Research Article

# Hepatoprotective Effect of *Wattakaka volubilis* Extract on Aluminium Sulphate Induced Liver Toxicity

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## ABSTRACT

*Wattakaka volubilis* (Linn) is used as a phytomedicine for liver diseases. In the present study the effect of methanolic extract of *Wattakaka volubilis* powder was carried out against the Aluminium toxicity in rat model. Various doses of *Wattakaka volubilis* extract (100, 200, 400 mg/kg/b.w) was given orally for 30 days to Aluminum sulphate toxicity exposed rats. They were observed each hour for 24 h and each day for 30 days for any changes in behavioural activity and mortality. Clinical observation, body weight, organ weight, biochemical enzymatic analysis and histological examination were carried out. The extract was found to possess hepatoprotective activity in a dose dependent manner and the effect was comparable with silymarin, a standard drug. The extract significantly reduce the toxicity of Aluminium sulphate caused in liver due to the presence of phytoconstituents such as alkaloids, sterols, tannins, triterpenoids and flavonoids which are known hepatoprotectant.

**Keywords:** *Wattakaka volubilis* extract, Aluminium sulphate, liver marker enzymes, histopathology.

## INTRODUCTION

Liver is one of the largest organs in human body and the chief site for intense metabolism and excretion. Therefore, it has an important role in the maintenance, performance and regulating homeostasis of the body. It is involved with almost all the biochemical pathways to growth, fight against disease, nutrient supply, energy provision and reproduction. [1] It is an important organ for detoxification of xenobiotics, environmental toxicants and liver damage is associated with distortion of several metabolic functions; hence liver diseases are of serious health problem. In the absence of reliable liver protective drugs in allopathic medical practices, naturally occurring compounds have been found to have major role in the management of various liver diseases. [2] Numerous pharmacological drugs and their formulations are used for liver disorders in ethnomedical practices and in traditional systems of medicine in India. However a satisfactory remedy for serious liver diseases is not still available, so search for effective hepatoprotective drugs are continued.

Aluminium, the third most abundant element of the earth's crust, is a non-essential and toxic metal in humans. [3-4] Aluminium and its salt are commonly used in daily life a

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wide spread use that was enhanced by the belief that it is non-toxic and is quickly excreted from the body in the urine. However, this element has a negative impact as human health. Due to its abundance, every organism contains small quantities of aluminium [5-6] and it can be found in practically all of the tissues of mammals, including the brain, liver, kidney, heart, blood and bones. There has been considerable debate as to whether chronic exposure to aluminium is involved in neuro-degenerative disorders, such as Alzhimer's disease [7-10], Parkinson's dementia [11-12] and hepatotoxicity. [13-14] The toxic effects of aluminium appear to be mediated at least in part, by free-radical generation. [15-16] The treatment commonly used in aluminium caused hepatotoxicity is silymarin which is associated with undesirable side effects, it is very expensive and it is only efficient when applied intraverously or subcutaneously. However a satisfactory remedy for serious liver diseases is not still available, so search for effective hepatoprotective drugs are continued.

*Wattakaka volubilis* (Linn) (Asclepiadaceae), is a tall woody climber with densely lenticulate branches, occurring throughout the warmer regions of India and Nicobar Islands. The parts of the plant have been traditionally used for medicinal purposes. The juice of the plant is used as a sternutatory and leaves are employed in application for boils and abscesses. [17] The roots and tender stalks are used as emetic and expectorant. It is reported that an alcohol (50%) extract of the plant showed activity on the central nervous

system as well as anti-cancer activity against Sarcoma 180 in mice. [18] The isolation and characterization of twelve polyhydroxy C/D cispregnane glycosides, β-sitosterol, kaempherol-3-galactoside, 2-deoxy sugar, drevogenin A, drevogenin P, D-cymarose and L-olendrose from the plant was also reported. [19-21] The pentacyclic triterpenoid, taraxerone isolated from this plant having anti-leishmanial and anti- cancer activity on K562 leukemic cell line. [22] The present study was aimed to study the role of methanolic extract of *Wattakaka volubilis* (leaf) (MEWV) against aluminium sulphate induced liver damage in rats.

## MATERIALS AND METHODS

### Plant material

The leaves of *Wattakaka volubilis* were collected from Trichirappalli. The plant material was taxonomically identified by Dr. John Britto Rabinet Herbanium. St. Joseph's College, Trichy.

### Preparation of extract

The leaves of *Wattakaka volubilis* were dried in shady condition and powdered. The 200 g of powdered material was dissolved with 250 ml of 95% methanol and extract was prepared using soxhlet apparatus for 48hr. The extract was filtered and concentrated in rotatory evaporator at 35-40ºC under reduced pressure (yield: 28.5% w/w) and was stored in refrigerated condition for further use.

### Drugs and chemicals

Aluminium sulphate and Silymarin were purchased from Sigma-Aldrich chemical company (St. Lousis mo, USA). The diagnostic kits required for enzymatic assays were purchased from Span Diagnostics, India.

### Experimental animals

Adult male Wistar Albino rats weighing 250-350 g were used for the present investigation. They were housed in a clean polypropylene cage and maintained under standard laboratory conditions (temperature 25±2ºC with dark/light cycle 12/12h).They were fed with standard pellet diet (Hindustan lever, Kolkata, india) and water *ad libitum*. The animals were acclimatized to laboratory conditions for one month to experiment. All procedures described were reviewed and approved by the animal ethics committee, IAEC No/232 Sastra Uuniversity, Tanjavur, Tamil Nadu, India.

### Experimental design

The animals were divided into 7 groups consisting of 6 animals in each group. Group I rats received saline (1 ml/kgb.wt), Group–II rats administered twice with Aluminium sulphate (50 mg/kg/day) dissolved in (1 ml/kgb.wt) saline will be injected intraperitoneally double dose per week to induce hepatotoxicity. Group III, IV and V will be administered with Aluminium sulphate same procedure like Group II and also treated with MEWV (100 mg/kg/b.w) (200 mg/kg/b.w), (400 mg/kg/b.w) dissolved in corn oil (1ml/kg b.wt) orally for 30days. Group VI, the hepatotoxicity induced rats were treated with silymarin (25 mg/kg/b.w) dissolved in corn oil (w/v) orally for 30 days. Group VII rats were treated with MEWV alone (200 mg/kg/b.w) dissolved in corn oil (1 ml/kg b.wt) orally for 30days.  The body weights of rats of each group were measured before the experimental trial and 30 days after the MEWV treatment. Liver weight of all rats was measured after the sacrifice.

Animals were sacrificed by injecting with sodium pentabarbitone and blood was collected in plain and heparinized tubes immediately after sacrifice for biochemical assays. Liver was removed and washed with saline. Blood samples centrifuged for 10min at 2500 rpm and the serum separated stored at 4ºC until further investigations. Alkaline phosphatase (ALP), aspartate transaminase (AST), alanine transaminase (ALT), lactate dehydrogenase (LDH), g‑Glutamyl Transferase (GGT) enzyme levels in serum and the levels of various other biochemical parameters such as serum albumin, protein, total bilirubin, creatinine, urea, uric acid and glucose were estimated by using standard kits available commercially.

The hepatoprotective action of MEWV was determined in aluminium sulphate induced hepatotoxic models by various biochemical parameters and serum marker enzymes.

### Histopathological studies

For histopathological study, the fresh liver tissues were collected and immediately fixed in 10% formalin dehydrated in gradual ethanol (50-100% v/v) cleared in xylene and embedded in paraffin. A paraffin embedding technique was carried out and sections were taken at 5µm thickness. Sections were prepared and then stained with hematoxylin–eosin dye for photo microscopic observations.

### Statistical analysis

The data were statistically analysed and all values were expressed as mean ± S.E.M. The data were also analyzed by one way ANOVA followed by Dunnet's t3-test. $p < 0.05$, $p < 0.01$, $p < 0.001$ was considered significant.

## RESULTS

The effect of MEWV on body weight and liver weight are reported in Table 1. Before sacrifice, the total body weight of each rat was measured. Rats from the normal control group (Group I) followed a normal pattern of growth and attained a normal weight gain reaching over 4 weeks. The $Al_2(SO_4)_3$ administered rats (Group II) suffered growth retardation and had a significantly ($p<0.05$) lower weight than other groups. Rats treated with the doses (100, 200 and 400 mg/kg) of MEWV (Group III, IV and V) gained more weight than those of Group II. Silymarin-treated rats (25 mg/kg) (Group VI) and MEWV alone treated rats (Group VII) attained weight gain equivalent to Group I, the normal rats.

The administration of $Al_2(SO_4)_3$ to the animals resulted in a significant rise in serum ALP, AST, ALT, LDH and GGT when the levels compared with normal control in Table 2. In groups III, IV and V, the toxic effect of $Al_2(SO_4)_3$ was gradually reversed in the animals by showing a significant decrease in the serum ALP, AST ALT, LDH and GGT levels. Group VI in comparison with silymarin (standard) showed a significant decrease in serum liver marker enzymes except in ALP levels.

Meanwhile, the changes in the biochemistry parameters were not statistically significant between the MEWV alone treated and control animals at the end of the experiment. These results show the non-toxic nature of MEWV. The biochemical parameter profiles such as creatinine, urea, uric acid, total protein, total bilirubin, albumin and glucose levels of the treated and control groups are shown in Table 3 & Fig. I. The administration of $Al_2(SO_4)_3$ cause significant changes in total protein, urea, uric acid, total bilirubin, albumin, glucose and creatinine when the comparison done with normal control group. These data demonstrated that the effects of toxicity induced by $Al_2(SO_4)_3$ on the liver function such as carbohydrate, protein and nucleic acid metabolism could be effectively counterbalanced by MEWV treatment.

**Table 1: Effect of MEWV on body weight & Liver weight in Al$_2$(SO$_4$)$_3$ induced rats**

| Groups | Dose (mg/kg b.wt) | Initial body wt. (g) | Final body wt. (g) | Final liver wt. (g) |
|---|---|---|---|---|
| Group I (Normal saline) | 1ml | 342.5±11.8 | 378.8±14.8 | 10.45±1.5 |
| Group II ( Al$_2$(SO$_4$)$_3$ induced group) | 50 | 291.5±10.1 | 297.0±10.2* | 7.39±0.45 |
| Group III ( Al$_2$(SO$_4$)$_3$ + MEWV) | 100 | 294.2±9.6 | 315.1±9.8# | 8.57±0.56 |
| Group IV ( Al$_2$(SO$_4$)$_3$ + MEWV) | 200 | 289.6±12.4 | 316.5±10.7# | 9.58±0.78 |
| Group V ( Al$_2$(SO$_4$)$_3$ + MEWV) | 400 | 281.6±10.9 | 309.4±11.6# | 9.6±0.89 |
| Group VI ( Al$_2$(SO$_4$)$_3$ + Silymarin) | 25 | 290.1±11.6 | 309.5±10.7# | 9.49±0.67 |
| Group VII (MEWV alone) | 200 | 285.6±10.6 | 309.4±13.5 | 9.86±0.59 |

Results are expressed as mean ± S.E.M, n = 6. *$p < 0.001$, statistically significant as compared with control rats and #$p < 0.001$ statistically significant as compared with Al$_2$(SO$_4$)$_3$ control group

**Table 2: Effect of MEWV on liver marker enzymes in Al$_2$(SO$_4$)$_3$ induced rats**

| Parameter (U/L) | Group I | Group II | Group III | Group IV | Group V | Group VI | Group VII |
|---|---|---|---|---|---|---|---|
| Alkaline phosphatase | 73.3±5.2 | 231.6±11.8* | 194.1±5.6[a] | 153.3±4.3# | 146.3±3.8# | 225.3±5.9 | 75.3±1.6 |
| Aspartate transaminase | 26.13±1.1 | 78.1±3.5* | 62.03±4.8[a] | 56.7±3.9# | 52.7±2.1# | 52.63±1.3# | 27.5±1.0 |
| Alanine transaminase | 42.0±1.6 | 76.5±3.7* | 63.4±4.3[a] | 51.8±2.8# | 48.5±1.9# | 46.7±2.6# | 36.2±1.4 |
| Lactate dehydrogenase | 74.4±2.8 | 283.7±11.7* | 266.9±12.8[a] | 233.7±10.5# | 190.0±11.0# | 141.9±8.5# | 91.5±7.3 |
| Gamma-glutamyl transpeptidase | 2.7±0.15 | 7.7±0.21* | 7.1±0.56[a] | 4.5±0.33# | 3.7±0.24# | 3.2±0.18# | 2.5±0.11 |

Results are expressed as mean ± S.E.M, n = 6. *$p < 0.001$, statistically significant as compared with control rats and [a]$p < 0.05$; #$p < 0.001$ statistically significant as compared with Al$_2$(SO$_4$)$^3$ induced group

**Table 3: Effect of MEWV on general biochemical parameters in Al$_2$(SO$_4$)$_3$ induced rats**

| Parameters | Group I | Group II | Group III | Group IV | Group V | Group VI | Group VII |
|---|---|---|---|---|---|---|---|
| Creatinine (mg/dl) | 1.20±0.06 | 2.37±0.18* | 2.20±0.19 | 1.92±0.11[b] | 1.56±0.09[c] | 1.35±0.10[c] | 1.13±0.08 |
| Urea (mg/dl) | 25.9±1.6 | 11.8±0.83* | 17.1±1.0[a] | 18.2±1.2[b] | 19.1±1.5[c] | 22.9±1.0[c] | 24.3±1.6 |
| Uric acid (mg/dl) | 3.10±0.18 | 1.86±0.16* | 1.89±0.15 | 2.53±0.22[b] | 2.66±0.24[c] | 2.63±0.25[c] | 3.02±0.19 |
| Glucose (mg/dl) | 106±7.6 | 68±5.4* | 82±4.6[a] | 94±3.9[b] | 92±4.1[c] | 92±1.7[c] | 111±5.4 |
| Protein(g/dl) | 8.0±0.79 | 12.3±0.93* | 10.8±0.63 | 10.6±0.51[b] | 8.8±0.48[c] | 9.3±0.41[c] | 8.3±0.56 |

Results are expressed as mean ± S.E.M, n = 6. *$p < 0.001$, statistically significant as compared with control rats and #$p < 0.001$ statistically significant as compared with Al$_2$(SO$_4$)$_3$ group
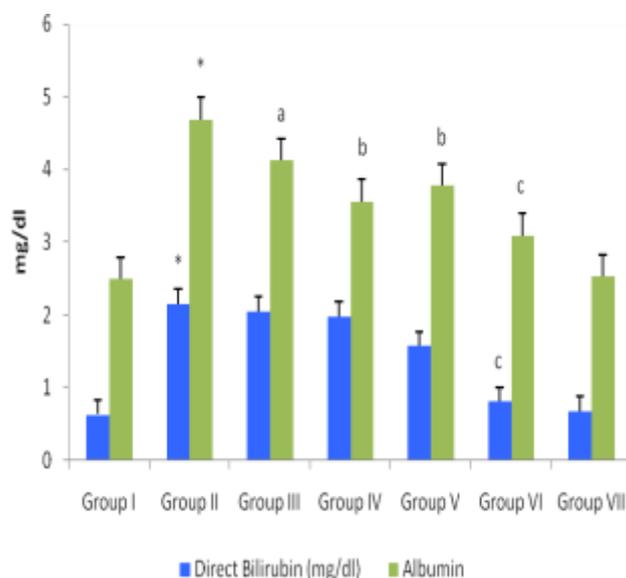


**Fig. I: Effect Of MEWV On Albumin And Direct Bilirubin In Al$_2$(So$_4$)$_3$ Induced Rats**
Results are expressed as mean ± S.E.M, n = 6. *$p < 0.001$, statistically significant as compared with control rats and [a]$p < 0.05$; [b]$p< 0.01$; [c]$p < 0.001$ statistically significant as compared with Al$_2$(SO$_4$)$_3$ group.

The histopathological analysis revealed no apparent changes in the liver tissue architecture from both control and MEWV treated rats. The microscopic structures of the organs depicted in Figure II A & II G show unnoticeable differences between the control and test groups. Pathological alteration such as hepatic lesions and neutrophil infiltration were recorded in the histological sections of the liver of the Al$_2$(SO$_4$)$_3$ induced group (Fig IIB). Animals treated with Al$_2$(SO$_4$)$_3$ and various doses (100, 200 and 400 mg/kg b.wt) of MEWV showing, gradual decrease in hepatic toxic lesions, reduced size of neutrophil infiltration and recovery of normal hepatocytes (Fig. II C, II D, II E). Sections from the animals treated with Al$_2$(SO$_4$)$_3$ and silymarin (standard drug) shows protection against the hepatic tissues with no pathological hepatic lesions caused by Al$_2$(SO$_4$)$_3$ (Fig. II F).

**DISCUSSION**

The hepatoprotective activity of MEWV were determined on the basis of liver marker enzymes, general biochemical parameters and histological sections using three different doses (100, 200 and 400 mg/kg b.wt) of extract and results were compared with respect to control, standard drug silymarin and Al$_2$(SO$_4$)$_3$ treated animals. The animals treated MEWV remained alive and did not manifest any visible toxicity at the doses used. Clinical observations and serum biochemistry did not show any significant differences between the control and the MEWV alone treated groups.

Histopathology results of liver did not show any significant differences between controls and the MEWV alone treated groups. The livers of the low dose MEWV-treated Group III showed less regeneration than those of the Silymarin-treated Group VI, but the improvements were not great as those seen in Groups VI and V. These histological evaluations provide further confirmation that MEWV treatment effectively protected the liver from further injury in a dose dependent manner.

Previous studies on aluminium toxicity have been based on biochemical analysis. [23-24] Thus it has been seen that aluminium accumulation within the liver is associated with a number of biochemical changes which include the release of enzyme markers of liver injury, and alteration in the oxidant status. [25] Al induced hepatic dysfunctions, DNA cross-linking in rat ascites hepatoma cells, MN and sister chromatid exchange (SCE) formations in human peripheral blood lymphocytes. [26] AST and ALT is more specific and predominant in the liver and myocardial injury, respectively. The modulations in transaminase are also influenced by the degree of hepatic decompensation of cell necrosis. [27] The LDH released into the medium provides an index of cell death and membrane permeability to LDH, and an increase in
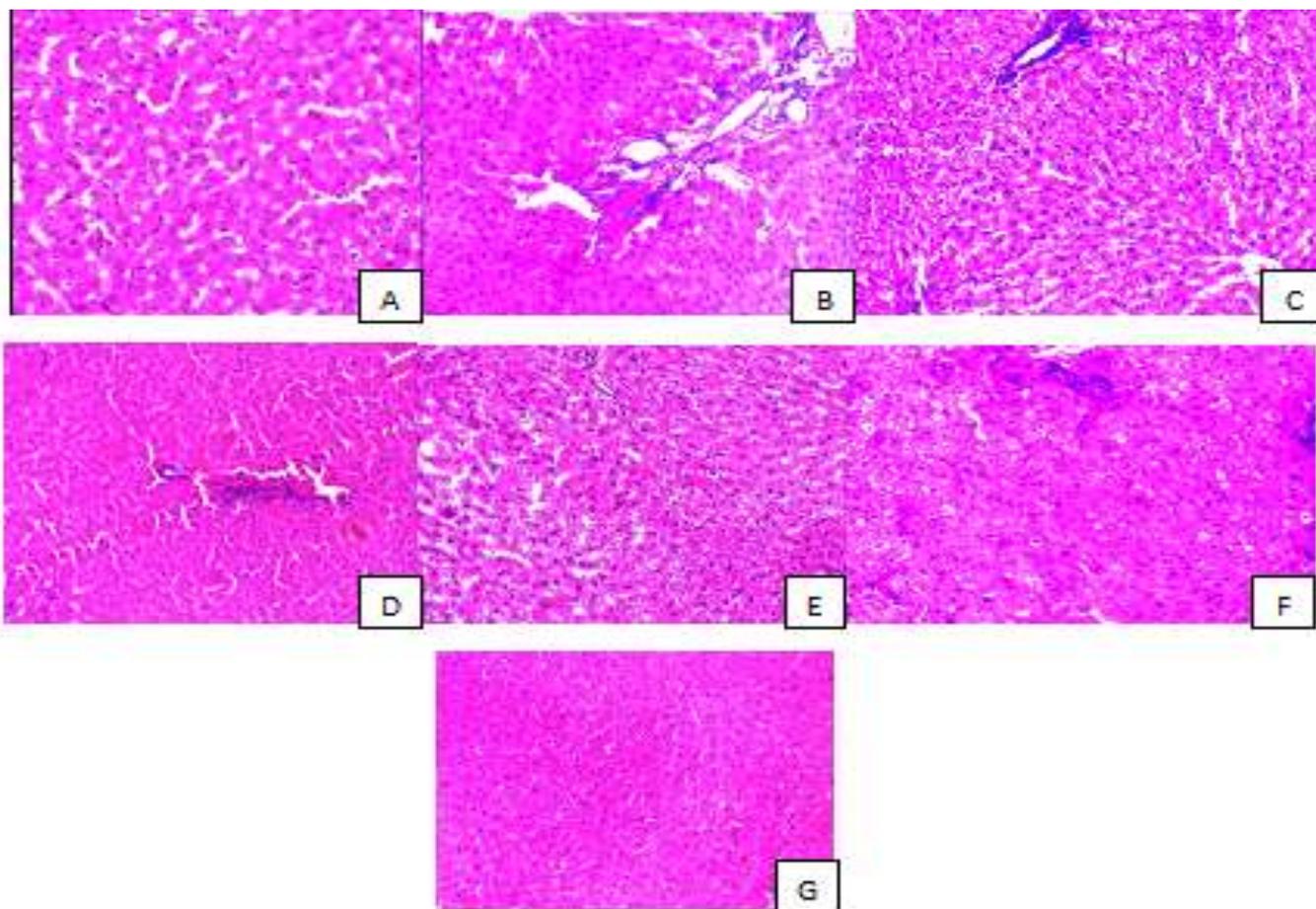
**Fig. II: Histological examination of Liver tissue section in control & experimental rats (hematoxylin & eosin, 20x)**
Control animal liver section showing normal hepatic architecture with normal hepatocytes with nucleus (Fig. 2A). Sections from the animals treated with $Al_2(SO_4)_3$ are showing hepatic lesions and large size of neutrophil infiltration (Fig.2B). Sections from the animals treated with $Al_2(SO_4)_3$ and various doses (100, 200 and 400 mg/kg b.wt) of MEWV showing, gradual decrease in hepatic toxic lesions, reduced size of neutrophil infiltration and recovery of normal hepatocytes (Fig. 2C, 2D, 2E). Sections from the animals treated with $Al_2(SO_4)_3$ and silymarin (standard srug) shows protection against the hepatic tissues with no pathological hepatic lesions caused by $Al_2(SO_4)_3$ (Fig. 2F). Sections from the animals treated with MEWV alone are showing normal tissue architecture and hepatocytes (Fig. 2G).

LDH activity in the medium occurs as a result of cell membrane disintegration and enzyme leakage. The present study also demonstrates that aluminium significantly affects LDH level in serum. The evidence reveals that the activities of LDH are used as a marker of Al toxicity. [28]

The treatment with MEWV ameliorated these changes and normalized the levels. This effect may be due to its stabilization of plasma membrane as well as repair of damage tissues. It can be concluded that the MEWV at a dose of 200 mg & 400 mg possess good hepatoprotective activity against aluminium sulphate caused toxicity in liver tissues than 100 mg when it is compared with standard silymarin hepatoprotection. It is always better to select optimum dose with better protection for human disease treatment. Phytochemical analysis of *Wattakaka volubilis* showed the presence of carbohydrate, saponins, flavonoids alkaloids, sterols, tannins, terphenoids, wax and resins. [29-30] these compounds are considered to be major contributors to the antioxidant action of plants. This plant extract trial has been conducted on various liver disorders which show good protection with no side effects. [31]

To conclude, from the overall result of the biochemical and histopathological examinations, it could be inferred that *Wattakaka volubilis* leaf extract showed the highest hepatoprotective activity in Aluminium sulphate induced hepatotoxicity. The possible action may be due to its hepatoprotective constituents and antioxidant compounds present in the extract.

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