Research article

Rapports De Pharmacie Vol.4 (4), 2018, 505-509
ISSN: 2455-0507

THERAPEUTIC INVESTIGATION OF GALIC ACID ON PACLITAXEL-INDUCED MOTOR-INCOORDINATION IN MUS MUSCULUS

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ABSTRACT

Gallic acid (GA) is a natural phenolic compound (3,4,5-trihydroxybenzoic acid). It has potential neuroprotective action via versatile antioxidant actions. Current research work is designed to explore the therapeutic role of GA in paclitaxel-induced alteration of neuromuscular function. Motor incoordination was developed by administration of paclitaxel (2 mg/kg, *i.p.* for 5 successive days) in mice. The GA (20 and 40 mg/kg; *i.v.*) was administered for 10 consecutive days. The motor-incoordination was assessed by the rota-rod test. The motor-incoordination was carried out at time points *i.e.*, 0, 4, 8, 12 and 16th day. GA treatment ameliorated the paclitaxel-induced motor-incoordination in a dose-dependent manner. Similarly, GA was also attenuated the tissue biomarkers *i.e.*, thiobarbituric acid reactive substances (TBARS), reduced glutathione (GSH) and myeloperoxidase (MPO) activity changes. The neuroprotective action of GA and prevention of motor-incoordination is due to its potential anti-oxidant, anti-lipid peroxidative, and anti-inflammatory actions.

Keywords: Calcium, Gallic Acid, Motor-incoordination, Paclitaxel, sciatic nerve.

INTRODUCTION

Motor coordination plays an essential role in day to day life activity. The motor activity mainly assessed by kinematic analysis with and without stimuli [1]. The reflux responses are based on the integration of proprioceptive signal in the neuromuscular system [2]. The major part of brain *i.e.*, cerebellum contributes the key role in the regulation of motor coordination [3]. Various cancer chemotherapeutic agents like oxaliplatin, cisplatin, vincristine, vinblastine, docetaxel, bortezomib including paclitaxel are induced the damage of sensory and motor neurons [4, 5]. Paclitaxel (PT) is widely used for breast, prostate, ovarian, lung, bladder, and esophageal cancers; and various types of solid tumors. Paradoxically, it also accelerates the peripheral neuropathy and myopathy (myalgia and arthralgia) [5]. The pathophysiological mechanism of PT induced neuromuscular dysfunction due to its ability of free radical induction, inactivation of tubulin proteins, raising of intracellular calcium ion concentration, the release of inflammatory cytokines, and alteration of mitochondrial events [6, 7]. The motor incoordination functions of rodents are mainly tested in rota rod instruments. The rotarod device provides information about motor coordination function neuromuscular acting agents, cerebral conditions, and muscular strength & fatigue situation of the muscular system [8]. Jones and Roberts (1968) demonstrated the rota-rod instruments for the assessment of motor coordination. Rotarod device functioning based on two methods like 1) *Accelerated speed:* it starts from 10 rpm and raised 5 revolutions per minutes (RPM) in every minute; and 2) *Constant speed:* rota rod working based on 25 rpm of revolving rod [9]. The injection of PT (2 mg/kg, *i.p.* for 5 successive days) causes the motor incoordination in rodents [10]. The roles of phytomedicines for the amelioration of PT induced motor in-coordination are limited. Gallic acid is natural polyphenolic compounds and it found in gallnuts, witch hazel, tea leaves, sumac, and oak bark. Further, it possesses free radical scavenging, anti-inflammatory, and neuroprotective actions [11, 12]. However, GA action in PT induced motor in-coordination not screened yet. Consequently, current research work delineated to test GA against paclitaxel-induced motor-incoordination in mice.

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MATERIALS AND METHODS

Animals

The disease-free male Swiss albino mice (20-25 g;

10 months old) was recruited in this research work. Mice were allowed to confiscate the standard laboratory diet and water *ad libitum.* The 37 °C temperature and 60 % humidity conditions of the animal house and 12 hours light-dark cycle were maintained throughout the experimental protocol. The experimental design was authorized by the Institutional Animal Ethics Committee (IAEC; No.: ATRC/09/I4). Mice care was taken as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Drugs and chemicals

1,1,3,3-tetramethoxy propane was obtained from Sisco Research Laboratories (SRL) Pvt. Ltd. India. Gallic acid and thiobarbituric acid were procured from Sigma Aldrich Mumbai. The remaining chemical reagents were obtained from S.D. Fine Chemicals, Mumbai, India with the analytical grade.

Experimental protocol

Five groups were recruited in this research work. Each groups comprising six Swiss albino mice (n = 6). *Group I:* Healthy mice were subjected to assessment of motor coordination test without any drug administration. *Group II:* Paclitaxel (PT) dose 2 mg/kg, *i.p.*; for 5 consecutive days was administered for the induction of motor incoordination [13]. *Group III (per se):* GA (40 mg/kg, *i.v.*) for 10 successive days administered to healthy Swiss albino mice. *Group IV and V:* GA (20 and 40 mg/kg, *i.v.*) for 10 successive days administered to PT treated animals. All five groups were computed for a motor coordination test and biochemical estimations. The motor coordination tests were assessed on different time points *i.e.,* 0, 4, 8, 12 and 16th day. On the 16th day, mice were sacrificed. The sciatic nerve and surrounding tissue samples were collected for further biochemical evaluation.

Rotarod test

The motor-coordination function of mice was computed as demonstrated by Jones and Roberts [9]. Concisely, mice were placed on a rotating rod after training (*i.e.,* 5 min walking on rotating rod before 30 minutes) period. The motor coordination of mice was assessed by two-speed pattern *i.e.,* at a constant speed (25 rpm); and accelerated speed (10 rpm followed by raise 5 rpm per minutes). The motor functions of mice were assessed as an index of fall of time from the revolving rod. The cut off time will be perpetuating for 5 minutes to avoid potential tissue injury.

Biochemical estimation

Sciatic nerve and thigh muscles of mice were stored in a humidity chamber and maintained at 85 % relative humidity at 37 ºC. The sciatic nerve (10 % w/v) tissue was homogenated with phosphate buffer (pH 7.4). The biomarkers *i.e.,* thiobarbituric acid reactive substances (TBARS), and reduced glutathione (GSH) and total protein were determined in an aliquot of sciatic nerve tissue. Further, muscular tissue was homogenated using phosphate buffer for the estimation of myeloperoxidase (MPO) activity.

Estimation of thiobarbituric acid reactive substance (TBARS):

The TBARS was quantified as described by Ohkawa *et al.* [14]. The absorbance was estimated by spectrophotometer method using UV-Vis spectrophotometer, Japan at 535 nm wavelength. A standard plot was obtained by using 1-10 nM of 1, 1, 3, 3-tetramethoxy propane as standard. The results of TBARS concentration were intimated as nM of MDA per mg of protein.

Estimation of reduced glutathione (GSH) content:

The GSH content was estimated as described by Beutler *et al.* [15]. The absorbance was estimated by using spectrophotometer at 412 nm wavelength. The standard plot was obtained by using 10-100 µg of GSH as standard. The results of GSH values were intimated as µg of GSH/mg of protein.

Estimation of myeloperoxidase (MPO) activity:

The MPO activity level of muscular tissue was estimated by Patriarca *et al.* [16] with little modification of Grisham *et al.* [17]. The changes of absorbance were noted at 460 nm wavelength. The results of MPO activity level were intimated as units/mg of protein/min.

Estimation of total protein content:

The total protein values were quantified by Lowry's *et al.* [18]. The absorbance changes were noted at 750 nm wavelength. The standard plot was prepared with 1-10 mg of bovine serum albumin. The total protein values were intimated as mg/ml of supernatant.

Statistical analysis

All the results were intimated as mean ± standard deviation (SD). Data obtained from motor coordination were analyzed using two-way analysis of variance (ANOVA) method. Further Bonferonni's *post-hoc* analysis was applied in Graph pad prism (Version 5.0) software. The tissue biomarker results were analyzed by one way ANOVA method. The Tukey's multiple range tests were applied in Sigmastat (Version 3.5) software. A probability value *i.e., p* less than 0.05 ($p < 0.05$) was contemplated as statistically significant.

RESULTS

Role of GA in the rotarod test

The administration of paclitaxel (PT; 2 mg/kg, *i.p.* for 5 consecutive days) resulted to ($p < 0.05$) decrease the fall of time as an indication of raising the motor incoordination when compared to normal control group. Administration of GA (20 and 40 mg/kg, *i.v.*) attenuated PT induced motor incoordination in a dose-dependent manner. However, vehicle and GA (40 mg/kg; *i.v.*) *per se* treated group did not allow any significant changes in PT induced motor incoordination in mice. The change of motor coordination with constant speed and accelerated speed were illustrated in figure 1 and 2 respectively.

Role of GA in tissue biomarker changes

The administration of paclitaxel (PT; 2 mg/kg, *i.p.* for 5 consecutive days) resulted to ($p < 0.05$) rise the TBARS & MPO levels; and decrease in GSH content as an indication of oxidative stress, inflammation, and neuronal damage when juxtaposed to the normal group. Administration of GA (20 and 40 mg/kg, *i.v.*) attenuated PT induced changes of above tissue biomarkers in a dose-dependent manner. However, vehicle and GA (40 mg/kg; *i.v.*) *per se* treatment did not allow any significant changes in PT induced tissue biomarker changes (Table 1).

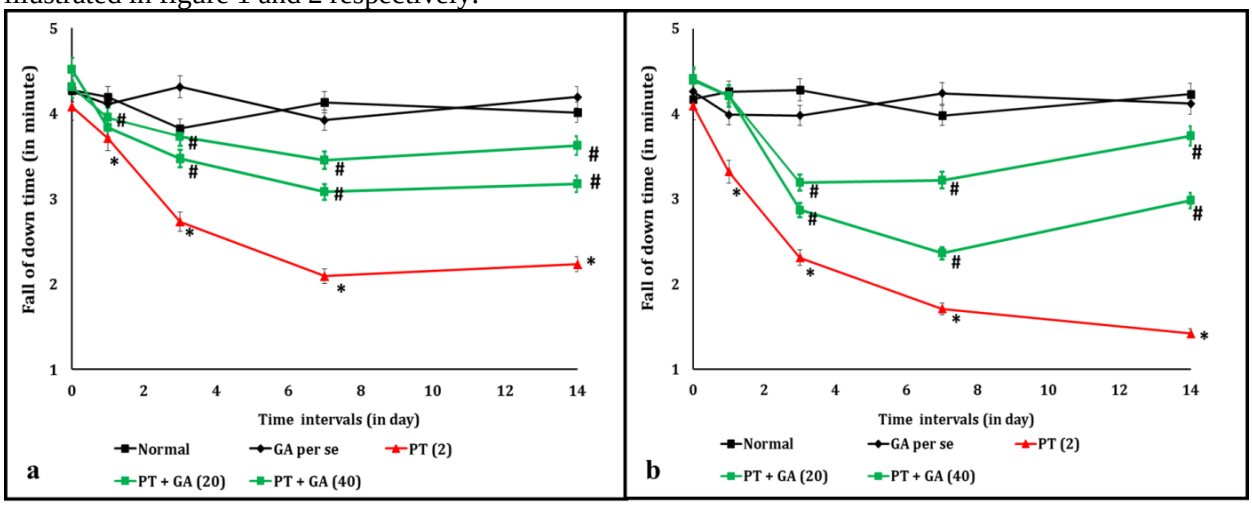

Figure 1: Role of GA on PT induced changes of motor in-ordination with a constant speed (a) with accelerated speed (b) of rota-rod. Digits in parenthesis indicate dose in mg/kg. Data were expressed as mean ± SD, n = 6 mice per group. $^{*}p < 0.05$ when compared to normal control group. $^{\#}p < 0.05$ when compared to PT control group. Abbreviation: PT, paclitaxel; and GA, gallic acid.

Table 1: Role of GA on PT induced biomarker changes in sciatic nerve tissue.

Groups	**TBARS** (nM/mg of protein)	**GSH** (µg/mg of protein)	**MPO** (unit/Min/mg of protein)
Normal	3.87 ± 0.53	81.24 ± 1.97	14.69 ± 1.42
GA *per se*	3.96 ± 0.68	83.19 ± 1.42	16.07 ± 0.98
PT	9.02 ± 0.48*	34.69 ± 1.74*	99.37 ± 2.47*
PT + GA (20)	4.85 ± 1.23$^{\#}$	74.83 ± 2.67$^{\#}$	56.84 ± 1.93$^{\#}$
PT + GA (40)	4.39 ± 1.06$^{\#}$	82.46 ± 2.72$^{\#}$	41.71 ± 2.13$^{\#}$

Table 1: Role of GA on PT induced biomarker changes in tissue supernatant. Digits in parenthesis indicate dose in mg/kg. Data were expressed as mean ± SD, n = 6 mice per group. $^{*}p < 0.05$ when compared to the normal group. $^{\#}p < 0.05$ when compared to PT control group. Abbreviation: PT, paclitaxel; GA, gallic acid; TBARS, thiobarbituric acid reactive substances; GSH, reduced glutathione and MPO, myeloperoxidase.

DISCUSSION

The data of current research work revealed that the administration of GA (20 and 40 mg/kg, *i.v.*) ameliorated PT induced motor incoordination. It indicates that GA holds the potent protective action of the neuromuscular system. Furthermore, it also attenuates the PT associated biomarkers changes. Further, GA reduced the TBARS and MPO levels; raise the GSH level due to its anti-lipid peroxidation, free radical scavenging, and anti-inflammatory actions. The administration of PT (2 mg/kg, *i.p.* for 5 consecutive days) is known to cause the motor incoordination via alteration of the neuromuscular motor unit neuron [13]. Clinically, the administration of PT causes the neuropathy and impairment of muscular tissue [19-21]. Furthermore, PT is also documented to cause the neuromuscular damage and neurodegeneration leads to develops the motor incoordination [22,23]. In addition, the PT induced motor incoordination occurs due to accumulation of free radicals, lipid peroxidation and synthesis & release of inflammatory cytokines via activation inflammatory marker enzymes like MPO [24, 25]. The primary events of PT associated motor incoordination and muscular fatigue is due to the raising of free radicals [26]. The data in hand, our previous research reports, and other laboratory reports are revealed that phytomedicines like *Ocimum sanctum* [27], *Acorus calamus* [28], *Vernonia cinerea* [29], *Bacopa monniera* [30]; and *Nymphaea lotus* [31] has potential protective action of the neuromuscular system. Current research data demonstrated that GA attenuates PT associated motor incoordination viz reduction of free radicals; oxidative stress and inflammatory reaction. Hence, it is concluded that gallic acid may be bioactive medicine for prevention of cancer chemotherapeutic agents associated with motor incoordination.

ACKNOWLEDGMENTS

The authors are thankful to the Akal Toxicology Research Centre, A unit of Akal College of Pharmacy & Technical Education, Mastuana Sahib, Sangrur-148001, Punjab (India) for supporting this study and providing technical facilities for this work.

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