International Journal of Pharmacology

ISSN 1811-7775

science alert

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Asian Network for Scientific Information

OPEN ACCESS International Journal of Pharmacology

ISSN 1811-7775
DOI: 10.3923/ijp.2019.837.843

Research Article
Synergistic Effect of Asiatic Acid and Madecassic Acid against Antioxidant Deficit in Rat Peripheral Nervous System

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Abstract

Background and Objective: Epidemiological studies have shown admiring protective roles of phytochemicals on peripheral nervous system, while the prevalent use of hypotensive drugs directly impacts normal functioning of the nervous system. Hence, the present study was assessed the synergistic role of Asiatic Acid (AA) and Madecassic Acid (MA) against the antioxidant deficit induced by the metoprolol tartrate. **Materials and Methods:** Wistar male rats (150 mg kg^{-1}) were divided into 4 groups. Group 1 as control with no treatment, group 2 rats with metoprolol tartrate (150 mg kg^{-1}/day, orally), group 3 rats pre-treated with AA (50 mg kg^{-1}, IP) and MA (30 mg kg^{-1}, IP) for 2 weeks prior to metoprolol tartrate and group 4 with AA and MA combined as drug control for 28 days. Blood pressure (BP), heart rate (HR) and other antioxidant parameters were monitored and the blood samples were collected for endocrine and biochemistry analysis. **Results:** Metoprolol administration demonstrated a significant reduction in body weight, systolic BP, heart rate and food intake, while the levels of lipid peroxidation was increased significantly compared to control rats. Also, a significant decrease ($p<0.01$) in the antioxidant levels such as; SOD, catalase, glutathione peroxidase, reduced glutathione were evidenced in metoprolol group. On the other hand, the neuronal markers enzyme acetylcholinesterase were reduced while the activity of Nitric Oxide Synthase (NOS), Cytochrome P450 Reductase (CPR) and Carbonic anhydrase were increased in metoprolol group compared to control. However, rats received AA and MA pre-treatment elicited the improved antioxidant enzymes with restored physiological parameters, food intake and reduced the marker enzymes activity. **Conclusion:** The results of present study demonstrated the protective role of AA and MA against antioxidant deficit induced by the metoprolol tartrate by improving the physiological functions.

Key words: Asiatic acid, madecassic acid, antioxidants, peripheral nervous system, metoprolol tartrate, antioxidant deficits, hypotensive agent

Citation: Peng Wang, Teng-Fei Long, Li-Ping Wang and Jie Song, 2019. Synergistic effect of asiatic acid and madecassic acid against antioxidant deficit in rat peripheral nervous system. Int. J. Pharmacol., 15: 837-843.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Centella asiatica is a homoeopathic herb that belongs to the family Apiaceae mostly found in Asian countries such as; China, Malaysia, Pakistan and India. It is cultivated and used as medicine for centuries in traditional Chinese drug and ayurvedic treatments[1]. *Centella asiatica* has been reported in several medicinal applications such as; boosting of memory performance, wound healing, reduce the inflammation and also improve cognitive performance in neurological degenerative diseases like Parkinson's and Alzheimer's diseases[2-4]. This plant contains several constituents, mainly, asiaticoside, madecassoside, asiatic acid, betulinic, thankunic and isothankunic acids[5]. Asiatic Acid (AA) and Madecassic Acid (MA) have been used for centuries in traditional medicine for longevity and improving mental function with potential antioxidant properties and neuroprotective effects. The *C. asiatica* is well known for its memory and cognitive enhancement and also neurological functions as well as nerve regeneration. Scientific investigators worked on the neuroregenerative capacity of *C. asiatica* on the CNS, mainly focusing on brain cells. The AA has the ability to promote the elongation of neurites via *in vitro* experimental technique and synergistic effect with AA perceived other active compounds found in *C. asiatica*[6]. Scientific evidences have shown that the defensive mechanism activated in cells by this herb is by exerting the antioxidant properties that has various functional aspects, such as; scavenging of the ROS (catechins and quercetin)[7] inhibiting the chain-breaking activity (p-coumaric acid), inhibition of the free radicals generation[8] and chelation of metals[8,9].

Metoprolol (MT), a selective β1-adrenergic receptor antagonist, a well known hypotensive drug which is often used in treating the clinical cases of myocardial infarction hypertension and arrhythmia[10]. Due to agathokakological effects of MT on cells, many intricate and functional structures of the cells are susceptible to many injuries and deregulation that reduces cognitive functions and life-threatening problems. Hence, there is need to exist in search of drugs that can overcome the side effects of existing drugs. Hence, in the present study, the active ingredients of *C. asiatica*, AA and MA were selected to test its potential on MT induced changes in the peripheral nervous system.

MATERIALS AND METHODS

All the work related to this study was conducted in affiliated institutes. The analytical part and animal study were conducted in the month of January till May, 2018 and other work was completed in November, 2018.

Experimental design: For the experimental purpose, Wistar strain albino male rats weighing about 150-180 g were taken under sustainable environmental conditions and supplied with water and proper diet. The experiment was conducted as per the ethical norms identified by the institution (CHTX201806). Drugs Metoprolol Tartrate (MT), Asiatic Acid (AA) and Madecassic Acid (MA) were obtained (Sigma Aldrich, St. Louis, MO, USA) and all other chemicals were used of analytical grade. Rats were divided into four groups with 6 rats in each group. The first group comprises of rats with normal saline control. These rats were fed with a proper diet and unlimited water for 28 days. Group 2 rats were administrated with metoprolol tartrate (150 mg kg^{-1}/day, orally) dissolved in water. The Group 3 rats were administrated with asiatic acid (50 mg kg^{-1}, IP) and madecassic acid (30 mg kg^{-1}, IP) dissolved in DMSO for 2 weeks prior to metoprolol administration for 28 days. Group 4 rats were administrated with asiatic acid (50 mg kg^{-1}, IP) and madecassic acid (30 mg kg^{-1}, IP) alone for drug controls. After the experimental procedures the rats were fasted overnight and the next day they were killed by cervical decapitation and the corresponding blood samples were collected for biochemical analysis.

Blood Pressure (BP) measurement: The action of metoprolol in the peripheral nervous system was evaluated from the measurement of mean arterial pressure and heart rate using the non-invasive method of tail-cuff plethysmography method. For the measurement of final blood pressure, all rats were trained for the period of 10 days for instrument adaptation prior to the final measurement and the actual BP was observed from 20th-25th day of the treatment period. The BP and the heart rate were calculated as the average of 5-7 readings/day for 5 consecutive days.

Biochemical analysis: The commercial enzyme assay kits from Cayman chemicals were employed for the following assays as per the manufacturer's instructions. The levels of malondialdehyde (MDA) as a measure of lipid peroxidation, protein carbonyl contents using 2, 4-dinitrophenylhydrazine (DNPH) method, antioxidant enzymes such as; CAT, SOD, GPx and glutathione were analyzed (Cayman Chemicals, USA). The assay such as; acetylcholinesterase, nitric oxide synthase and Cytochrome P450 reductase activity were carried out by

using the assay kits from Abcam (Abcam, USA) and Carbonic anhydrase assay were carried out by using Biovision (Biovision Inc., CA, USA).

Statistical analysis: GraphPad Prism tool was used for the statistical analysis. The results are presented as means with standard error mean. The differences between groups were determined by student's t-test. The probability of $p<0.05$ was considered as significant value.

RESULTS

Physiological parameters: Upon administrating metoprolol for 28 days, reduction ($p<0.05$) in the body weight (Fig. 1a) was observed with a significant reduction in mean arterial pressure (97±3 mmHg) (Fig. 1b) and heart rate (Fig. 1c) compared to control. While rats pretreated with AA and MA elicited an unaltered arterial pressure and body weight which were equivalent to control vehicle-treated rats. While growth characteristics, food intake were monitored before and after the experiment and the results demonstrated a marginal reduction (<5%) in food intake (Fig. 1d) in metoprolol administration, but no significant difference in the activities of rats were observed during the study (Fig. 1).

Oxidative markers: Lipid peroxidation is the effect of free radical-mediated tissue damage and is considered as an indicator for oxidative damage by a series of chain reactions also a standard measure for free radical-mediated cellular injury. Figure 2a showed the concentration of MDA released in the plasma of control and clinical trial rats. The mean level of MDA in the metoprolol induced rats (Group 2) exhibits a significant increase than the control rats ($p<0.05$). In contrary, the concentration of MDA released was found to be significantly reduced in AA and MA-treated rats (Group 3). Additionally, the oxidative protein damage was measured by an upsurge in protein carbonyl content (Fig. 2b). The amount of carbonyl content in the serum samples was increased in rats on metoprolol induction while the increased carbonyl content of the metoprolol exposed group was significantly restored in AA and MA administered groups ($p<0.05$). Conversely, AA and MA administrated rats did not show any significant change in the carbonyl levels as compare to control.

Antioxidant enzymes: Figure 3a-d depicted the enzymatic antioxidants activities such as; CAT, SOD, GPx and glutathione in the plasma of control and clinical trial rats, respectively. The enzymes activities were significantly diminished in the

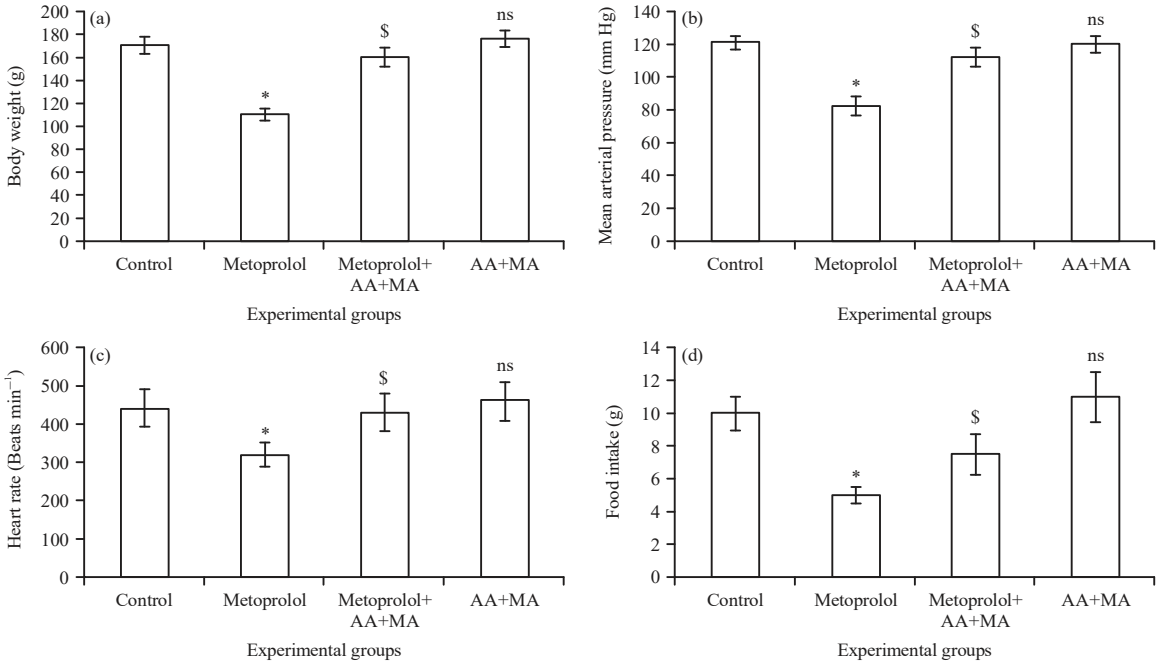

Fig. 1(a-d): Effect of metoprolol on (a) Body weight, (b) Arterial pressure, (c) Heart rate and (d) Food intake of control and experimental groups of rats

Values are expressed as Means±SE (n = 6), statistical significance expressed as *$p<0.05$ compared to vehicle-treated controls, $^{\$}p<0.05$ compared to metoprolol treatment, ns: Non-significant compared with vehicle-control rats

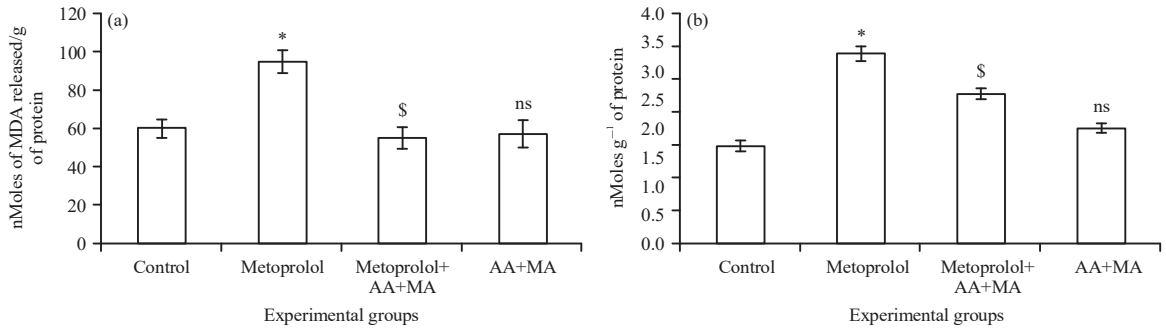

Fig. 2(a-b): Levels of (a) Lipid peroxides and (b) Protein carbonyls of control and experimental groups of rats

Values are expressed as Means±SE (n = 6), statistical significance expressed as *p<0.05 compared to vehicle-treated controls, $^{\$}$p<0.05 compared to metoprolol treatment, ns: Non-significant compared with vehicle-control rats

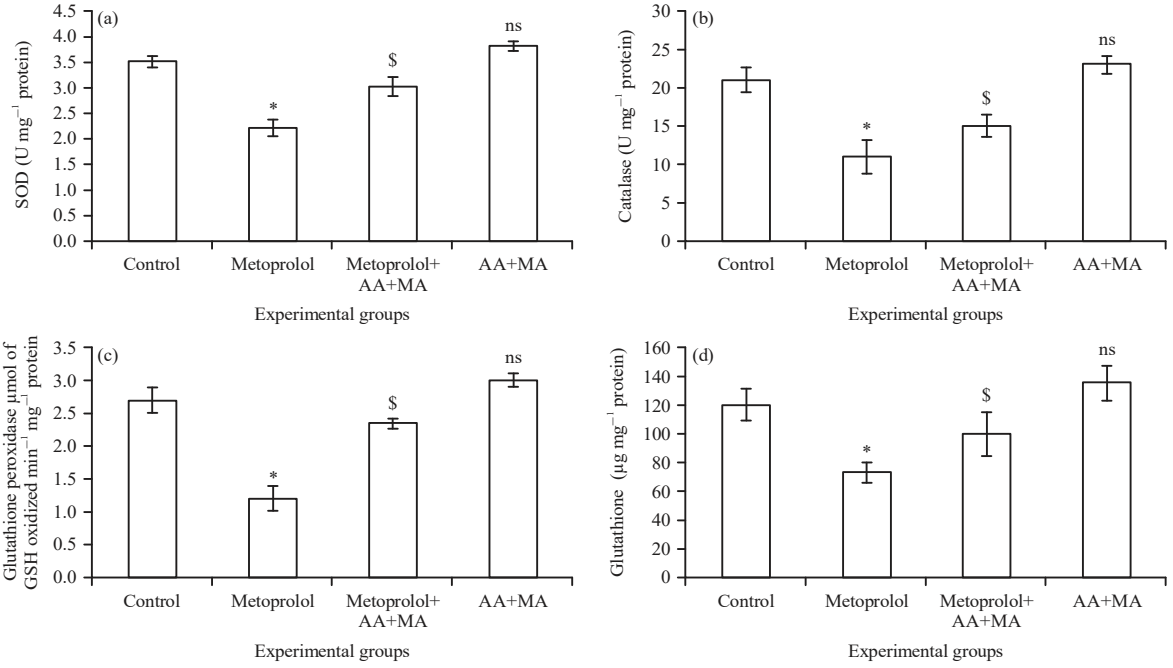

Fig. 3(a-d): Effect of AA and MA on the levels of antioxidant enzymes such as (a) Superoxide dismutase (SOD), (b) Catalase (CAT), (c) Glutathione peroxidase (GPx) and (d) Glutathione, respectively in the control and metoprolol administered groups of rats

Values are expressed as Means±SE (n = 6), statistical significance expressed as *p<0.05 compared to vehicle-treated controls, $^{\$}$p<0.05 compared to metoprolol treatment, ns: Non-significant compared with vehicle-control rats

metoprolol induced rats (p<0.05). On treatment of AA and MA, the animals showed a significant increase in the enzymatic antioxidants activities to near normal compared to beta-blocker administered rats (p<0.05). However, no significant statistical variations were found in rats treated with AA and MA compared to control rats.

Marker enzymes: The expression of marker enzymes related to metabolite catabolic pathways gives a clue on the direct role of drugs in cellular system. The marker enzymes activities like acetylcholinesterase, nitric oxide synthase, cytochrome P450 reductase and carbonic anhydrase were assayed in the clinical trial and normal rats. On administration of metoprolol, a significant decrease in the level of acetylcholinesterase (Ach) (Fig. 4a) with increased nitric oxide synthase (NOS) (Fig. 4b), cytochrome P450 reductase (Fig. 4c) and carbonic anhydrase activity (Fig. 4d) compared to control rats (p<0.05). While AA and MA administered rats demonstrated the restored enzymatic levels of acetylcholinesterase, nitric oxide synthase, cytochrome P450 reductase and carbonic anhydrase equivalent to vehicle-treated and drug-treated control rats (Fig. 4).

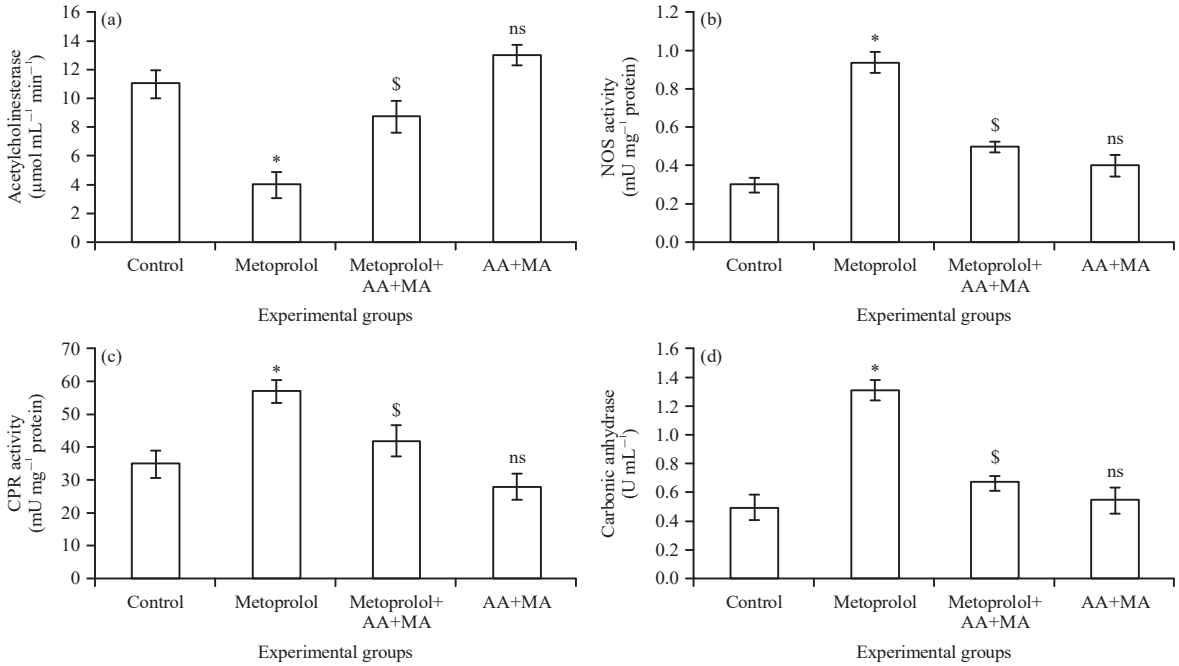

Fig. 4(a-d): Levels of (a) Acetylcholinesterase, (b) Nitric oxide synthase, (c) Cytochrome P450 reductase (CPR) and (d) Carbonic anhydrase in control and metoprolol administered groups of rats

Values are expressed as Means±SE (n = 6), statistical significance expressed as *p<0.05 compared to vehicle-treated controls, $p<0.05 compared to metoprolol treatment, ns: Non-significant compared with vehicle-control rats

DISCUSSION

The present study attempted to elucidate the neuroprotective effects of AA and MA using rats as experimental model by administering Metoprolol, a well known hypotensive drug with serious side effects in peripheral nervous system. The study results demonstrated the synergistic effect of AA and MA in restoring the physiological functions, antioxidant deficit while improving the functional enzymes of nervous system. In Ayurveda, *C. asiatica* is used alone or in combination with other phytochemicals that are considered as vital ingredients of various medicine formulations for the treatment of Central Nervous System (CNS) problems[11-13]. Peripheral nervous system is a dynamic part of the body, because it transfers information to maintain every cellular action.

It is well stated that the onset of lipid peroxidation is a biochemical process that induces oxidative degeneration of polyunsaturated fatty acids, which causes impaired membrane structure and functions. The MDA level, which is a major indicator of lipid peroxidation, indirectly reflects the extent of cellular injury *in vivo*[14]. The present study showed that lipid peroxides and oxidative protein carbonyl contents were significantly raised in metoprolol administered rats. Several studies have also shown that MDA content significantly increased in different tissues of animals exposed to various stresses[15,16]. Though, in the present study, co-administration of metoprolol along with AA and MA retain the lipid peroxidation activity at a safe level, the mechanism associated with the AA and MA is suggested to be mediated via the free radical scavenging activity which is in accordance with previous reports on the protective role of AA against MDA level induced by ISO mediated cardiotoxicity[12]. Furthermore, the observed results correlated with the previous research work on Pre and co-administration of AA exhibited reduced heart weight, MDA concentration, heart to body weight ratio in the isoproterenol (ISO) induced cardiotoxicity[17]. While the directive effect of centella in reducing the malondialdehyde (30-50%), reactive oxygen species (32-42%) and hydroperoxide levels (30-35%) were reported in the brain regions further strengthens the present study results[18].

Consequently, the anti-oxidative or anti-inflammatory properties of *C. asiatica* showed the beneficial effect in patients suffering from long-term Alzheimer's disease by enhanced secretase pathway or degraded the enzyme pathway[19]. The antioxidant activities are facilitated by a cascade of enzymes that can sequester ROS and convert them into a lesser toxic and non-reactive ROS molecules. The primary defence antioxidant activities responsible for

SOD activity is the enzyme dismutation reaction of the superoxide anion to yield H_2O_2 and role of catalase[20,21] is to degrade H_2O_2 to H_2O and O_2. In the present investigation, the endogenous antioxidants such as; CAT, SOD, GPx and glutathione was illuminated and the results showed that decrease antioxidant levels in metoprolol treated animals and the animals co-treated with AA and MA showed increased level of antioxidant enzyme activity. From these results, it is suggested that AA and MA have an antioxidant resorting capacity in cells that has got major attention due to its multiple pharmacological and therapeutic potential in various ailments[22]. Reports have suggested that AA demonstrated the antioxidant and free radical scavenging activities through connecting numerous pathways dose-dependent free radical scavenging potential by contradicting hydroxyl radicals and superoxide anions. It is not only the pure compounds of centella that exerts the action, but also the crude extracts of *C. asiatica* also possess the potential to inhibit the powerful ROS called hydroxyls[23].

In the present study, metoprolol was used and the basic mechanism of its catabolism take place in liver that can be co-metabolised by the oxidative reaction that could be predicted to be the mediator of various signal activation of metabolising enzyme system in the liver. Hence in the present study, Acetylcholinesterase, Nitric Oxide Synthase, Cytochrome P450 Reductase and Carbonic anhydrase enzymes were estimated in metoprolol induced animals. These enzymes were directly involved in the signal transduction pathways of the neurons and also act as signal transmitters in the case of xenobiotics transformation. Acetylcholinesterase (Ach), an enzyme presents in the CNS, that catalysis the neurotransmitter ACh to choline. Any disturbances in the level and activity of these enzyme leads to the partial dysfunction of the cellular system. For instance, the use of reversible and irreversible acetylcholinesterase inactivating compounds is being commonly applied in neurodegenerative disorders treatment[24]. In the present study, the activity of Ach is reduced significantly in metoprolol group while AA and MA treatment restored the level. The observed findings of the study correlated with the previous report on neuroprotective effects of *Centella asiatica* against colchicine-induced cognitive impairment in rats demonstrating the restored enzyme level with persevered loco motor activity[25,26].

The observed results were in concordance with the study demonstrated the protective role of AA against metabolic syndrome through its antioxidant and anti-inflammatory activities with restored regulation of endothelial nitric oxide synthase (eNOS)/inducible nitric oxide synthase (iNOS) expression[24]. Adding a note to comply the results of the present study, the restored levels of carbonic anhydrase correlates with the study using the phytochemical arjunolic acid as a cardio protective agent through inhibition of zinc independent inhibition of carbonic anhydrase activity[27,28]. Thus, the study recapitulated the protective effect of AA and MA in rat peripheral nervous system even at physiological functional level; thus a little more molecular analysis would help in identifying the cellular signaling behind its role.

CONCLUSION AND RECOMMENDATION

The study results demonstrated the protective effect of AA and MA against metoprolol (betablocker) induced cellular distortion by restoring the physiological functions and activity of acetylcholinesterase, nitric oxide synthase, cytochrome P450 reductase and carbonic anhydrase enzymes suggest the directive evidence of AA and MA in peripheral nervous system provides new insight into the pharmacological actions of these drugs which needs further molecular investigation to confirm its downstream signalling.

SIGNIFICANCE STATEMENT

This study discovered the potential role of Asiatic and madecassic acids in rat peripheral nervous system that can be beneficial to overcome the drug related toxicities. This was evidenced from the present findings that hypotensive drug metoprolol mediated cellular deregulation and the combination of AA and MA administration restored it. Thus the present study will help the researchers to uncover the critical areas that plant based treatments could help in near future as an alternate medicine which is an unmet need of the hour. Hence, further investigations can bring the novel findings to treat ailments that exist as necessary evil in modern drug treatments.

ACKNOWLEDGMENT

This study was supported by the Program of the Shanghai Municipal Health Commission (grant No: 20134Y105).

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