



Contents lists available at ScienceDirect

Journal of Traditional and Complementary Medicine

journal homepage: <http://www.elsevier.com/locate/jtcm>

Original article

Hepatoprotective potential of ethanolic extract of *Aquilaria agallocha* leaves against paracetamol induced hepatotoxicity in SD rats



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ARTICLE INFO

Article history:

Received 28 September 2015

Received in revised form

25 December 2015

Accepted 30 December 2015

Available online 3 February 2016

Keywords:

Aquilaria agallocha

Hepatoprotective potential

Oxidative stress

Paracetamol

Hepatotoxicity

ABSTRACT

Many traditional systems of medicines employ herbal drugs for the hepatoprotection. Aim of the study was designed to evaluate the hepatoprotective potential of 'ethanolic extract of *Aquilaria agallocha* (沉香 *Chen Xiang*) leaves' (AAE) against paracetamol (PCM) induced hepatotoxicity in SD rats. Group I animals were treated with 1% CMC for 8 days. Group II, III, IV and V animals were first treated with '1% CMC' 1 ml/kg/day, AAE 200 mg/kg/day, AAE 400 mg/kg/day and silymarin 100 mg/kg/day respectively for 7 days and then, orally administered with PCM 3 g/kg b. wt. on 8th day in a single dose. 24 h after the last dosing by PCM, the blood was obtained through the retro-orbital plexus under light anesthesia and the animals were sacrificed. Hepatoprotective potential was assessed by various biochemical parameters such as ALT, AST, ALP, LDH, bilirubin, cholesterol, TP and ALB. Group IV rats showed significant ($p < 0.01$) decrease in ALT, AST, ALP, LDH, cholesterol, bilirubin, liver wt. and relative liver wt. levels while significant ($p < 0.01$) increase in final b. wt., TP and ALB levels as compared to group II rats. Hepatoprotective potential of AAE 400 mg/kg/day was comparable to that of standard drug silymarin 100 mg/kg/day. Results of the study were well supported by the histopathological observations. This study confirms that AAE possesses hepatoprotective potential comparable to that of standard drug silymarin as it exhibited comparable protective potential against PCM induced hepatotoxicity in SD rats.

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1. Introduction

Aquilaria agallocha (沉香 *Chen Xiang*) belonging to the family thymelaeacea is known as Agarwood in English, Agar in Hindi and Aguru in Sanskrit.¹ Agarwood has been utilized for varied purposes all over the world for thousands of years. Its utilization has been reported in Ayurveda, Tibetan and traditional East-Asian medical practice including *Susruta Samhita* and *Shahih Muslim*.^{2,3} Traditionally the bark, root and leaves of the heartwood of Agarwood are used for their medicinal properties like aphrodisiac, anodyne, acrid, astringent, aromatic, cardiogenic, bitter, carminative, fragrant and stimulant. Agarwood is also used as appetizer, carminative and mouth freshener and used to treat colic pain, toothache, severe pains and headache during the pregnancy.⁴

Agar is a pathological product formed by fungal attack of the host.⁵ Phytochemical constituents obtained from the Agarwood are agarol, aquillochin; agarospiranes (sesquiterpenes): agarospirol, baimuxinic acid, baimuxinal, vetaspira-2(11)-6-dien-14-al, vetaspira-2(11),6(14)-dien-7-ol, 2,14-epoxyvestipir-6(14)-7-diene, 2,14-epoxyvestispir-6-ene, 11-hydroxyspirovetiv-1(10)-en-2-one; agarofurans (sesquiterpenes): baimuxifuranic acid, dihydro-3,4-dihydroxyagarofuran, norketoagarofuran, dihydro-4-hydroxyagarofuran, dihydroagarofuran, β -agarofuran, α -agarofuran, epoxy- β -agarofuran, selina-4,11-dien-14-oic acid, selina-3,11-dien-14-al, neopetasane and dehydrojinkoheremol.^{6–15} The most remarkable bioactive constituents of Agarwood are alkaloids, steroids, saponins, tannins, terpenoids, flavonoids and phenolic compounds.¹⁶

The plant *A. agallocha* has several pharmacological activities and shows antioxidant, antidiabetic, analgesic, antipyretic, anti-inflammatory, antihistaminic, laxative, anticancerous, antidiarrhoeal, antidiabetic, antihistaminic, antimicrobial, antibacterial, anxiolytic, sedative effect, anticonvulsant and ulcer protective

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Peer review under responsibility of The Center for Food and Biomolecules, National Taiwan University.

activities.¹⁷ Hence, present study was undertaken to evaluate the hepatoprotective potential of ethanolic extract of *A. agallocha* leaves against paracetamol (PCM) induced hepatotoxicity in SD rats, as the drugs having antioxidant and antiulcer activities can show hepatoprotective activity too.

2. Materials and methods

2.1. Chemicals and instrumentation

All the chemicals used were of analytical grade, silymarin (Sigma chemicals, USA), paracetamol (SD fine chemical Ltd Mumbai, India), ALT test kits (Span Diagnostics Ltd Surat), AST test kits (Span Diagnostics Ltd Surat), LDH (UV kinetics Accurex Biochemical Pvt Ltd., Mumbai), albumin test kit (Span Diagnostics Ltd Surat), bilirubin test kit (Span Diagnostics Ltd Surat), alkaline phosphatase test kits (Merk Specialities Ltd Mumbai), total protein test kit (Span Diagnostics Ltd Surat), mini centrifuge (Spinwell), UV spectrophotometer (PharmaSpec UV-1700, Shimadzu).

2.2. Collection and authentication of the plant specimen

A. agallocha (沉香 Chen Xiang) leaves were procured from Assam Aromas (police line, GRB road, Fauzdaripatty, Nagaon-782001, Assam) and authenticated by the botanists, authentication office, Faculty of Pharmacy, Integral University Lucknow (ref. no.: IU/PHAR/HRB/15/22). A voucher specimen was deposited to authentication office for future reference.

2.3. Preparation of plant extract and evaluation of percentage yield

The dried leaves were subjected to size reduction to a coarse powder with the help of grinder and powdered material was then subjected to Soxhlet extraction employing ethanol as solvent. The extract was concentrated to dryness with the help of rotavapor and finally air dried thoroughly to remove all traces of the solvent. The obtained dried extract (AAE) was weighed and extractive value was calculated. It was kept in an air tight container in a refrigerator below 4 °C and used for pharmacological investigation.¹⁸

2.4. Experimental animals

Sprague-Dawley (SD) rats (150–200 g) were procured from Animal House, Central Drug Research Institute (CDRI) Lucknow and kept in departmental animal house, Integral University Lucknow (IUL). All the rats were housed separately in polypropylene cages at the 50–60% relative humidity and temperature of 23 ± 2 °C with a 12 h light/dark cycle for seven days before and during the commencement of the experiment. The rats were kept on standard pellet diet and drinking water throughout the housing period. All the experiments were conducted in accordance with the guidelines 'Committee for the Purpose of Control and Supervision' (CPCSEA) and ethical clearance was obtained from Institutional Animal Ethics Committee (IAEC) of Faculty of Pharmacy, Integral University Lucknow (Approval No.: IU/PHARM/IAEC/15/06).

2.5. Experimental protocol

The animals were randomly divided into five groups each consisting of five rats ($n = 5$). Group I animals were treated with vehicle (1% CMC) for 8 days and served as normal control. Group II animals were first treated with vehicle for 7 days and then, hepatotoxicity was tried to induce on 8th day by oral administration of PCM 3 g/kg b. wt. in a single dose.¹⁹ Group III, IV and V animals were first treated with AAE 200, AAE 400 and silymarin 100 mg/kg/day

respectively for 7 days and then, hepatotoxicity was tried to induce on 8th day by oral administration of PCM 3 g/kg b. wt. in a single dose. 24 h after the last dosing by PCM, the blood was obtained through the retro-orbital plexus under light anesthesia and the rats were sacrificed. Serum for the analysis of various biochemical parameters was separated by centrifugation at 3000 rpm at 4 °C for 20 min. The isolated liver tissues were washed twice with ice cold saline, blotted, dried and then weighed of each group. The relative liver weight was calculated as percentage ratio of liver weight to body weight. A small portion of tissue was fixed in formalin for histopathological examination.

2.6. Assessment of liver function test

Serum alanine transaminase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), bilirubin, cholesterol, albumin (ALB) and total Protein (TP) were estimated by using standard kits from Span Diagnostic Ltd Surat, India. Serum lactate dehydrogenase (LDH) was estimated by using standard kits from Accurex biochemical Pvt Ltd, Mumbai, India. All the enzymatic estimations were assessed as per standard kit methods using UV spectrophotometer and the standard kit methods were obtained in detail from the leaflets provided in the commercially kits.

2.7. Histopathological examination

Slices of liver were stored in 10% neutral formalin solution to preserve them. The tissues were mounted by embedding in paraffin wax in the laboratory and sections of the size of 6 μm were cut. The sections were stained with eosin and haematoxylin dyes. The slides were observed under light microscope and photomicrographs were captured by using camera. These were observed for fibrosis, fatty infiltration, centrilobular necrosis and lymphocyte infiltration.²⁰

2.8. Statistical analysis

The data was expressed as mean ± standard error of mean (mean ± SEM). Student's 't' test was followed by individual comparison by Newman-Keuls test using graph pad prism software for determination of level of significance. The value of probability less than 5% ($p < 0.05$) was considered statistically significant.

3. Results

The percentage yield was found to be 23.29% w/w.

Effects of AAE on different liver specific variables in control and experimental groups of animals are shown in Table 1. PCM treatment significantly ($p < 0.01$) increased the relative liver weight to $5.52 \pm 0.51/100$ g body weight (b. wt.) as compared to the normal control (group I) with that of $3.12 \pm 0.81/100$ g b. wt. Administration of AAE 200 and 400 mg/kg significantly (AAE 200 mg/kg, $p < 0.05$ and AAE 400 mg/kg $p < 0.01$) reduced the relative liver weight to 3.57 ± 0.27 and $2.95 \pm 0.35/100$ g b. wt. respectively as compared to PCM treatment with that of $5.52 \pm 0.51/100$ g b. wt. AAE treated group IV was more close to standard silymarin treated group V rats at the concentration used. The rats treated with AAE 400 mg/kg exhibited significant change ($p < 0.01$) in the final b. wt. and relative liver wt. as compared to group II rats.

PCM treated group II rats showed increased serum AST (315.78 ± 0.99 U/L, $p < 0.01$), ALT (179.41 ± 8.0 U/L, $p < 0.01$), ALP (199.13 ± 1.60 U/L, $p < 0.01$) and LDH (701.32 ± 2.77 U/L, $p < 0.01$) as compared to normal control group I rats (93.26 ± 0.58 U/L, 42.06 ± 1.77 U/L, 65.83 ± 1.26 U/L and 344.60 ± 0.60 U/L respectively). The AAE treated groups III and IV significantly decreased AST (210.68 ± 1.128 U/L, $p < 0.05$ and 136.75 ± 1.89 U/L, $p < 0.01$)

Table 1Effects of ethanolic extract of *A. agallocha* leaves (AAE) on different liver specific variables in control and experimental groups of animals.

Treatment groups and liver specific variables	I (Normal control: 1% CMC 1 ml/kg b. wt.)	II (Hepatotoxic control: 1% CMC 1 ml/kg b. wt.+PCM 3 g/kg b. wt.)	III (AAE 200 mg/kg b. wt.+PCM 3 g/kg b. wt.)	IV (AAE 400 mg/kg b. wt.+PCM 3 g/kg b. wt.)	V (Silymarin 100 mg/kg b. wt.+PCM 3 g/kg b. wt.)
AST (U/L)	93.26 ± 0.58	315.78 ± 0.99 [#]	210.68 ± 1.128 [*]	136.75 ± 1.89 ^{**}	99.08 ± 2.57 ^{**}
ALT (U/L)	42.06 ± 1.77	179.41 ± 8.0 [#]	91.46 ± 2.027 [*]	48.25 ± 4.961 ^{**}	49.79 ± 1.98 ^{**}
ALP (U/L)	65.83 ± 1.26	199.13 ± 1.60 [#]	102.04 ± 2.86 [*]	89.27 ± 1.18 ^{**}	76.42 ± 3.08 ^{**}
LDH (U/L)	344.60 ± 0.60	701.32 ± 2.77 [#]	533.18 ± 28.34 [*]	439.14 ± 27.73 ^{**}	376.16 ± 7.63 ^{**}
Bilirubin (mg/dl)	0.19 ± 0.007	1.17 ± 0.069 [#]	1.072 ± .059 [*]	0.41 ± .004 ^{**}	0.21 ± 0.109 ^{**}
Cholesterol (mg/dl)	41.06 ± 4.98	77.2 ± 4.06 [#]	60.4 ± 2.85 [*]	52.4 ± 3.18 ^{**}	39.1 ± 3.40 ^{**}
ALB (g/dl)	4.04 ± 0.02	2.19 ± 0.07 [#]	3.50 ± 0.16 [*]	4.08 ± 0.06 ^{**}	4.11 ± 0.05 ^{**}
TP (g/dl)	6.21 ± 0.265	3.57 ± 0.13 [#]	4.63 ± .072 [*]	5.07 ± 0.40 ^{**}	5.71 ± 0.13 ^{**}
Initial b. wt. (g)	180 ± 5.99	163.8 ± 5.19	180 ± 4.04	199 ± 3.13	193.2 ± 1.62
Final b. wt. (g)	205 ± 10.92	157 ± 5.90 [#]	190.2 ± 4.09 [*]	210 ± 7.24 ^{**}	229.2 ± 6.68 ^{**}
Liver wt. (g)	6.09 ± 0.32	8.64 ± 0.62 [#]	6.98 ± 0.59 ^{ns}	6.21 ± 0.23 ^{**}	6.08 ± 0.40 ^{**}
Relative liver wt. (Liver wt./100 g b. wt.)	3.12 ± 0.08	5.52 ± 0.51 [#]	3.57 ± 0.27 [*]	2.95 ± 0.35 ^{**}	2.54 ± 0.092 ^{**}

Values were expressed as mean ± SEM ($n = 5$), where, [#] indicates $p < 0.01$ as compared with respective control group I; ^{ns} indicates $p > 0.05$, ^{*} $p < 0.05$, ^{**} $p < 0.01$ as compared with respective group II.

ALT (91.46 ± 2.027 U/L, $p < 0.05$ and 48.25 ± 4.961 U/L, $p < 0.01$), ALP (102.04 ± 2.86 U/L, $p < 0.05$ and 89.27 ± 1.18 U/L, $p < 0.01$) and LDH (533.18 ± 28.34 U/L, $p < 0.05$ and 439.19 ± 27.73 U/L, $p < 0.01$ respectively) as compared to group II. Silymarin at 100 mg/kg significantly ($p < 0.01$) declined the elevated levels of AST, ALT, ALP and LDH to the levels of 99.08 ± 2.57 U/L, 49.79 ± 1.98 U/L, 76.42 ± 3.08 U/L and 376.16 ± 7.84 U/L respectively as compared to PCM treated group II.

PCM treated group II showed significantly ($p < 0.01$) decreased serum ALB (2.19 ± 0.07 g/dl) and TP (3.57 ± 0.13 g/dl) while, increased bilirubin (1.17 ± 0.069 mg/dl) and cholesterol (77.2 ± 4.06 mg/dl) as compared to control group I (4.04 ± 0.02 g/dl, 6.21 ± 0.265 g/dl, 0.19 ± 0.007 mg/dl and 41.06 ± 4.98 mg/dl respectively). The AAE treated groups III and IV significantly increased serum ALB (3.50 ± 0.16 , $p < 0.05$ and 4.08 ± 0.06 g/dl, $p < 0.01$) and TP ($4.63 \pm .072$, $p < 0.05$ and 5.07 ± 0.40 g/dl, $p < 0.01$) while, decreased bilirubin ($1.172 \pm .059$, $p < 0.05$ and $0.41 \pm .004$ mg/dl, $p < 0.01$) and cholesterol (60.4 ± 2.85 , $p < 0.05$ and 52.4 ± 3.18 mg/dl, $p < 0.01$ respectively) as compared to group II. Silymarin at 100 mg/kg significantly ($p < 0.01$) increased the ALB (4.11 ± 0.05 g/dl) and TP (5.71 ± 0.13 g/dl), while decreased the

bilirubin (0.21 ± 0.109 mg/dl) and cholesterol (39.1 ± 3.40 mg/dl) as compared to PCM treated group II.

The effects of AAE on the gross anatomy of liver are shown in Fig. 1 and on histopathology of liver in Fig. 2.

4. Discussion

Paracetamol (PCM) is an analgesic and antipyretic drug which, when taken in at toxic doses, becomes a potent hepatotoxic substance liberating fulminated renal tubular and hepatic necrosis lethal to experimental animals and humans.^{21,22} Its overdose can cause liver function failure, centrilobular hepatic necrosis and even death in experimental animals as well as human.²³ The laboratory features of PCM induced hepatotoxicity is similar to other kinds of acute inflammation and liver ailment with major increase of AST, ALP, ALT, LDH, cholesterol, bilirubin and decrease of ALB and TP.²⁴ Results of the study clearly demonstrated that the serum level of hepatic enzymes AST, ALP, ALT, LDH, cholesterol, bilirubin were increased, and ALB, TP were decreased in the present study, reflecting the hepatocellular damage in the PCM induced hepatotoxicity in animal model.

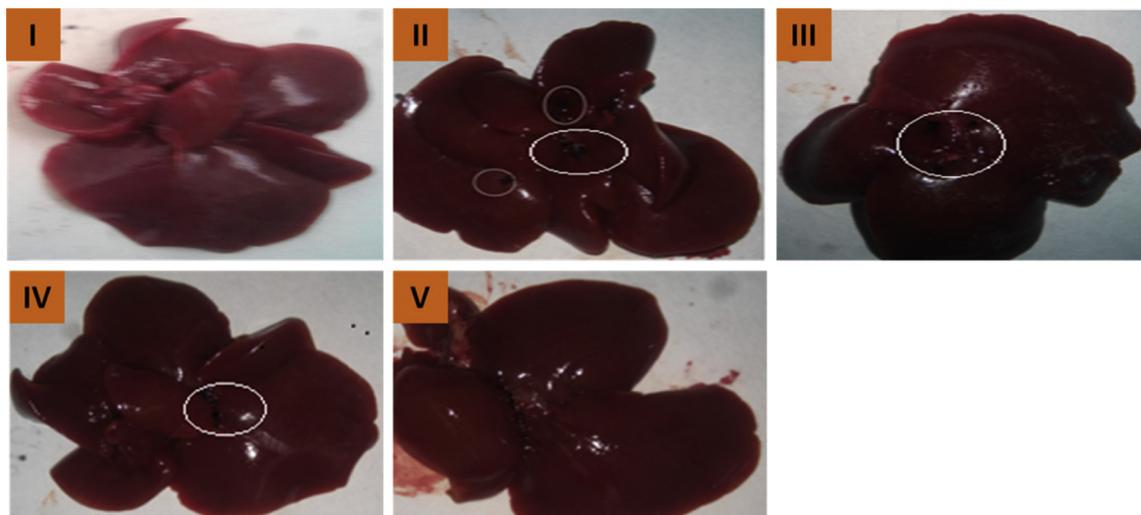


Fig. 1. Gross anatomy of liver in control and experimental groups of rats: Group I is with normal appearance. Marks in group (II) indicated damage of liver cells. Marks in rest of the groups (III, IV and V) indicated recovery of damage cells towards normal.

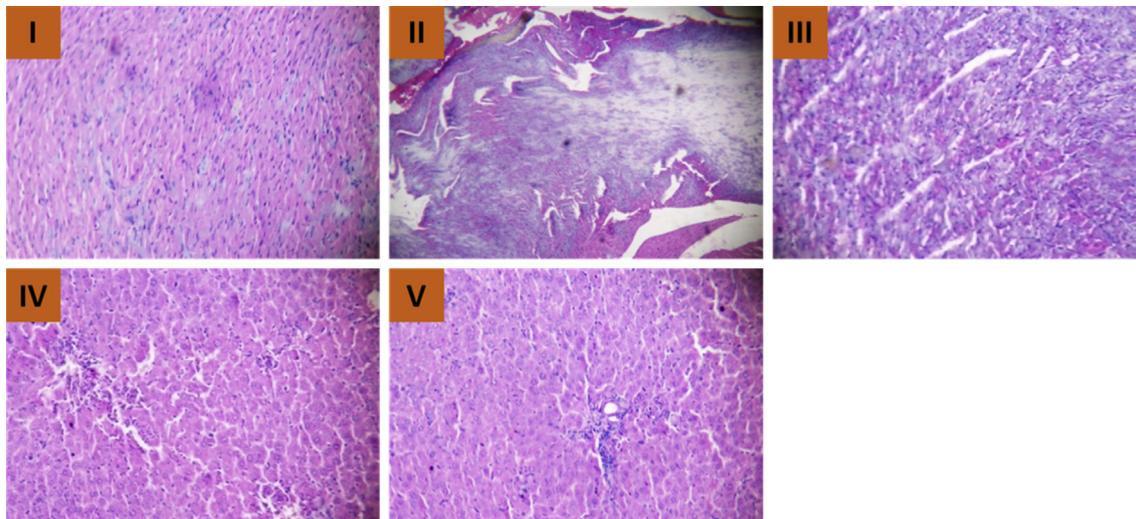


Fig. 2. Histopathology of liver tissues in control and experimental groups of rats. (I) Normal control group: showed the well defined architecture and vesicular nuclei. (II) Hepatotoxic group: showed degenerative changes in hepatocytes and necrosis occurred. (III) Group treated with AAE 200 mg/kg: showed proliferative hepatocytes with mild degenerative changes and increased inter cellular space. (IV) Group treated with AAE 400 mg/kg: showed normal architecture and well defined nuclei when compared with group I. (V) Group treated with silymarin 100 mg/kg: section showed normal architecture with well defined nucleus.

The ethanolic extract of *A. agallocha* leaves (AAE) screened for acute toxicity according to OECD (Organization for Economic Co-operation and Development) 423 guidelines upto the dose of 2000 mg/kg b. wt. was found to be non-toxic and safe.²⁵ Hence, two dose levels of as low 200 mg/kg and high 400 mg/kg of AAE were selected here for the experiment.

AAE at the doses of 200 and 400 mg/kg significantly ($p < 0.05$ to $p < 0.01$) lowered the AST, ALP, ALT, LDH, cholesterol and bilirubin in these PCM intoxicated rats. The results of liver function tests were correlated to the histopathological changes from photomicrographs taken. The centrilobular hepatic necrosis, cell degeneration and infiltrating lymphocytes were well displayed in PCM intoxicated group II. Treatment with AAE prevented these PCM induced histopathological changes. These results suggested that the inhibition of elevated hepatic damage and hepatic function markers may participate in the protective effect of the AAE against PCM induced hepatotoxicity.

The hepatoprotection by AAE may be due to antioxidant property of the phytochemicals present in AAE which reduce the oxidative stress imposed by PCM and other like anti-inflammatory and analgesic properties preventing the inflammatory hepatic damage.^{26,27}

5. Conclusion

Ethanolic extract of *A. agallocha* leaves (AAE) possesses hepatoprotective effect as it exhibited protective effect against paracetamol induced hepatotoxicity in SD rats demonstrated by significant decrease in AST, ALT, ALP, LDH, cholesterol, bilirubin and increase in ALB, TP concentration, and prevention of PCM induced histopathological changes in liver.

Conflicts of interest

None to declare.

Acknowledgements

Authors are thankful to the Faculty of Pharmacy, Integral University for providing all the necessary facilities related to the present research work.

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