potency in preventing cyclophosphamide- induced dyslipidemia; such as, Zingerone, a polyphenolic alkalone found mostly in dry zinger (Mir et al., 2018); Cucurmine, a polyphenol from *Curcuma longa*; Piperine, an alkaloid from *Piper nigrum* (Chakraborty et al., 2017). and Lupeol, a pentacyclic triterpene from *Crataeva nurvala* (Sudharsan et al., 2005). The biological activity imparted by these extracts and bio- active components can be attributed to their free-radical scavenging and anti- oxidative properties, which counteract with the pro- oxidant behaviour of chemotherapeutic drug, cyclophosphamide. Anti- hyperlipidemic potential of leaves of *Allium scorodoprasum* can be due to its strong anti-oxidant and anti- inflammatory effects resulting from its phenolic and flavonoid content (Gungor et al., 2023). Lipoic acid, an ideal anti- oxidant, has been reported to counteract cyclophosphamide induced disturbances in lipid status of serum as well as cardiac tissue (Mythili et al., 2006).

Table 2. Value of atherogenic index in control and treatment groups and estimation of protection percentage against cyclophosphamide in *Achyranthes aspera* extract treated groups

Groups	Treatment and Dose (mg/kg body weight)	Atherogenic Index (Cardiac risk ratio)	Protection (%)
I	Vehicle Control (Distilled Water)	1.16 ± 0.17	NA
II	Cyclophosphamide (CYP) (i.p., 50)	3.42 ± 0.19$^{a\$}$	NA
III	AAML (i,g., 400)	1.69 ± 0.04^{a*}	NA
IV	AAMS (i,g., 400)	1.68 ± 0.24	NA
V	AAML (i,g., 400) + CYP (i.p., 50)	1.89 ± 0.28$^{b\#}$	44.73
VI	AAMS (i,g., 400) + CYP (i.p., 50)	2.26 ± 0.22$^{b\#}$	33.99

*Values were Mean ± S.D (n=5); a showed significant difference from Group I; b showed significant difference from Group II; d showed significant difference from Group V; *****p=.05, #p=.01, **$**p<.001*

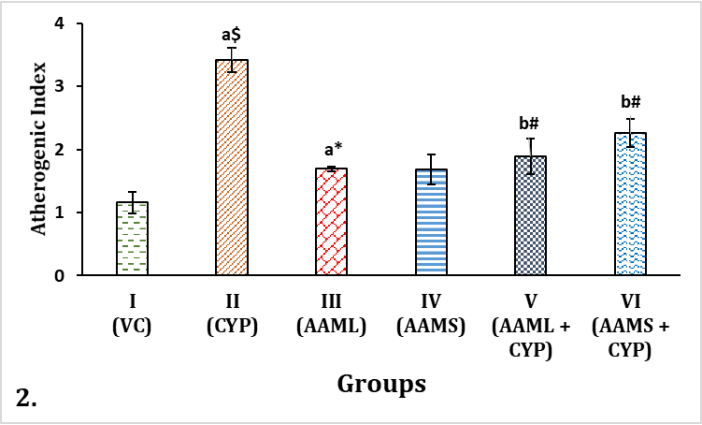


Fig. 2. Graph showing atherogenic index (cardiac risk ratio) in control and experimental group rats

3.2 Effects on Atherogenic Index

The excess of cholesterol may deposit along the wall of arteries leading to narrowing of the vessels. It may disturb the flow of blood to the organs. High cholesterol in the blood may lead to many heart diseases, especially atherosclerosis. The extent of damage to the heart can be estimated in terms of ratio of total cholesterol and HDL- cholesterol (Singh et al., 2022).

HDL- cholesterol is good cholesterol, which helps in the elimination of excess cholesterol from the circulation. It also acts as an anti- oxidant agent which reduces free radical activity on LDL (Singh et al., 2022). A high amount of HDL- cholesterol lowers the risk of atherosclerosis (Singh et al., 2022). Table 2 showed the value of atherogenic index (cardiac risk ratio) in various control and experimental groups. Single dose administration of cyclophosphamide resulted in a significant elevation in atherogenic index ($p<0.001$). However, pre- treatment with leaf (group V) and seed (group VI) extracts of *A. aspera* significantly inhibited this elevation in atherogenic index in cyclophosphamide intoxicated rats ($p=0.01$). Elevation in atherogenic index showed lower value of HDL- cholesterol in the circulation and vice- versa. Percentage (%) protection provided by the extracts have also been calculated (Table 2; Fig. 2). Pre- treated with leaf extract of *A. aspera* showed greater protection (44.73 %) against cyclophosphamide induced cardiac damage, when compared with seed extract pre-treated group (33.99 %). The atherogenic index in extract control group rats (III & IV) was nearly normal, suggesting non- toxic effects of the *A. aspera*. Present findings are consistent with the study made by Moirangthem et al, (2016), who documented lowered value of atherogenic index in experimental animals administered with aqueous extract of leaves of *Phyllanthus fraternus* along with cyclophosphamide. They further quoted that an increase in serum level of HDL- c can be considered as a protective sign against atherosclerosis, as it facilitates transport of cholesterol back to the liver where it can be secreted as bile acids (Moirangthem et al., 2016) Reduction of atherogenic index was also reported in animals models administered with aqueous extract of *Ipomoea aquatica* (Das et al., 2017) hydroethanolic extract of *Chenopodium album* (Verma et al., 2018) and aqueous extract of leaf of *Lophira lanceolata* (Ogbe et al., 2018). In all the cases, the cardioprotective efficacy of the plant- based extracts can be attributed to the anti- oxidative potential of their phyto-constituents. Phytochemical analysis of methanolic leaf extract of *Achyranthes aspera* showed presence of alkaloids, flavonoids, phenols, saponins, terpenoids and many other bioactive components (Samdershi, 2023). Most of these phytoconstituents have been reported to possess anti- oxidant, anti- cancer, vasodilating, hypocholesterolmenic, anti- inflammatory and many other pharmacologically beneficial properties (Koche et al., 2016). Capsaicin, an alkaloid derived from chilli peppers, has been documented to exert protective effects against cyclophosphamide- induced cardiotoxicity by inhibition of generation of free radicals in rats (Ahmed et al., 2023). The role of antioxidants in the amelioration of cardiotoxicity induced by cyclophosphamide has been reported by Ayza et al, (2020). Phytochemical analysis Different extracts of *Achyranthes aspera* have also been reported to be rich in various phytochemicals responsible for their anti- oxidative potential (Manandhar et al., 2021).

4. CONCLUSION

From the findings of present investigation, it can be concluded that the pre- treatment with leaf and seed extracts of *Achyranthes aspera* can prevent the cyclophosphamide induced dyslipidemia and cardio- toxicity to a greater extent. On account of pro- oxidative behaviour of cyclophosphamide, the protective efficacy of the extract can be attributed to an abundance of bio-active phyto- constituents, contributing to their anti- oxidative and other pharmacological properties. However, further detailed investigation is required to know about specific bioactive agent responsible for anti- dyslipidemic and cardioprotective potential of *Achyranthes aspera* against cyclophosphamide induced toxicity. Systematic scientific investigations regarding the mode, dose and duration of administration of plant extracts are also advised for confirmation of pharmacological benefits.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

ETHICAL APPROVAL

The maintenance and handling of the experimental animal was done as per the

guidelines of institutional ethical committee of Ranchi University, Ranchi, Jharkhand, India (2/511/2022).

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COMPETING INTERESTS

Author has declared that no competing interests exist.

REFERENCES

Afsar, T., Razak, S., & Almajwal, A. (2019). Effect of *Acacia hydaspica* R. Parker extract on lipid peroxidation, antioxidant status, liver function test and histopathology in doxorubicin treated rats. *Lipids in Health and Disease, 18*(1), 126. https://doi.org/10.1186/s12944-019-1051-2

Afsar, T., Razak, S., Almajwal, A., & Rashid Khan, M. (2017). Modulatory influence of *Acacia hydaspica* R. Parker ethyl acetate extract against cisplatin inveigled hepatic injury and dyslipidemia in rats. *BMC Complementary and Alternative Medicine, 17*(1), 307. https://doi.org/10.1186/s12906-017-1824-y

Ahmed, R. A., Alam, M. F., Alshahrani, S., et al. (2023). Capsaicin ameliorates cyclophosphamide-induced cardiotoxicity by inhibiting free radicals generation, inflammatory cytokines, and apoptotic pathway in rats. *Life, 13*(3), 786. https://doi.org/10.3390/life13030786

Alam, A., Farooq, U., Singh, R., Dubey, V. P., Kumar, S., Kumari, R., et al. (2018). Chemotherapy treatment and strategy schemes: A review. *Open Access Journal of Toxicology, 2*(5). https://doi.org/10.19080/OAJT.2018.02.555600

Al-Snafi, A. E. (2022). Blood lipids lowering effect of medicinal plants. *GSC Biological and Pharmaceutical Sciences, 19*(03), 015-043. https://doi.org/10.30574/gscbps.2022.19.3.0213

Ayza, M. A., Zewdie, K. A., Tesfaye, B. A., Wondafrash, D. Z., & Berhe, A. H. (2020). The role of antioxidants in ameliorating cyclophosphamide-induced cardiotoxicity. *Oxidative Medicine and Cellular Longevity, 2020*, 4965171. https://doi.org/10.1155/ 2020/4965171

Ayza, M. A., Zewdie, K. A., Yigzaw, E. F., et al. (2022). Potential protective effects of antioxidants against cyclophosphamide-induced nephrotoxicity. *International Journal of Nephrology, 2022*, 5096825. https://doi.org/10.1155/2022/5096825

Bin-Hafeez, B., Ahmad, I., Haque, R., & Raisuddin, S. (2001). Protective effect of *Cassia occidentalis* L. on cyclophosphamide-induced suppression of humoral immunity in mice. *Journal of Ethnopharmacology, 75*(1), 13-18. https://doi.org/10.1016/s0378-8741(00)00382-2

Chakraborty, M., Bhattacharjee, A., & Kamath, J. V. (2017). Cardioprotective effect of curcumin and piperine combination against cyclophosphamide-induced cardiotoxicity. *Indian Journal of Pharmacology, 49*(1), 65-70. https://doi.org/10.4103/0253-7613. 201015

Das, R., Devi, K. S., Dutta, S., Das, A., Das, P., & Devi, K. K. P. (2017). Effects of *Ipomoea aquatica* Forsk. in cyclophosphamide-induced dyslipidaemia in albino rats. *International Journal of Basic and Clinical Pharmacology, 6*(11), 2743–2748. https://doi.org/10.18203/2319-2003.ijbcp20174799

DeChiara, J. R., Birch, E. M., & Harper, H. (2023). Low-dose cyclophosphamide associated with hyponatremia and hepatotoxicity. *Cureus, 15*(9), e45375. https://doi.org/10.7759/cureus.45375

Ebrahimi, Y., Hasanvand, A., Valibeik, A., Ebrahimi, F., & Abbaszadeh, S. (2019). Natural antioxidants and medicinal plants effective on hyperlipidemia. *Research Journal of Pharmacy and Technology, 12*(3), 1457-1462. https://doi.org/10.5958/0974-360X.2019.00242.7

Espinosa, E., Zamora, P., Feliu, J., & Baron, M. G. (2003). Classification of anticancer drugs: A new system based on therapeutic targets. *Cancer Treatment Reviews, 29*, 515–523. https://doi.org/10.1016/S0305-7372(03)00116-6

Fraiser, L. H., Kanekal, S., & Kehrer, J. P. (1991). Cyclophosphamide toxicity: Characterizing and avoiding the problem. *Drugs, 42*, 781–795.

Gungor, H., Ekici, M., Karatas, O., & Dik, B. (2023). Protective effect of *Allium scorodoprasum* L. ethanolic extract in cyclophosphamide-induced hepatotoxicity model in rats. *Journal of Pharmacy and Pharmacology, 75*(5), 625-634. https://doi.org/10.1093/jpp/rgad002

Habeeb, A. A. (2018). *Moringa oleifera* leaf extract: A potent ameliorator of cyclophosphamide induced liver toxicity in rat model. *Journal of Bioscience and Applied Research, 4*(1), 22-38. https://doi.org/10.21608/JBAAR.2018.129732

Jali, A. M., Alam, M. F., Hanbashi, A., Mawkili, W., Abdlasaed, B. M., Alshahrani, S., et al. (2023). Sesamin's therapeutic actions on cyclophosphamide-induced hepatotoxicity, molecular mechanisms, and histopathological characteristics. *Biomedicines, 11*(12), 3238. https://doi.org/10.3390/biomedicines11123238

Kamel, S., Abdelbaky, N., Sayed-Ahmed, M. M., Karkeet, R. M., Osman, A. M., & Fouad, M. A. (2022). Cyclophosphamide-induced cardiotoxicity. *Azhar International Journal of Pharmaceutical and Medical Sciences, 2*(2), 1–8. https://doi.org/10.21608/aijpms.2022.114213.1103

Khan, N., Akhtar, M. S., Khan, B. A., Braga, V. A., & Reich, A. (2015). Antiobesity, hypolipidemic, antioxidant and hepatoprotective effects of *Achyranthes aspera* seed saponins in high cholesterol fed albino rats. *Archives of Medical Science, 11*(6), 1261-1271. https://doi.org/10.5114/aoms.2015.56353

Koche, D., Shirsat, R., & Kawale, M. (2016). An overview of major classes of phytochemicals: Their types and role in disease prevention. *Hislopia Journal, 9*(1/2), 1-11.

Krishnakumari, S., & Priya, K. M. (2006). Hypolipidemic efficacy of *Achyranthes aspera* on lipid profile in sesame oil fed rats. *Ancient Science of Life, 25*(3&4), 49-56.

Manandhar, N., Bajgain, K., & Neupane, A. (2021). Study on phytochemical profile and antioxidant activity of *Achyranthes aspera* whole plant. *International Journal of Biochemistry Research & Review, 30*(2), 16-23. https://doi.org/10.9734/IJBCRR/2021/v30i230251

Mbong, M. A., Djiokeng, G., Ntentie, R., Dimodi, H., Ngondi, L., & Oben, J. (2014). Protective effect of hydroethanolic extracts of *Solanum scabrum* and *Cola verticillata* against cyclophosphamide-induced toxicity in female rats. *Journal of Food Research, 3*(3). https://doi.org/10.5539/jfr.v3n3p18

Mir, B. A., Amin, I., Rehman, M. U., Razak, R., Ali, A., Baba, O., et al. (2018). Chemoprotective potential of zingerone (vanillyl acetone) in cyclophosphamide-induced hepatic toxicity. *Pharmacognosy Magazine, 14*(57s), S434–S439.

Moirangthem, R. S., Jitendra, S. S. V. J., Devi, H. C., Devi, K. S., Gunindro, N., & Devi, N. M. (2016). Effect of aqueous extract of *Phyllanthus fraternus* leaf against cyclophosphamide-induced dyslipidemia and aortitis in Wistar albino rats. *International Journal of Contemporary Medical Research, 3*(9), 2703–2706.

Mythili, Y., Sudharsan, P. T., Sudhahar, V., & Varalakshmi, P. (2006). Protective effect of DL-alpha-lipoic acid on cyclophosphamide induced hyperlipidemic cardiomyopathy. *European Journal of Pharmacology, 543*(1-3), 92-96. https://doi.org/10.1016/j.ejphar.2006.06.007

Ogbe, R. J., Agbese, S. P., & Abu, A. H. (2020). Protective effect of aqueous extract of *Lophira lanceolata* leaf against cisplatin-induced hepatorenal injuries and dyslipidemia in Wistar rats. *Clinical Phytoscience, 6*(4). https://doi.org/10.1186/s40816-019-0149-4

Ogino, M. H., & Tadi, P. (2023). Cyclophosphamide. In *StatPearls*. StatPearls Publishing.

Olowe, T. G., Oyovwi, M. O., Nwangwa, K. E., Ohwin, E. P., & Oghenetega, O. B. (2024). Cytotoxic properties of cyclophosphamide: A focus on its mechanistic impacts on male gonadal functions. *Journal of Exploratory Research in Pharmacology, 9*(2), 106–115. https://doi.org/10.14218/JERP.2023.00025S

Rashid, N. A., Halim, S. A. S. A., Teoh, S. L., Budin, S. B., Hussan, F., Ridzuan, N. R. A. R., & Jalil, N. A. A. (2021). The role of natural antioxidants in cisplatin-induced hepatotoxicity. *Biomedicine & Pharmacotherapy, 144*. https://doi.org/10.1016/j.biopha.2021.112328

Samdershi, D. (2023). Protective effect of methanolic leaf extract of *Achyranthes aspera* (Linn) against cyclophosphamide induced suppression of specific immunity in albino rats. *CIBTech Journal of Zoology, 12*, 88-100.

Samdershi, D., Kumar, A., & Besra, S. (2023). A comprehensive study on ethno-pharmacological exploration of *Achyranthes aspera* Linn. *International Journal of Zoological Investigations, 9*(1), 69–86. https://doi.org/10.33745/ijzi.2023.v09i01.007

Sarvesh, C. N., & Fernandes, J. (2017). Evaluation of antihyperlipidemic activity of leaves of *Achyranthes aspera* Linn. using hyperlipidemic rats. *Asian Journal of Pharmaceutical and Clinical Research, 10*(4), 211–215. https://doi.org/10.22159.ajpcr.2017.v10i4.16576

Sarvesh, C. N., Fernandes, J., Janadri, S., Yogesh, H. S., & Swamy, S. K. (2017). Antihyperlipidemic activity of *Achyranthes aspera* Linn. leaves on cholesterol induced hyperlipidemia in rats. *Research Journal of Pharmacy and Technology, 10*(1), 200-204. https://doi.org/10.5958/0974-360X.2017.00043.9

Singh, B. M. (2020). Prophylactic action of flaxseed oil against cyclophosphamide induced oxidative stress in mice liver. *International Journal of Science and Research, 9*(4), 1709-1713. https://doi.org/10.21275/SR20423102445

Singh, R., Tripathi, B. D., Kumari, S., & Tiwari, S. (2022). The ameliorative role of potential herbal plants for managing cholesterol. *Systematic Review Pharmacy, 13*(6), 405-409. https://doi.org/10.31858/0975-8453.13.6.405-409

Skrovankova, S., Misurcova, L., & Machu, L. (2012). Antioxidant activity and protecting health effects of common medicinal plants. *Advances in Food and Nutrition Research, 67*, 75–139. https://doi.org/10.1016/B978-0-12-394598-3.00003-4

Srivastava, R., & Srivastava, P. (2018). Lipid lowering activity of some medicinal plants: A review of literature. *Biomedical Journal of Scientific & Technical Research, 9*(1). https://doi.org/10.26717/BJSTR.2018.09.001738

Sudharsan, P. T., Mythili, Y., Sudhahar, V., & Varalakshmi, P. (2005). Role of lupeol and its ester on cyclophosphamide-induced hyperlipidaemic cardiomyopathy in rats. *The Journal of Pharmacy and Pharmacology, 57*(11), 1437-1444. https://doi.org/10.1211/jpp.57.11.0009

Verma, M. K., Ahmad, A. H., Pant, D., Kumar, N., & Patwal, P. C. (2018). *Chenopodium album* ameliorates cyclophosphamide-induced hyperlipidemia in Sprague Dawley rats. *The Pharma Innovation Journal, 7*(12), 423-426.

Vijayabhaskar, K., Ramadevi, V., Chaitanyaprasad, K., Kumar, S. R., Sripada, D., & Himabindu, G. (2014). Evaluation of anti-cholesteremic and anti-lipidemic activity of seed extract of *Achyranthes aspera* in diet induced hyperlipidemia model in rats. *Journal of Chemical and Pharmaceutical Research, 6*(5), 1247-1250.

Yadav, V., Krishnan, A., Zahiruddin, S., Ahmad, S., & Vohora, D. (2023). Amelioration of cyclophosphamide-induced DNA damage, oxidative stress, and hepato- and neurotoxicity by *Piper longum* extract in rats: The role of γH2AX and δ-OHdG. *Frontiers in Pharmacology, 14*, 1147823. https://doi.org/10.3389/fphar.2023.1147823

Zambare, M., Bhosale, U. A., Somani, R. S., Yegnanarayan, R., & Talpate, K. A. (2011). Effect of treatment with *Achyranthes aspera* (Agadha) ethanol extract on various haematological and biochemical parameters in alloxan induced diabetic rats. *International Journal of Pharmaceutical Frontier Research, 1*(1), 42-52.

Zhang, Z., Pan, T., Liu, C., et al. (2021). Cyclophosphamide-induced physiological and biochemical changes in mice with an emphasis on sensitivity analysis. *Ecotoxicology and Environmental Safety, 211*, 111889. https://doi.org/10.1016/j.ecoenv.2020.111889

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