

## Effects of *Ipomoea aquatica* Forsk. in cyclophosphamide induced dyslipidaemia in albino rats

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### ABSTRACT

**Background:** Cyclophosphamide (CP) is commonly used as anticancer and immuno suppressant agent. It induces hyperlipidemia and myocardium damage. *Ipomoea aquatica* Forsk. is traditionally used for cardiovascular disease, paralysis and general debility. The present study was done to evaluate the protective effect of the plant against CP induced dyslipidaemia in albino rats.

**Methods:** Twenty albino rats were divided into 4 groups of 5 animals each. Group I (normal group) received normal saline intraperitoneally. Groups II to IV received CP (200mg/kg body weight, intraperitoneally) single dose on day 1 of experimental period. Groups I and II (toxic group) animals were given 2% gum acacia per orally daily for 10 days. Groups III and IV received aqueous extract of stem and leaves of *Ipomoea aquatica* 200mg/kg and 400mg/kg per orally respectively daily for 10 days. On 11th day, blood samples were collected for estimation of triglycerides, total cholesterol, LDL-cholesterol and HDL-cholesterol and heart tissues were sent for histopathology examination (HPE).

**Results:** CP administration significantly ( $P<0.05$ ) increased the levels of triglyceride, total cholesterol, LDL-cholesterol and decreased the level of HDL-cholesterol in toxic group compared to normal group. Treatment with aqueous extract of *Ipomoea aquatica* significantly ( $P<0.05$ ) reversed the status of lipid profile compared to toxic group. HPE of myocardium showed reversal of the toxic effects of CP in the extract treated groups.

**Conclusions:** *Ipomoea aquatica* showed protective effects against CP induced dyslipidaemia in albino rats.

**Keywords:** Albino rats, Cyclophosphamide, Dyslipidaemia, *Ipomoea aquatica* Forsk.

### INTRODUCTION

Cyclophosphamide (CP) is one of the most widely used alkylating agents. It can be administered orally and intravenously. It is mainly used for breast cancer, ovarian cancer, non-Hodgkin's lymphoma, chronic lymphocytic leukemia, soft tissue sarcoma, neuroblastoma, Wilms' tumor, rhabdomyosarcoma and as immunosuppressant agent. It is activated by hepatic cytochrome P450 mixed-function oxidase system and converted to 4-hydroxycyclophosphamide, which is in equilibrium with aldophosphamide. Aldophosphamide, delivered to both

tumour and normal tissue is converted to cytotoxic form phosphoramidate mustard and acrolein by non-enzymatically.<sup>1</sup>

Acrolein damages many cell types by producing reactive oxygen species and lipid peroxidation. Oxidative stress is associated with the pathogenesis of cardiovascular disorder including ischemic heart disease, heart failure and atherosclerosis. Acrolein also decreases cholesterol clearance by reducing hepatic lipase activity. Reduced cholesterol clearance is associated with dyslipidemia. Acrolein induced dyslipidemia is characterized by changes

in the lipoprotein composition especially in VLDL cholesterol and triglycerides and induces pro-atherogenic changes in lipoproteins. It was found that, acrolein can induce apoptosis by activation of the mitochondrial pathway or death receptor signalling. In some studies, it was proved that acrolein can exerts detrimental effects in adult mice heart tissues through apoptotic cell death.<sup>2,3</sup>

*Ipomoea aquatica* Forsk. belongs to the family *Convolvulaceae*. It is commonly known as Water spinach, Kolamani (Manipuri), Kalmisak (Bengali) etc. This plant has a wide distribution and grows in moist soils, side-lines of fresh water, ditches, lakes, ponds, marshes and wet rice field usually all-round the year. The plant is commonly found in Manipur, Bihar, Orissa, Bengal and South India. It is generally consumed as vegetable.<sup>4,5</sup> *Ipomoea aquatica* is traditionally used for cardiovascular disease, paralysis, general debility and as purgative. Juice of plant is use for ring worm (with salt), opium and arsenic poisoning. Leaves of plant relieve pain due to insect bites.<sup>6</sup>

The non-toxic nature of *Ipomoea aquatica* as well as its multiple beneficial effects has made it one of the most attractive compounds to explore for its protective role. Therefore, the present attempt has been made to investigate its role in dyslipidaemia induced by cyclophosphamide.

## METHODS

### Chemicals and reagents

Cyclophosphamide (CYPHOS<sup>TM</sup>) was purchased from Getwell Pharmaceuticals, Haryana, India. Biochemical estimation kits (Benesphera<sup>SM</sup>) were obtained from Avantor Performance Materials India Ltd, Uttarakhand. Other chemicals and solvents used were of highest purity and analytical grade.

### Study Setting

The study was conducted at Department of Pharmacology and Department of Pathology, Regional Institute of Medical Sciences (RIMS), Imphal, Manipur, India.

### Preparation of aqueous extract of *Ipomoea aquatica* Frosk.

The fresh plant of *Ipomoea aquatica* was collected from local market in the month of October, 2016. They were identified and authenticated by Professor P. K. Singh of the Life Sciences Department, Manipur University, Imphal, India. The leaves and stems were separated, cleansed with water, cut into small pieces and dried in shade. It was then coarsely powdered by mixer grinder. Aqueous extract of *Ipomoea aquatica* (AEIA) was prepared by soxhlet extraction method described by Verma SCL and Agrawal SL.<sup>7</sup> Brownish crude dry extract was obtained. The yield was 19.5%. The extract was

freshly dissolved in 2% gum acacia before giving each dose to animals.

### Acute oral toxicity study

For acute oral toxicity study, Limit test was done in albino rats according to OECD guideline 423. Rats were healthy and there was no mortality observed at the dose of 2000mg/kg till 14 days. So, doses were selected 200mg/kg and 400 mg/kg for the study.<sup>8</sup>

### Experimental animals

Healthy adult albino wistar rats of either sex weighing 110-210gm were housed in standard polypropylene cages, maintained under controlled room temperature ( $21 \pm 2^\circ\text{C}$ ), relative humidity ( $55 \pm 5\%$ ) and under 12:12 h artificial light and dark cycle in the well-ventilated animal house, Department of Pharmacology, RIMS, Imphal. Animals were acclimatized for five days. All the rats were provided with commercially available standard pellet diet, water ad libitum. The animals were maintained under standard conditions in the animal house as per the guidelines of Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA).<sup>9</sup>

### Experimental protocol

The animals comprising 20 albino rats were divided into four groups, five animals each. Group I served as normal control receiving single intraperitoneal (i.p.) injection of 0.5ml/100gm of 0.9% normal saline. Groups II, III and IV received one dose of CP (200mg/kg body weight) dissolved in 0.9% normal saline through i.p. injection on day 1. Groups I and II received 2% gum acacia suspension (1ml/100gm, per orally daily for 10 days). Groups III and IV were received 200mg/kg and 400mg/kg of AEIA (1ml/100gm, per orally daily for 10 days) dissolved in 2% gum acacia. Group II served as CP control group.

### Serum lipid profile estimation

Blood samples were collected on 11<sup>th</sup> day from retro-orbital venous sinus by using capillary tube under ether anaesthesia. Serum was separated by centrifugation at 3000 rpm for 10 mins and used in biochemical estimation of triglycerides (TG), total cholesterol (TC) and HDL-cholesterol (HDL-c). Serum estimation of triglyceride was done as per Sullivan DR et al, total cholesterol was done as per Allian CC et al, HDL-cholesterol was done as per Grub F et al, method.<sup>10-12</sup> Direct colorimetric method was used for these methods. LDL-cholesterol (LDL-c) was calculated by using Friedwald and Fredrickson's formula  $\text{LDL-c} = \text{Total cholesterol} - (\text{Triglyceride}/5 + \text{HDL-c})$ .<sup>13</sup>

### Atherogenic index (AI) values

AI or cardiac risk ratio was calculated by using formula  $\text{AI} = \text{total cholesterol}/\text{HDL-cholesterol}$ . Percentage of protection was calculated by  $[(\text{AI of toxic control group} - \text{AI of treatment group}) / \text{AI of toxic control group}] \times 100$ .

AI of extract treated group)/AI of toxic control group]×100.<sup>14</sup>

**Histopathological evaluation**

After blood collection, all the animals were sacrificed with high dose ether. A midline incision was performed at the thoracic region. The heart was dissected out, fixed in 10% formalin for approximately 48 hours. The paraffin sections were prepared on a glass slide and stained with haematoxylin and eosin for subsequent microscopic evaluation.<sup>15-17</sup>

**Statistical analysis**

Data was computed in SPSS version 21 software. Results were expressed as mean±SEM. One-way analysis of

variance (ANOVA) followed by Dunnett’s-t test was used for comparisons between different groups. P value <0.05 was considered to be significant.

**RESULTS**

**Effects on lipid profile**

CP control group demonstrated a significant (P<0.05) increase in serum levels of TG, TC, LDL-c and decrease in serum level of HDL-c compared to normal control group. Treatment with *Ipomoea aquatica* (AEIA 200mg/kg and 400mg/kg), after inducing toxicity by CP there was significant (P<0.05) decrease in the levels of serum TG, TC, LDL-c and increase in the levels of serum HDL-c compared to CP control group (Table 1).

**Table 1: Effect of *Ipomoea aquatica* on serum level of lipid profile in CP-induced dyslipidaemia in rats.**

Group	Triglyceride (mg/dL)	Total cholesterol (mg/dL)	LDL-cholesterol (mg/dL)	HDL-cholesterol (mg/dL)
Normal control	59.30±3.16	75.76±5.41	22.48±3.35	41.42±2.71
CP control	81.91±7.12*	131.06±5.60*	83.62±7.40*	31.06±3.62*
CP + AEIA 200mg/kg	62.84±4.58 <sup>#</sup>	87.02±3.29 <sup>#</sup>	36.85±2.54 <sup>#</sup>	37.58±2.02 <sup>ns</sup>
CP + AEIA 400mg/kg	55.70±4.52 <sup>#</sup>	78.60±4.61 <sup>#</sup>	25.48±4.73 <sup>#</sup>	41.98±2.01 <sup>#</sup>

All the values are in mean±SEM, n=5, ns=not significant, \*P<0.05 when compared to normal control group, <sup>#</sup>P<0.05 when compared to CP control group. AEIA=Aqueous extract of *Ipomoea aquatica*.

**Effects on atherogenic index (AI)**

Atherogenic index (cardiac risk ratio) was also significantly (P<0.05) increased in CP control group as compared to normal control group. AEIA treatment significantly (P<0.05) reduced the ratio compared to CP control group. High dose of the plant extracts showed higher protection percentage (Table 2).

**Table 2: Comparison of atherogenic index in group wise and estimated protection percentage.**

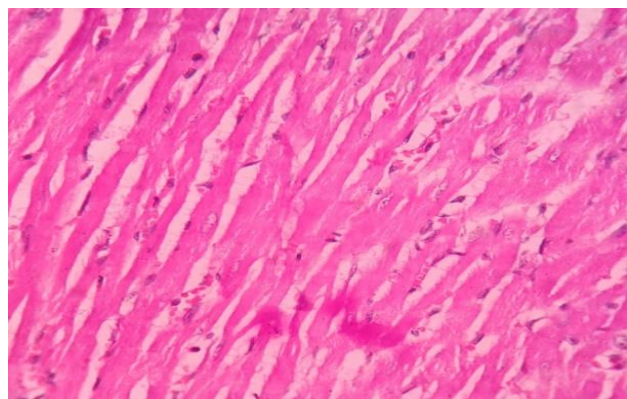
Treatment	Atherogenic Index (Cardiac risk ratio)	Protection (%)
Normal control	1.83	-----
CP control	4.68*	-----
CP + AEIA 200mg/kg	2.33 <sup>#</sup>	50.21
CP + AEIA 400mg/kg	1.89 <sup>#</sup>	59.62

All the values are in mean±SEM, n=5, \*P<0.05 when compared to normal control group, <sup>#</sup>P<0.05 when compared to CP control group. AEIA=Aqueous extract of *Ipomoea aquatica*.

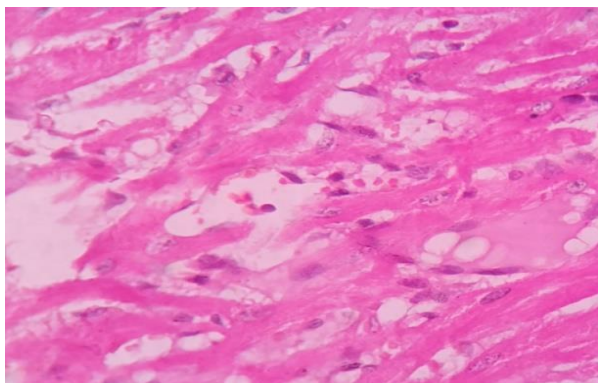
**Histopathology of heart**

In normal control group cardiac muscle fibres were uniform in size, shape and configuration (Figure 1). CP

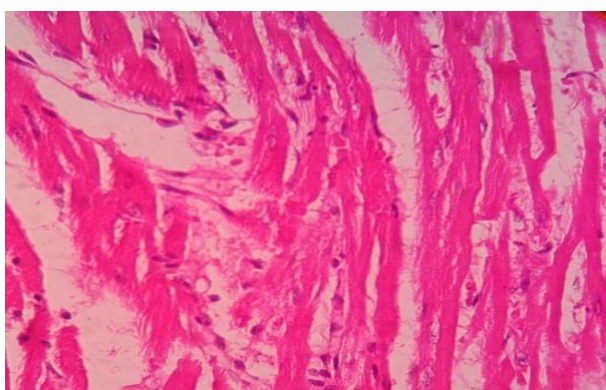
control group showed loss of normal architecture of myocardium, necrosis and degeneration of myocardial fibres, vacuole changes and fragmentation of cardiac muscle fibres (Figure 2) compared to normal control group. Group III (CP + AEIA 200 mg/kg) showed moderate myocardial damage with less myofibrillar degeneration (Figure 3) and group IV (CP + AEIA 400 mg/kg) showed near normalization of cardiac muscle fibres (Figure 4). The protection of heart tissue offered by AEIA was strongly correlated with normalization of dyslipidaemia by AEIA treatment.



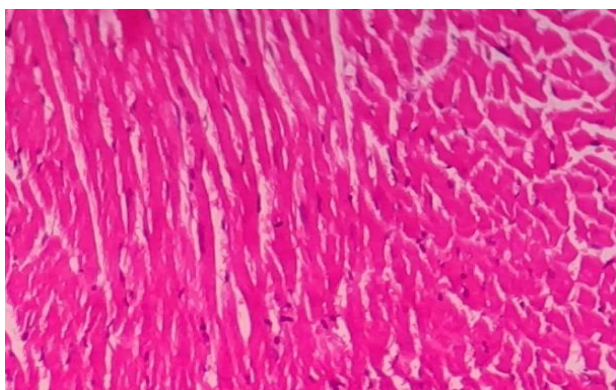
**Figure 1: Histopathology (H and E, 400X) of normal control group showing normal texture of cell.**



**Figure 2: Histopathology (H and E, 400X) of CP control group showing vacuolization, degeneration, fragmentation of cardiac muscle fibres.**



**Figure 3: Histopathology (H and E, 400X) of group III (CP+ AEIA 200mg/kg) showing less myofibrillar degeneration.**



**Figure 4: Histopathology (H and E, 400X) of group IV (CP+ AEIA 400mg/kg) showing near normalization of cardiac muscle fibres.**

## DISCUSSION

Dyslipidaemia is the presence of elevated or abnormal levels of lipids and lipoproteins, and low levels of HDL-cholesterol. Serum lipids and lipoproteins abnormalities are regarded as a highly modifiable risk factor for cardiovascular diseases.<sup>18</sup> Cyclophosphamide induces

oxidative stress through its toxic metabolite acrolein. It can cause dyslipidaemia by increasing cholesterol biosynthesis, decreasing cholesteryl ester hydrolysis and reducing cholesterol efflux. So, cholesterol accumulation occurs by increase in biosynthesis and decrease in its utilization. Triglycerides are degraded by the lipoprotein lipase (LPL) to fatty acids. Alterations in LPL activity by CP may increase the level of triglycerides.<sup>19,20</sup>

In the study, CP treatment caused hypertriglyceridemia, hypercholesterolemia, increased in LDL-cholesterol level and decreased in HDL-cholesterol level resulting in dyslipidaemic changes in blood. AI or cardiac risk ratio also increased in CP control rats compared to normal control rats. Post treatment with AEIA after CP injection caused reduction in the elevated levels of triglycerides, total cholesterol, LDL-cholesterol and atherogenic index. There was also increased in HDL-cholesterol in AEIA treated group. Higher dose of AEIA caused more favourable changes in lipid profile. So, it proves that AEIA alleviate CP induced lipid profile derangements. In Moirangthem RS et al, study, there was similar changes in the lipid profile and atherogenic index by CP and treatment with plant extract reverses the changes in albino rats.<sup>14</sup> Sivaraman D et al, in his study also found that, *Ipomoea aquatica* has lipid lowering activity in rats.<sup>21</sup>

In histopathology examination, CP caused massive change in the myocardium like necrosis, vacuolization, degeneration and fragmentation of cardiac muscle fibres. Treatment with AEIA effectively inhibits CP induced cardiac damage by reversal of the above changes. Protective changes in myocardium are strongly correlated with favourable changes of lipid profile by AEIA treatment. Small animals like rat are generally resistant to the development of vascular atherosclerotic changes,<sup>14</sup> so more studies with long duration and higher animal like rabbit should be done. Swamy AHMV et al, in his study also found the similar cardiac damages by CP and there was reversal of the damages by plant extract treatment in rats.<sup>20</sup>

Flavonol glycoside like 7-O- $\beta$ -D-glucopyranosyl-dihydroquercetin-3-O- $\alpha$ -D-glucopyranoside constituent of *Ipomoea aquatica* has potent antioxidant activity. Carotenoids like violaxanthin, lutein and  $\beta$ -carotene, flavonoids, tannins, poly-phenolic constituents present in the plant have antioxidant activity. Among them, flavonoids are known for their diverse activities including hypolipidemic and antioxidant activity. These phytoconstituents along with others in *Ipomoea aquatica* may be providing the protective effect against CP induced dyslipidaemia.<sup>22,23</sup>

## CONCLUSION

From the present study, it can be concluded that cyclophosphamide treatment causes dyslipidaemia and tissue damage in the myocardium. Administration of aqueous extract of leaves and stems *Ipomoea aquatica*

protects the CP-induced dyslipidaemia. Biochemical and histopathological studies confirm the protective role of *Ipomoea aquatica* Frosk.

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