Chemo-Herbal Potentials of Fractionalized Extract of Mimosa pudica in Cadmium-Induced Hepatocellular Tumor with Associated Alpha-Fetoprotein and Gamma-Glutamyl Transferase Elevation

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SUMMARY

The plant Mimosa pudica (M. pudica) has been reported by researchers as an anti-inflammatory herb, amongst other properties, but its use as an anticancer herb is still sketchy. This study was aimed at evaluating the n-hexane, butanol and aqueous fractions of M. pudica leaf extract as a chemo-herbal therapy in cadmium-induced hepatocellular tumor. Forty-five (n = 45) adult rats of Sprague Dawley strain were used for this research. The rats were randomly assigned into nine different clusters (groups A-I), of five rats of Sprague Dawley strain each; hepatocellular tumor was induced using 0.4 mg/ml cadmium administered through drinking water to groups B–I for 50 days. M. pudica fractionalized extracts were administered orally at the dose of 25 mg/kg and 50 mg/kg to groups D and I for 14 days, respectively. Meanwhile, group C received 2.5mg/kg of Mesotheroxate (standard cancer drug) for 10 days. Histological slides for groups C-I showed a notable histomorphological improvement in liver tissue as well as markedly reduced degeneration when compared with the damage control group (group B). The AST, ALT, ALP, γGT and AFP levels in group B (285.30 ± 4.61 IU/l, 137.30 ± 12.72 IU/l, 424.70 ± 33.5 IU/l, 6.80 ± 0.26 IU/l and 1.82 ± 0.28 ng/ml, respectively) were significantly increased (p < 0.05) when compared to the control group values (123.30 ± 5.81 IU/l, 85.33 ± 2.40 IU/l, 253.70 ± 4.91 IU/l, 0.96 ± 0.35 IU/l and 0.37 ± 0.05 ng/ml) and other treated groups. This study reveals that M. pudica demonstrated some prospective anti-carcinogenic activity. Hence, it could be used as a potential chemo-herbal therapy.

Key words: chemo-herbal, hepatocellular tumor, Mimosa pudica, mitotically active cell, cancer

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INTRODUCTION

The use of herbal medicines by humans for treatment of various diseases predates written history. The major reason for cancer treatment is to achieve palliation where cure is out of reach. Research has shown that only 30% of all cancers are cured routinely. Plants with anticancer properties have been acknowledged for centuries now. The National Cancer Institute (NCI) has screened approximately 35,000 plant species for potential anticancer activities. Among them, about 3,000 plant species have demonstrated reproducible anticancer activity. Natural products are the most important anti-cancer agents and approximately three quarters of anti-tumor compounds used in medicine are natural products or related to them. Mimosa pudica (M. pudica) has a rapid movement and undergoes changes in the orientation of its leaf, which is called nyctinastic movement or "sleep". The foliage closes during darkness and reopens in light. The leaflets also close when stimulated by touch, warmth, shaking or blowing. Mimosa pudica has an anticancer alkaloid, and phytochemical studies of this plant reveal the presence of nor-epinephrine, α-pinitol, β-sitosterol, mimosine and 5,7,4-trihydroxyl 8-C-[alpha-L-rhamnopyranosyl-(2)-]-beta-D glucopyranosylflavone. Other researchers have reported on its antioxidant, antibacterial, antifungal, anti-inflammatory, hepatoprotective, antinoceptive, anticonvulsant, antidepressant, antidiarrheal, hypolipidemic activities, as well as diuretic, antiparasitic, antimalarial, and hypoglycemic which have properties to different parts of the plant and forms of use; how-ever, its use as an anticancer herb is still sketchy. Hence, this study was aimed at evaluating the fractionalized leaf extracts of M. pudica as a chemo-herbal therapy in cadmium-induced hepatocellular tumor.

MATERIAL AND METHODS

Animals

Forty-five adult Sprague Dawley rats weighing an average of 185 g were used for this study. The animals were obtained from the Department of Pharmacology, Faculty of Basic Medical Sciences, College of Health Sciences, Niger Delta University, Wilberforce Island, Bayelsa State of Nigeria. The Sprague Dawley rats were randomly distributed into nine clusters (groups A-I), 5 rats each, and were placed in their respective cages. The cages were made up of aluminum frame with metal net-tings, and a plastic base and a cover. The Sprague Dawley rats were allowed to acclimatize for one week. They were fed growers mash manufactured by Grand cereals limited, Jos, plateau state, Nigeria. Water was provided ad libitum. The Sprague Dawley rats were exposed to natural lighting condition (12-hour light-dark cycle) and room temperature, and were handled in accordance to standard protocols and guidelines for the care, treatment and use of laboratory animals for research (National Institute of Health).

Cadmium chloride

Cadmium chloride hemihydrate with batch number GB/T 1288-94 XK 13-201000130, CN: 61504 manufactured by Xilong Scientific cooperation limited, China was used to induce a hepatocellular tumor.

Plant collection, extraction and fractionalization

A bulk of fresh M. pudica leaves used for this study was collected in the month of June, 2017 from different axis of the Garden city, Rivers State, Nigeria, located between latitude 50 60 1 W and longitude 60 101 E. The plant was authenticated and deposited with voucher number NDUP210 at herbarium of the Department of Pharmacognosy and Herbal Medicine, in the above mentioned University. The leaves of M. pudica were dried in an oven for 300 C for 4 days, and thereafter pulverized to coarse powder weighing 893 g. The powder was macerated in ethanol for 72 hours. The concentrated ethanolic extracts were further subjected to partial fractionation with solvents of increasing polarity using n-hexane, butanol and aqueous fractions.

Experimental design

Forty-five (n = 45) adult male Sprague Dawley rats weighing an average of 185 g were randomly distributed into nine groups of five animals each. Group A served as a normal control, group B served as a damage control. Groups B-I were treated with cadmium chloride (0.4 mg/ml) for 50 days, after which group C was treated for 10 days with 2.5 mg of Mesotheroxate and groups D-I were treated using an oral gastric tube for 14 days. The layout of the treatment groups was as follows:

Group A - Control (water ad libitum)
Group B - CdCl2 0.4 mg/ml
Group C - CdCl2 0.4 mg/ml + Mesotheroxate (2.5mg)
Group D - CdCl2 0.4 mg/ml + N-Hexane fraction of M. Pudica (25mg/Kg)

Group E - CdCl2 0.4 mg/ml + N-Hexane fraction of M. Pudica (50mg/Kg)

Group F - CdCl2 0.4 mg/ml + butanol fraction of M. Pudica (25mg/Kg)

Group G - CdCl2 0.4 mg/ml + butanol fraction of M. Pudica (50 mg/Kg)

Group H - CdCl2 0.4 mg/ml + aqueous fraction of M. Pudica (25mg/Kg)

Group I - CdCl2 0.4 mg/ml + aqueous fraction of M. Pudica (50mg/Kg)

Biochemical studies

Animals from the different treatment groups were sacrificed and blood samples were collected immediately through the jugular vein and placed in plain sample bottles. The samples were allowed to clot for about 30 minutes and then centrifuged at 3000 rpm for 15 minutes. The resultant supernatants (serum) were aliquoted and stored in the refrigerator at -20°C for biochemical analysis of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and gamma-glutamyltransferase (γGT). These biochemical parameters were determined using SELETRA PRO S chemistry analyzer manufactured by ELITech Group.

The alpha-feto protein (AFP) was analyzed using the Accu®Bind Elisa Test system method for the determination of hepatocellular tumor. In 1996, Henry JB stated that AFP is the most useful marker in the diagnosis and management of hepatocellular carcinoma (15).

Histological study

The rats in group A and B were sacrificed after the 50th day, while rats in groups C-I were sacrificed after treatment on the 65th day, using chloroform inhalation method. The liver of each animal of control and treated groups were collected and fixation was carried out in 10% formal saline fixative for twenty-four hours. The tissues were processed through dehydration, clearing and impregnation, using automatic tissue processor – Histokinet (LEICA TP 1020). The tissues were embedded in paraffin wax in tissue embedder (LEICA EG 1160), trimmed and sectioned in a rotary microtome (LEICA RM 2125 RTS) with gauge set at 20 and 5 microns thickness, respectively. The tissues were further attached to grease free slides and afterward dewaxed in xylene and stained using haematoxylin and eosin to demonstrate the general tissue structure (16). The stained slides were examined using a bright field light compound microscope at x 100 magnifications.

Statistical analysis

The data obtained were analyzed using one-way analysis of variance (ANOVA) and the means (± standard error of mean) were compared with each other using Dunnett’s Multiple Comparison Test. The probability value of 0.05 was considered significant. Statistical analysis were performed using the JMP statistical discovery™ software version 12.1 (SAS Institute, Cary, NC, USA).

RESULTS

Biochemical parameters

The biochemical parameters of the experimental animals by treatment group is given in Table 1. AST was significantly increased (p < 0.05) in groups B, F and H (285.30 ± 4.61 IU/l, 206.00 ± 40.38 IU/l and 211.30 ± 16.90 IU/l), respectively, when compared to the control group A (123.30 ± 5.81 IU/l). However, there was no significant (p > 0.05) difference between groups C, D, E, G and I (173.70 ± 8.99 IU/l, 170.00 ± 29.50 IU/l, 158.00 ± 13.65 IU/l, 162.00 ± 11.14 and 160.00 ± 21.59 IU/l, respectively) and the control group (123.30 ± 5.81 IU/l). Although ALT increased significantly (p < 0.05) in group B (137.30 ± 12.72 IU/l) when compared to the control group (85.33 ± 2.40 IU/l), we observed no significant (p > 0.05) difference in ALT in groups C-I (93.67 ± 2.40 IU/l, 67.33 ± 10.35 IU/l, 58.33 ± 10.93 IU/l, 76.33 ± 6.17 IU/l, 70.67 ± 5.20 IU/l, 81.67 ± 10.53 IU/l and 73.33 ± 1.76 IU/l, respectively) and the control group. Similarly, ALP (424.70 ± 33.53 IU/l) and γGT (6.80 ± 2.6 IU/l) also increased significantly (p < 0.05) in group B, while other groups (C-I) [ALP (279.30 ± 52.19 IU/l, 259.30 ± 8.68 IU/l, 252.70 ± 6.64 IU/l, 239.70 ± 14.88 IU/l, 247.70 ± 24.44 IU/l, 263.00 ± 13.01 IU/l and 225.70 ± 20.02 IU/l, respectively) and γGT (5.76 ± 0.31 IU/l, 1.06 ± 0.37 IU/l, 3.07 ± 2.96 IU/l, 0.57 ± 0.33 IU/l, 0.46 ± 0.38 IU/l, 1.06 ± 0.21 IU/l and 0.66 ± 0.27 IU/l, respectively] were not significant (p > 0.05) when compared with the controls (253.70 ± 4.91 IU/l and 0.96 ± 0.35 IU/l), but were significant (p < 0.05) when compared to group B.

Graph 1 shows the alpha feto protein in the experimental animals by treatment group. In the same vein, serum alpha feto protein was increased in group B (1.82 ± 0.28 ng/ml) when compared to the control (0.37 ± 0.05 ng/ml) and other treatment groups C-I (0.54 ± 0.23 ng/ml, no.05)
0.34 ± 0.14 ng/ml, 0.12 ± 0.03 ng/ml, 0.27 ± 0.08 ng/ml, 0.19 ± 0.04 ng/ml, 0.24 ± 0.03 ng/ml and 0.28 ± 0.07 ng/ml).

**Histopathological studies**

Figure 1 shows the photomicrograph of the liver of group A (control) with normal central vein, with well radiating hepatocytes and sinusoids. Group B representative slide is characterized by mixed inflammatory and mitotically active cells and periportal necrosis. Groups C, D, E and F show heavy infiltration of inflammatory cells, while group H shows degenerating and necrotic liver stroma. However, groups G and I are similar to the control.

**Table 1. Biochemical parameters of experimental animals by treatment group**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>AST (IU/l) Mean ± SEM</th>
<th>ALT (IU/l) Mean ± SEM</th>
<th>ALP (IU/l) Mean ± SEM</th>
<th>γGT(IU/l) Mean ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>123.30 ± 5.81ab</td>
<td>85.33 ± 2.40a</td>
<td>253.70 ± 4.91a</td>
<td>0.96 ± 0.35a</td>
</tr>
<tr>
<td>Group B</td>
<td>285.30 ± 4.61b</td>
<td>137.30 ± 12.72b</td>
<td>424.70 ± 33.53b</td>
<td>6.80 ± 0.26b</td>
</tr>
<tr>
<td>Group C</td>
<td>173.70 ± 28.99a</td>
<td>93.67 ± 2.40a</td>
<td>279.30 ± 52.19a</td>
<td>5.76 ± 0.31a</td>
</tr>
<tr>
<td>Group D</td>
<td>170.00 ± 29.50a</td>
<td>67.33 ± 10.35a</td>
<td>259.30 ± 8.68a</td>
<td>1.06 ± 0.37a</td>
</tr>
<tr>
<td>Group E</td>
<td>158.00 ± 13.65a</td>
<td>58.33 ± 10.93a</td>
<td>252.70 ± 6.64a</td>
<td>3.07 ± 2.96a</td>
</tr>
<tr>
<td>Group F</td>
<td>206.00 ± 40.38ab</td>
<td>76.33 ± 6.17a</td>
<td>239.70 ± 14.88a</td>
<td>0.57 ± 0.33a</td>
</tr>
<tr>
<td>Group G</td>
<td>162.00 ± 11.14a</td>
<td>70.67 ± 5.20a</td>
<td>247.70 ± 24.44a</td>
<td>0.46 ± 0.38a</td>
</tr>
<tr>
<td>Group H</td>
<td>211.30 ± 16.90ab</td>
<td>81.67 ± 10.53a</td>
<td>263.00 ± 13.01a</td>
<td>1.06 ± 0.21a</td>
</tr>
<tr>
<td>Group I</td>
<td>160.00 ± 21.59a</td>
<td>73.33 ± 1.76a</td>
<td>225.70 ± 2.02a</td>
<td>0.66 ± 0.27a</td>
</tr>
</tbody>
</table>

SEM=Standard error of mean; within each parameter, means ± SEM with different superscripts (a, b and ab) are significantly different at p < 0.05. Treatment groups: Group A = Normal control; Group B = Damage control (Cadmium chloride); Group C= Methotrexate; Group D= N-Hexane fraction (25mg/kg); Group E= N-Hexane fraction (50mg/kg); Group F= Butanol fraction (25mg/kg); Group G= Butanol fraction (50mg/kg); Group H=Aqueous fraction (25mg/kg); Group I = Aqueous fraction (50mg/kg).

**Graph 1. Alpha-feto protein (ng/ml) in experimental animals by treatment group**
Group A (control) shows normal central vein (CV), with well-radiating hepatocytes (dash arrow) and sinusoids (bold arrow).

Group B is characterized by mitotically active cells, inflammatory cells, and periportal necrosis. However, groups C, D, E, and F show heavy infiltration of inflammatory cells (IC).

Group H shows degenerating and necrotic liver stroma (black circle), while groups G and I are similar to the control (group A). H&E x100 magnification.

**Figure 1. Photomicrograph of the liver of experimental animals by treatment group**

**DISCUSSION**

The present study showed the potentials of fractionalized extract of *M. pudica* in ameliorating the hepatocellular tumor induced by cadmium chloride in adult male Sprague Dawley rats. There was a significant (p < 0.05) elevation in the values of serum AST, ALT, ALP, and γGT in group B when compared to the control group (Table 1). Increased values of serum AST and ALT have been attributed to any condition involving necrosis of hepatocytes, while increased serum ALP occur as a result of hepatobiliary diseases characterized by
intra- or extrahepatic cholestasis among others. However, γGT are markedly increased in lesions which cause intrahepatic and/or extrahepatic obstruction of bile ducts, including liver diseases with a major cholestatic component. Lesser elevations of gamma-GT maybe seen in other liver diseases, while drugs causing hepatocellular degeneration and cholestasis may elevate the gamma-GT serum level (17). Our result is similar to that of Renugadevi and Prabu (18) and Kang et al. (19) where all liver specific enzymes were significantly elevated when induced with cadmium. The elevated enzyme reported in our current study corroborated with the histological findings of the hepatocellular tumor, which were basically characterized by mitotically active cells, inflammatory cells and periportal necrosis.

Once there is a damage to the liver, the injured endothelial cell obstruct capillary lumen and produce ischemia which may then initiate a number of molecular and cellular events that result in a subsequent activation of Kupffer cells, release of inflammatory mediators and recruitment of inflammatory cells, mainly polymorphonuclear neutrophils (PMN) and leukocytes (20). In the liver, the inflammatory cells accumulate in the sinusoid and adhere to endothelial cells (20, 21). Our result was further validated by a significant elevation of AFP tumor marker. Alpha fetoprotein is a marker of hepatocellular carcinoma and germ cell carcinoma (22). It has been reported to be the most useful in determining prognosis, diagnosis and management of hepatocellular carcinoma (22, 15). Elevated AFP levels indicate the presence of metastasis. Falling or rising AFP levels after therapy may determine the success or failure of the treatment regimen. A significant increase of AFP in patients considered free of metastatic tumor may indicate the development of metastasis (22).

Studies have revealed that cadmium exerts its toxic effects via oxidative damage to cellular organelles by inducing the generation of reactive oxygen species (ROS) (23, 24). ROS are capable of causing damage to biomolecules, including proteins, lipids, and nucleic acids, leading to cell and tissue injury. However, the molecular mechanism of this process is not fully understood, but reports have shown that cadmium does it via an indirect phenomenon (25).

The level of serum liver specific enzymes (AST, ALT, ALP and γGT), hepatocellular carcinoma and germ cell carcinoma tumor marker (AFP) in the groups treated with different fractions of M. pudica leaf extract decreased significantly, especially with the high doses (50mg/kg) of n-hexane, butanol and aqueous fractions. These findings were supported by the histological architecture of slides G and I (Figure 1), which were similar to the control in haematoxylin-stained sections when viewed under a light compound microscope. A significant decrease in the biochemical parameters level and the histological findings of our present study support previous conclusions (26-31) that M. Pudica has hepatoprotective and antioxidant effects. The hepatoprotective effect of M. Pudica has been attributed to the activities of its constituents like alkaloid, tannins, glycosides, terpenoids, flavonoids and saponins (31). Mimosine, which is a phytochemical component of M. Pudica, known to be a tyrosine, has the ability to chelate transition metals, such as Fe(3+) (32) resulting in iron deficiency, which in turn could alter folate metabolism in mammals and interfere with tumor cell growth (33). Thus, these responses tend to inhibit an intense mitotic activity of cancer cells (34, 35).

**CONCLUSION**

The histological and biochemical findings of the present research demonstrated that n-hexane, butanol and aqueous fractions of M. pudica may have some anti-carcinogenic activities, which was evident in the improvement of experimentally induced hepatocellular tumor in Sprague Dawley rats, decreasing the serum level of increased alpha-feto protein and gamma-glutamyl transferase. It is therefore recommended that a detailed molecular research on its mechanism of action, chemical constituent(s) and safe dose be carried out.
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Potencijal frakcionisanog ekstrakta biljke *Mimosa pudica* kao biljnog hemoterapeutika kod hepatocelularnog tumora izazvanog kadmijumom sa povišenim vrednostima alfa-fetoproteina i gama-glutamila transferaze

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**SAŽETAK**

Istraživači su do sada opisali da biljka *Mimosa pudica* (*M. pudica*) poseduje i antiinflamatorna svojstva, dok su njena antikancerogena svojstva još uvek nedovoljno ispitana. Cilj ove studije bio je ispitivanje heksanske, butanolne i vodene frakcije ekstrakta lista *M. pudica* kao biljnog hemoterapeutika kod hepatocelularnog tumora izazvanog kadmijumom. Za potrebe ovog istraživanja korišćeno je 45 odraslih pacova soja Sprague Dawley. Pacovi su nasumično podeljeni u devet grupa (grupe A do I), pri čemu se svaka grupa sastojala od pet pacova. Hepatocelularni tumor bio je izazvan primenom kadmijuma u dozi od 0,4 mg/ml, kroz pijacu vodu, u grupama B do I, u trajanju od 50 dana. Frakcionisani ekstrakti biljke *M. pudica* davani su oralno u dozi od 25 mg/kg i 50 mg/kg grupama D i I u trajanju od 14 dana. U međuvremenu, grupa C primila je 2,5mg/kg metoteroksata (uobičajeni lek za lečenje kancera) u narednih deset dana. Histološki slajdovi za grupe C do I pokazali su značajni histomorfološki pomak u tkivu jetre, kao i značajno smanjenje degeneracije u poređenju sa kontrolnom grupom (grupa B). Nivoi AST, ALT, ALP, γGT i AFP u grupi B (285,30 IU/l ± 4,61 IU/l, 137,30 IU/l ± 12,72 IU/l, 424,70 IU/l ± 33,5 IU/l, 6,80 IU/l ± 0,26 IU/l i 1,82 ng/ml ± 0,28 ng/ml) bili su značajno povećani u poređenju sa vrednostima kontrolne (123,30 IU/l ± 5,81 IU/l, 85,33 IU/l ± 2,40 IU/l, 253,70 IU/l ± 4,91 IU/l, 0,96 IU/l ± 0,35 IU/l i 0,37 ng/ml ± 0,05 ng/ml) i drugih tretiranih grupa. Ova studija je pokazala da biljka *Mimosa pudica* poseduje moguća antikancerogena svojstva. Stoga se može koristiti u potencijalnoj biljnoj hemoterapiji.

**Ključne reči:** biljni hemoterapeutik, hepatocelularni tumor, *Mimosa pudica*, mitotički aktivna ćelija, kancer