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PROTECTIVE EFFECT OF METHANOLIC EXTRACT OF RHIZOMES OF *ACORUS CALAMUS* AGAINST ALUMINIUM CHLORIDE INDUCED OXIDATIVE STRESS IN RAT LIVER

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ABSTRACT

The present work investigated the Hepatoprotective and antioxidant potential of methanolic extract of *Acorus calamus* rhizomes on Wistar rats against $AlCl_3$-induced liver injury. *In vitro* free radical scavenging activity of methanolic extract of *Acorus calamus* was estimated using Hydrogen peroxide scavenging and Nitric oxide (NO) radical scavenging methods. Hepatotoxicity was induced by oral administration of $AlCl_3$ at 40 mg/kg body weight of Wistar rats daily for a period of 5 weeks. Experimental rats received MEAC orally at the doses of 100, 200 and 400 mg/kg daily for 5 weeks. Vitamin-E, a routine Hepatoprotective drug, was used as a reference drug. Hepatoprotective effects were investigated by assessment of serum biochemical enzymes such as alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP) total bilirubin (TB), malondialdehyde (MDA), and antioxidant enzymes (SOD, CAT, GPx and GSH), along with histopathological studies. Hydrogen peroxide scavenging and Nitric oxide (NO) radical scavenging activity of MEAC exhibits the IC_{50} value of 157.61 and 302µg/ml respectively. Oral treatment with MEAC exhibited a significant decrease in the levels of AST, ALT, ALP and TB. The extract significantly diminished MDA levels. The activities of the antioxidant enzymes were significantly augmented in rats pretreated with MEAC 100, 200 and 400mg/kg respectively. Histopathological examination demonstrated lower liver damage in MEAC-treated groups as compared to $AlCl_3$ groups.

INTRODUCTION

Absorption and accumulation of Al in humans occurs via diet as in some food products and additives medication like antacids vaccines and parenteral fluids, adding to cosmetics, inhaled fumes, and particles from occupational exposures [1]. Aluminum gets access to the body via the gastrointestinal and respiratory tracts and accumulates in many tissues, such as kidney, liver, heart, blood, bone and brain [2]. The toxic effect of aluminum has been suggested to be mediated by reactive oxygen species generation resulting in the oxidative deterioration of cellular lipids, proteins and DNA and also induces changes in the activities of tissue antioxidant enzymes [3]

altered gene expression and apoptosis. The induced oxidative stress by aluminum and its salts is responsible for hepatotoxicity [4], nephrotoxicity, cardiac toxicity, reproductive toxicity and also neurodegenerative disease and Alzheimer like neurofibrillary tangle formation. Therefore, the external supply of antioxidants is important to suppress caspase activation and for the defense against the deleterious effects of oxidative stress [5]. Several chelating agents and antagonists are developed to reduce the metal toxicity, some of them are burned with undesirable side effects. To avoid intrinsic limitations and variability of efficacy of heavy metal chelating agents, metal intoxication therapy now development is focused on phytochemical actions.

Acorus calamus commonly known as Bach has been used in the Indian and Chinese system of medicine for hundreds of years to cure disease especially the CNS abnormalities [6]. Ethanolic extract of this plant traditionally used for antidiabetic antiproliferative and immunosuppressive, Antidiarrheal and Hypolipidemic activities [7-10]. It is reportedly useful in clearing speech in children. In Ayurvedic medicine, it is used for the treatment of insomnia, melancholia, epilepsy, hysteria, loss of memory remittent fever and neurosis [11]. Recently, *Acorus calamus* has been reported to possess high antioxidant activity [12]. Based on this background, the present study was carried out to investigate the possible In-vitro Antioxidant activities and Hepatoprotective activity of AC against $Alcl_3$ induced intoxication.

MATERIALS AND METHODS

Materials

All chemicals, used in this study, were of analytical grade. Aluminum chloride ($AlCl_3$) was purchased from Sigma Chemical Company, St. Louis, Missouri, USA. The fresh rhizomes of *Acorus calamus* were collected from the outskirts of Maisammaguda situated in the state of Telangana (India) and the methanol extract was prepared using soxhlet extraction process.

Animals: An ethical approval of this experimental study was obtained from the Institutional Animal Ethical Committee of Malla Reddy College of pharmacy, Hyderabad with Reg. No 1217/PO/Re/S/08/CPCSEA. Thirty six albino rats with average body weight from 150 to 250 g were utilized in this study. They were procured from Teena labs, Plot no 41, SV cooperative industrial estates, Bachupally (V), Quthbullapur. The rats were housed in polypropylene cages and maintained under standard conditions (12 h light and dark cycles at 25 ± 3°C and35-60 % humidity). Standard pelletized feed and tap water were provided *ad-libitum*.

In-vitro Antioxidant activity Experiments Procedure:

1) Hydrogen peroxide (H_2O_2) scavenging activities: The ability of the hydroxyl radical to remove or add hydrogen molecules to unsaturated hydrogen bonds of organic lipids makes it potentially one of the most reactive oxidants in biological systems. It has very short half-life. The procedure involved UV-spectrophotometric determination of Hydrogen peroxide radical scavenging. Three solutions i.e. Standard, Test and Control were prepared.

Preparation of standard ascorbic acid solutions: Different concentrations (50–250 µg/ml) of the ascorbic acid were prepared in distilled water. 1 ml of each solution of ascorbic acid was mixed with 2 ml of 0.1 M phosphate buffer solution and 600 µl of 100 mM H_2O_2 solution. After 10 minutes absorbance of different concentration of ascorbic acid solutions was taken at 230 nm.

Preparation of Test solutions: Various concentrations (50, 100, 150, 200, 250 µg/ml) of the Extract were prepared in distilled water. 1ml of each solution of extract was mixed with 2ml of 0.1 M phosphate buffer solution and 600 µl of 100 mM H_2O_2 solution. After 10 minutes (approximately) absorbance of different concentration of extract solutions were taken at 230 nm.

Preparation of Control solution: For control 2 ml of 0.1 M phosphate buffer solution was mixed with 600 µl of 100 mM H_2O_2 solution. After 10 minutes absorbance of control was taken at 230 nm.

Percentage Hydrogen peroxide radical scavenging activity of MEAC and ascorbic acid was calculated by using the formula:

I% = Ac - (At - Ab) /Ac X 100

Where, I% = Percentage inhibition, Ac = Absorbance of control (0.1 M phosphate buffer solution and H_2O_2), At =Absorbance of ascorbic acid / plant extract with H_2O_2 after 10 min., Ab=Absorbance of ascorbic acid / plant extract without H_2O_2.

The results were expressed as percentage (%) inhibition exhibited by the test substances and the standard IC50 value was calculated in each case.

2) Nitric oxide scavenging activity: Nitric oxide radical scavenging activity was determined according to the method reported by (Garrat). Sodium nitro prusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions, which can be determined by the use of the Griess Illosvoy reaction. 2 ml of 10 mM sodium nitroprusside in 0.5 ml phosphate buffer saline (pH 7.4) was mixed with 0.5 ml of extract at various concentrations and the mixture incubated at 25℃ for 180 min. From the incubated mixture 0.5 ml was taken out and added into 1.0 ml sulfanilic acid reagent (33% in 20% glacial acetic acid) and incubated at room temperature for 5 min. finally, 1.0 ml naphthylethylenediaminedihydrochloride (0.1% w/v) was mixed and incubated at room temperature for 30 min before measuring the absorbance at 540 nm was measured with a spectrophotometer. The nitric oxide radicals scavenging activity was calculated. Methanolic extract of *Acorus calamus* exhibited good nitric oxide scavenging activity. The results were expressed as percentage (%) inhibition exhibited by the test substances and the standard IC50 value was calculated in each case.

Experimental Design: Rats were randomly divided into six groups (n=6 each). The first group (Group-I) included the control rats which received distilled water, in second group (Group-II) $AlCl_3$ treated group-injected with $AlCl_3$ daily at a dose 40 mg/kg b.w/p.o for 5 weeks, in third group (Group-III) Vitamin-E treated group-injected with $AlCl_3$ 40 mg/kg b.w/p.o and Vitamin-E 100 mg/kg b.w, in fourth group (Group-IV) $AlCl_3$ + MEAC group- injected with $AlCl_3$ 40 mg/kg b.w and methanol extract of *Acorus calamus* 100 mg/kg b.w/p.o. daily for 5 weeks,in fifth group (Group-V) $AlCl_3$ + MEAC group- injected with $AlCl_3$ 40 mg/kg b.w/p.o and methanol extract of *Acorus calamus* 200 mg/kg b.w/p.o. daily for 5 weeks and in sixth group (Group-VI) $AlCl_3$ + MEAC group- injected with $AlCl_3$ 40 mg/kg b.w/p.o and methanol extract of *Acorus calamus* 400 mg/kg b.w/p.o. daily for 5 weeks were administered. At the end of the experiment, rats were fasted overnight. The body weight of the rats was measured. The animals were sacrificed on 35^{th} day by carbon dioxide inhalation through euthanasia chamber and blood was immediately collected by carotid bleeding method. Blood was centrifuged using Remi centrifuge at 4000 rpm for 15 mins & the resultant serum was collected and stored at -20℃ till analysis. Livers were dissected rapidly, a part of these tissues were minced and then homogenized with phosphate buffer using tissue homogenizer. Homogenates were centrifuged at 10,000 x g for15minutes at 4 ℃ and the resultant supernatant was removed and stored at -80℃ until used for antioxidant enzyme activities and lipid peroxidation (MDA)assays. The other part of tissue was promptly washed with normal saline and stored in 10%v/v buffered formalin and then processed further for histopathological studies.

Biochemical Assessment: Serum Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were calorimetrically assessed according to the method of Reitman and Frankel while serum alkaline phosphatase (ALP) was measured according to the method described by Belfeld. [13]Total bilirubin was determined calorimetrically according to Schmidt and Eisenburg method. [14]

Biochemical estimation of markers of oxidative stress: Reduced glutathione (GSH) level was determined in the liver and kidney tissue homogenates according to Ellman method [15], enzymatic antioxidant

catalase (CAT) activity were also assessed according to Aebi *et al.*, method [16]. Glutathione peroxidase was assayed according to the procedure of Hafeman *et al.*, method [17] with some modifications. Malondialdehyde (MDA) level was analyzed by estimation of the produced thiobarbituric acid reactive substances (TBARS) by the method of Buege and Aust.[18] Glutathione reductase activity was measured according to previous reports.[19]

Histopathological study:

On completion of the regimen animals were sacrificed the liver was dissected out and fixed in 10% buffered neutral formalin solution and further processed to obtain of paraffin blocks. Five-micron thick paraffin sections were prepared and routinely stained with Hematoxylin and Eosin (H&E) and examined for the histopathological changes using the light microscope.

Statistical analysis: The obtained results were analyzed for statistical significance using one way ANOVA followed by Dunnet test using the graph pad statistical software for comparison between different experimental groups. P-values < 0.001 were considered statistically significant.

RESULTS AND DISCUSSION

The most likely mechanism of antioxidant protection is direct interaction of the extract (or compounds) and the hydrogen peroxide rather than altering the cell membranes and limiting damage. Hydrogen peroxide is a weak oxidizing agent that inactivates a few enzymes directly, usually by oxidation of essential thiol (- SH) groups. It can cross cell membranes rapidly; once inside the cell, it can probably react with Fe^{2+} and possibly Cu^{2+} ions to form hydroxyl radicals and this may be the origin of many of its toxic effects. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. However, the activities of antioxidants have been attributed to various mechanisms such as prevention of chain initiation, decomposition of peroxides, reducing capacity and radical scavenging.Nitric oxide is a free radical produced in the mammalian cells and is involved in regulation of various physiological processes. However excess production of nitric oxide is associated with several diseases. Methanolic extract of *Acorus calamus* rhizomes has demonstrated dose dependent radical scavenging activity against NO free radicals. *Acorus calamus* demonstrated dose dependent antioxidant activity comparable with Ascorbic acid. The antioxidant activity of methanol extract of *Acorus calamus* rhizomes was compared with L-Ascorbic acid which is a well known antioxidant. IC50 value of L-Ascorbic acid by Hydrogen peroxide method was found to be 123.94 µg/ml (Table-1) comparatively IC50 value of MEAC was found to be 157.61 µg/ml (Table-2). IC50 value of L-Ascorbic acid by Nitric oxide method was found to be 258 µg/ml (Table-3), the rhizomes extract of Acorus calamus showed prominent IC50 value of 302.0 µg/ml (Table-4)

Biochemical results: The body weight of rats from toxin group were significantly ($p<0.0001$) decreased when compared with normal control group. Treatment with extract of rhizomes of *Acorus calamus* prior to $AlCl_3$ intoxication has shown a dose dependant protection. (Table-5) Liver weight of rats from toxin group were significantly ($p<0.0001$) decreased when compared with normal control group. Treatment with extract of rhizomes of *Acorus calamus* prior to $AlCl_3$ intoxication has shown a dose dependant protection. (Table-6) Oral administration of $AlCl_3$ to Wistar albino rats for 35 days resulted in significant increase ($P<0.001$) in serum activities of AST, ALT, ALP and Total bilirubin concentration. Interestingly, the levels of all these parameters showed significant improvement toward their normal levels seen in the control rats received the vehicle when MEAC and Vitamin-E were concomitantly administered with $AlCl_3$. (Table-7).The effects on some components of the oxidative system in the liver of Wistar albino rats after 35 days of exposure to $AlCl_3$ and MEAC are represented in (Table-8). There was a significant depletion of GSH level, GPx, Catalase and Glutathione reductase activities with concurrent significant elevation ($P< 0.05$) in the MDA level in the liver tissues of

orally exposed rats to $AlCl_3$ when compared with the other experimental groups. Vitamin-E, MEAC administration along with $AlCl_3$ ameliorate the deleterious effects produced by Al via improvement of antioxidant status, causing elevation of GPx , GSH, GR and Catalase levels and a significant decrease ($P < 0.05$) in the levels of MDA when compared with $AlCl_3$ treated group. Notably, enhanced antioxidant level in the extract treated groups than normal indicates the high antioxidant potential of MEAC.

MEAC is able to inhibit the free radical generation, could further reduce the oxidative threat caused by aluminum, which could mitigate the consumption of endogenous enzymatic and non enzymatic antioxidants and increased their levels and markedly reduces the hepatic and renal LPO. In view of the present study, it can be concluded that MEAC played an important role as an antioxidant, which includes free radical scavenging and metal-chelating property and thereby improved the detrimental state of liver cells which unraveled its use as a possible mitigator/attenuating agent in aluminum induced hepatotoxicity.

Table- 1: % Inhibition of H_2O_2 by Ascorbic acid

S. No	Conc(µg/ml)	Absorbance (control) A_c	Absorbance (test) A_f	% inhibiton	IC50(µg/ml)
1	50	0.71	0.42	39.86	123.94
2	100		0.372	47.61	
3	150		0.322	54.65	
4	200		0.269	62.11	
5	250		0.191	73.10	

Values represent the mean ± SEM; number of readings in each group = 3

Table -2: % Inhibition of H_2O_2 by Methanol Extract

S. No	Conc(µg/ml)	Absorbance (control) A_c	Absorbance (test) A_f	% inhibition	IC50 (µg/ml)
1	50	0.71	0.470	33.82	157.61
2	100		0.424	40.28	
3	150		0.394	44.50	
4	200		0.344	51.54	
5	250		0.290	59.15	

Values represent the mean ± SEM; number of readings in each group = 3

Table -3: % Inhibition of Nitric oxide by Ascorbic acid

S. No	Conc(µg/ml)	Absorbance (control) A_c	Absorbance (test) A_f	% inhibition	IC50 (µg/ml)
1	50	0.543	0.157	25.39	258
2	100		0.122	31.83	
3	150		0.103	35.33	
4	200		0.073	40.86	
5	250		0.052	44.72	

Values represent the mean ± SEM; number of readings in each group = 3

Table 4: % Inhibition of Nitric oxide by Methanol extract

S. No	Conc(µg/ml)	Absorbance (control) A_c	Absorbance (test) A_f	% inhibition	IC50(µg/ml)
1	50	0.543	0.185	20.23	302
2	100		0.151	26.49	
3	150		0.127	30.91	
4	200		0.102	35.52	
5	250		0.089	37.91	

Values represent the mean ± SEM; number of readings in each group = 3

Table -5: Effect of Methanolic Extract of Rhizomes of *Acorus calamus* on Body weight in Aluminium chloride Induced Oxidative stress in Rats:

S. No	Group	Initial weight(gm)	Weight After treatment(gm)	Change in body weight(gm)
I	Control	197±4.47	199±1.32	±2
II	$AlCl_3$ treated (40mg/kg (oral))	226±4.41$^{\#}$	195±4.49$^{\#}$	-31$^{\#}$
III	Vitamin E + $AlCl_3$l(100mg/kg+ 40mg/kg (oral))	239±3.99***	228±5.89***	-11***
IV	Meth. ext. *A.calamus* + $AlCl_3$ (100mg/kg+40mg/kg (oral))	140±4.58*	98±7.28*	-42*
V	Meth. ext. *A.calamus* + $AlCl_3$ (200mg/kg+40mg/kg (oral))	180±3.47**	163±4.25**	-17*
VI	Meth. ext. *A.calamus* + $AlCl_3$ (400mg/kg+40mg/kg (oral))	200±6.96**	192±5.93**	-8*

Values are expressed as mean ±SEM, n=6. Using t-test, the intergroup variation between various groups was conducted by graph pad Prism software & Data were analyzed by using one way analysis of variances (ANOVA). Values are expressed as mean ± SEM. $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$ as compared to $AlCl_3$ treated group (Group II) [Groups III to VI were compared with Group II], # $p < 0.001$ as compared to Control group (Group I) [Group II was compared with Group I].

Table-6: Effect of Methanolic Extract of Rhizomes of *Acorus calamus* on Organ weight in Aluminium chloride Induced Oxidative stress in Rats:

S. No	Group	Liver(gm)
I	Control	8gm
II	$AlCl_3$ treated (40mg/kg (oral))	7.51gm$^{\#}$
III	Vitamin E + $AlCl_3$l(100mg/kg+ 40mg/kg (oral))	7.5gm***
IV	Meth. ext. *A.calamus* + $AlCl_3$ (100mg/kg+40mg/kg (oral))	8.42gm*
V	Meth. ext. *A.calamus* + $AlCl_3$ (200mg/kg+40mg/kg (oral))	8.11gm**
VI	Meth. ext. *A.calamus* + $AlCl_3$ (400mg/kg+40mg/kg (oral))	6.84gm***

Values are expressed as mean ±SEM, n=6. Using t-test, the intergroup variation between various groups was conducted by graph pad Prism software & Data were analyzed by

using one way analysis of variances (ANOVA). Values are expressed as mean ± SEM. $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$ as compared to $AlCl_3$ treated group (Group II) [Groups III to VI were compared with Group II], # $p < 0.001$ as compared to Control group (Group I) [Group II was compared with Group I].

Table-7: Effect of Methanolic Extract of Rhizomes of *Acorus calamus* in Serum Biochemical Parameters for Hepatoprotective activity in Aluminium chloride induced Oxidative stress in Rats:

S. No	Group	SGOT(U/L)	SGPT(U/L)	ALP(U/L)	Bilirubin (mg/dl)
I	Control	8.66±0.22	10.35±0.22	110.29±0.23	0.20±0.04
II	$AlCl_3$ treated (40mg/kg (oral))	19.35±0.22#	22.35±0.22#	274.58±0.17#	1.62±0.17#
III	Vitamin E + $AlCl_3$l(100mg/kg+ 40mg/kg (oral))	7.21±0.07***	11.98±0.57**	120.70±0.12***	0.80±0.04***
IV	Meth. ext. *A.calamus* + $AlCl_3$ (100mg/kg+40mg/kg (oral))	11.51±0.48*	10.54±0.18*	154.70±0.47*	1.23±0.09*
V	Meth. ext. *A.calamus* + $AlCl_3$ (200mg/kg+40mg/kg (oral))	9.54±0.09**	8.72±0.09**	137.30±0.18**	1.09±0.09**
VI	Meth. ext. *A.calamus* + $AlCl_3$ (400mg/kg+40mg/kg (oral))	7.35±0.09***	5.68±0.17****	124.60±0.15***	0.65±0.02***

Values are expressed as mean ±SEM, n=6. Using t-test, the intergroup variation between various groups was conducted by graph pad Prism software & Data were analyzed by using one way analysis of variances (ANOVA). Values are expressed as mean ± SEM. $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$ as compared to $AlCl_3$ treated group (Group II) [Groups III to VI were compared with Group II], # $p < 0.001$ as compared to Control group (Group I) [Group II was compared with Group I].

Table 8: Effect of Methanolic Extract of Rhizomes of *Acorus calamus* on Antioxidant Parameters in Aluminium chloride Induced Oxidative stress in rat Liver.

S. No	Group	MDA(nm/gm)	GSH(µg/mg)	Catalase (K/min)	GR(u/ml)	GP_x (µg/mg)
I	Control	155.7±0.84	27.5±0.5	21±1.0	20.83±0.60	38.33±0.5
II	$AlCl_3$ treated (40mg/kg (oral))	429.1±1.4#	13.5±0.5#	11.5±0.5#	11.33±0.49#	21.16±0.47#
III	Vitamin E + $AlCl_3$l(100mg/kg+ 40mg/kg (oral))	155.6±1.08***	35.7±0.5***	18.91±0.5**	24.66±0.92**	34.33±0.55**
IV	Meth. ext. *A.calamus* + $AlCl_3$ (100mg/kg+40mg/kg (oral))	178.1±1.48*	28.53±0.5*	15±0.5*	17.51±0.42*	28.01±0.36*
V	Meth. ext. *A.calamus* + $AlCl_3$ (200mg/kg+40mg/kg (oral))	159.35±0.84**	31.52±0.5**	18.52±1.0**	18.2±0.5**	30.35±0.33**
VI	Meth. ext. *A.calamus* + $AlCl_3$ (400mg/kg+40mg/kg (oral))	148.48±0.93***	33.84±0.5***	25.15±0.5**	24.53±0.42***	35.31±0.5***

Values are expressed as mean ±SEM, n=6. Using t-test, the intergroup variation between various groups was conducted by graph pad Prism software & Data were analyzed by using one way analysis of variances (ANOVA). Values are expressed as mean ± SEM. $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$ as compared to $AlCl_3$ treated group (Group II) [Groups III to VI were compared with Group II], # $p < 0.001$ as compared to Control group (Group I) [Group II was compared with Group I].

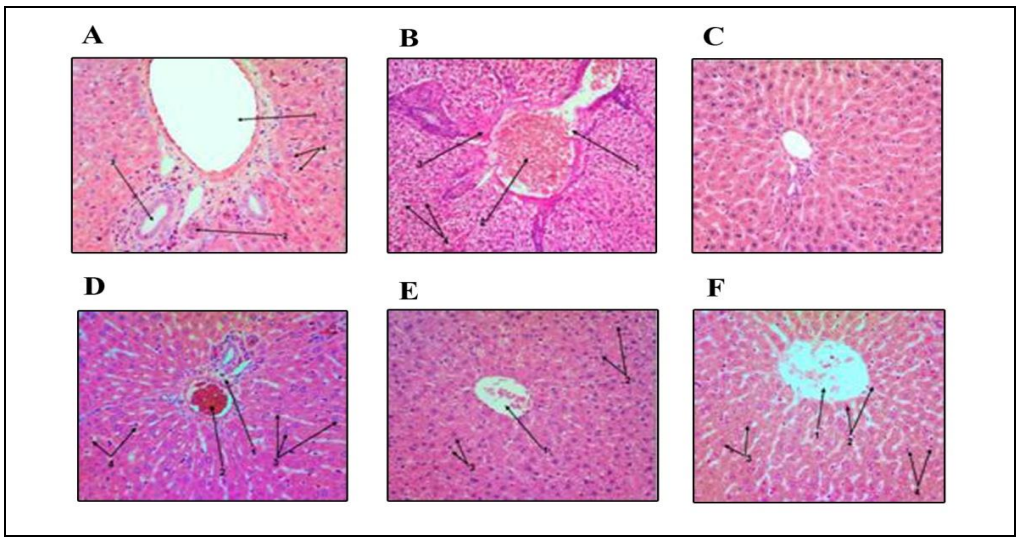

Figure 1: Histopathological changes in the liver tissue of experimental groups

Histopathological Results:

Histology of the Liver in the control rats (Fig.1A) was normal with prominent portal vein, hepatic artery and hepatocytes arranged in sheets. Rats receiving $AlCl_3$ (Fig.1B) showed distorted portal area distorted portal area and oedema, bleeding in the portal vein, fibrosis around portal area, and vacuolar degeneration of hepatocytes. The study of histological structures of $AlCl_3$ +Vit-E ((Fig.1C) showed normal hepatocytes lining of central vein, hepatocytes with normal nucleus and less atrophy of hepatocytes nucleus. The histomorphology of rats treated with $AlCl_3$ then MEAC (100 mg/kg) (Fig.1D) showed bleeding in the portal vein and atrophy of some hepatocytes. The histomorphology of rats treated with $AlCl_3$ then MEAC (200 mg/kg) (Fig.1 E) showed slight expansion of sinusoids and moderate atrophy. Rats receiving $AlCl_3$ then MEAC (400 mg/kg) (Fig1E) showed normal lining of the central vein and less atrophy

CONCLUSION

Acorus calamus has strong antioxidant activity and this activity may be responsible for the Hepatoprotective activity against Aluminium chloride induced liver damage. However, further studies on the active compounds and their biochemical mechanisms which may attribute to the Antioxidant and Hepatoprotective effects of *Acorus calamus* are necessary to be done.

Conflicts of Interest: The author declares that they have no conflicts of interest.

REFERENCES:

1. Inan-Eroglu and A. Ayaz, "Is aluminum exposure a risk factor for neurological disorders" Journal of Research in Medical Sciences 2018; 2: 1-51.
2. Al Kahtani, AM, Abdel-Moneim, MA, El-Sayed, MW (2014). The influence of taurine pretreatment on aluminum chloride induced nephrotoxicity in Swiss albino mice. Histopathology 2014;29: 45-55
3. Farina, M; Rotta, L N, Soares, F A. A, Jardim, F, Souza, DO, Rocha, J BT. Hematological changes in rats chronically exposed to oral aluminum. Toxicology 2005; 209(1):29-37
4. Mailloux, R J; Lemire, J, Appama, V D. Hepatic response to aluminum toxicity:

dyslipidemia and liver disease. Exp. Cell. Res 2011; 317(16): 2231-8.
5. Nan, Z, Ping, L, Lina, H. Protection of aluminum chelating agent against cardiac toxicity in rats exposed to aluminum chloride. J. Shandong Univ. (Health Sci.) 2009; 4(8): 125-7.
6. Schulze-Bergkamen H, Schuchmann, M, Fleischer, B, Galle, PR. The role of apoptosis versus oncotic necrosis in liver injury: facts or faith. Journal of Hepatology 2006; 44:984.
7. Lai XY, Liang H, et al. A survey of the studies on chemical constituents and pharmacological activities of Acorus plants. Zhongguo Zhong Yao Za Zhi 2002; 27: 198.
8. Maiti K, Mukherjee K, et al. Enhanced therapeutic benefit of quercetin-phospholipid complex in carbon tetrachloride induced acute liver injury in rats: a comparative study. Iranian Journal of .Pharmacology 2005; 4: 84–90.
9. Cesspooch L Native American Traditional Medicine and Diabetes: Acorus calamus L. A Sacred Medicinal Plant of the Native Cree 2005.
10. Mehrotra S, Mishra K, et al. Anticellular and immunosuppressive properties of ethanolic extract of Acorus Calamus rhizome. International Immunopharmacology 2003; 3: 53–61.
11. Shoba FG and Thomas M .Study of antidiarrhoeal activity of four medicinal plants in castor oil induced diarrhoea. Journal of Ethno pharmacology 2001; 76: 73 – 6.
12. Parab RS and Mengi SA. Hypolipidemic activity of Acorus Calamus L. in rats. Fitoterapia 2002; 73: 451–455.
13. A. Belfeld and D. Goldberg, "Revised assay for serum phenyl phosphatase activity using 4- amino - antipyrine," Enzyme Research 1971; 12(5): 561–573.
14. Schmidt, M, Eisenburg, J. Serum bilirubin determination in new born infants. A new micro method for the determination of serum of plasma bilirubin in new born infants. Fortschr. Med 1975; 93:1461-1466
15. Ellman, G L. Tissue sulfhydryl groups. Archives of Biochemistry and Biophysics 1959; 17: 214-226
16. H. Aebi, "Catalase in vitro," Methods in Enzymology 1984; 105: 121–126.
17. Rotruck, J.T., Pope, A.L. and Ganther, H.E., Swanson, A.B., Hafeman, D.G., and Hoekstra, W.G. Selenium: Biochemical role as a component of glutathione peroxidase. Science 1973; 179: 588-590.
18. Buege, J A, Aust, S D (1978). Microsomal lipid peroxidation. Methods Enzymology 1978; 52: 302-310.
19. Mavis, R.D. and Stellwagen, E. Journal of Biological Chemistry 1968; 243:809-814.