The International Journal of Indian Psychology
ISSN 2348-5396 (e)
ISSN: 2349-3429 (p)
Volume 4, Issue 1, No. 76,
DIP: DIP: 18.01.005/20160476
ISBN: 978-93-86162-13-7
http://www.ijip.in
October, 2016

Role of Mushroom in Maintaining Mental Health with Special Reference to Anti-Convulsant Activity

Deepak Vyas[1]*, Imtiyaz Ahmad Seikh[2], Gyanesh Kumar Tiwari[3]

ABSTRACT

Various mushroom proteins, such as lectins, fungal immunomodulatory proteins, ribosome inactivating proteins, ribonucleases, laccases and other proteins have interesting biological activities. These have become popular sources of natural antitumor, antiviral, antimicrobial, antioxidative and immunomodulatory agents. This paper updated the present status of bioactive compounds in *Ganoderma lucidum* a mushroom with biomedical potential. *Ganoderma lucidum* collected from botanical garden growing on *Mimosops elangi* as pathogen was investigate for the bioactive compounds and anticonvulsant activity. Its aqueous extract was injected in wistar albino rats. Phytochemical analysis was done by chemical, FTIR and mass spectrometric methods. Acute toxicity was determined using Lorke's method. The anticonvulsant activity of the extract was assessed in pentylenetetrazole (PTZ) induced and maximal electroshock (MES) induced convulsion in rats, with valproic acid and phenytoin as positive control (PC), respectively. Gamma amino butyric

[1] *Laboratory of Microbial Technology, Department of Botany, Doctor Harisingh Gour Vishwavidyalaya, Sagar, M. P., India*
[2] *Laboratory of Microbial Technology, Department of Botany, Doctor Harisingh Gour Vishwavidyalaya, Sagar, M. P., India*
[3] *Department of Psychology, Doctor Harisingh Gour Vishwavidyalaya, Sagar, M. P., India*
**Responding Author*

acid (GABA) estimation of rat brain was carried out by standard high performance liquid chromatography (HPLC). Phytochemical analysis showed the presence of polysaccharides, flavonoids, terpenoids and phenolic acids in particular. Six bioactive compounds were identified in GAE by FTIR and LC-MS characterization which included three triterpenoids and three phenolic compounds/flavonoids. Extracts were found non-lethal even at the doses over 5000 mg/kg intraperitoneally. GAE at higher dose (500mg/kg) and PC produced nearly similar effects (100% protection) against MES induced generalized tonic hind limb extension (THLE) and PTZ induced absence seizures. GAE showed significant effects against both convulsion models in a dose dependent manner. None of the deaths were recorded in MES rats while in PTZ rats higher doses of test reduced mortality to 16% along with the valproic acid, which also produced 16% deaths. GABA content was also found improved in test groups and standard in PTZ rats. GABA appeared non-essential in MES induced convulsions. These results suggest that *Ganoderma* aqueous extract possess anticonvulsant potential due to the presence of biologically active components.

Keywords: *Polypore Mushrooms, Ganoderma Lucidum, Trametes Hirsuta, Epilepsy, Mental Health.*

Mushrooms have become attractive as a well balanced food and pharmaceutical stuff with some advantages over plants providing definite nutrition and health benefits for human. Traditionally, wild and cultivable edible mushrooms are used by most of the Asian and other countries worldwide as good source of pharmacologically active compounds (1). Wild and cultivated mushrooms are rich in dietary fiber, minerals, and vitamins and low in fat (2). Over the past several decades, scientific research has been intensified and focused on analyzing varied nutritional and medicinal properties of mushrooms. Elaborative studies report that mushrooms accumulate variety of secondary metabolites, including phenolic compounds,

polysaccharides, terpenoids and steroids responsible for the enormous therapeutic activities. They are consistently being studied for their immense pharmacological properties which include hepatoprotective (3), immune-modulating (4), neuroprotective (5), anticancer (6), antidiabetic (7), antimicrobial (8), antiviral (9), and antioxidant (10; 8) activities. *Ganoderma* (Curtis) P. Karst is a polyporous medicinal mushroom belonging to family Ganodermataceae, predominantly found on broad leaved tree trunks and dead stumps in Central Indian sub-tropical forests (11,12). *Ganoderma lucidum* commonly known as Lingzhi or Reishi contains biologically active components like triterpenoids, polysaccharides, ganoderic acids and phenolic compounds and so on. Substantial reports are available in literature indicating vast array of pharmacological effects of *Ganoderma* species conferred by its bioactive components. There are several reports regarding the central nervous system (CNS) protective effects of *Ganoderma* (13; 14; 15).

Very little is known about ameliorative effects of *Ganoderma* against epilepsy. Epilepsy is a central nervous system disorder characterized by recurrence of paraoxysmal neurological or behavioral manifestations commonly termed seizures. Seizures or convulsions result from chronic imbalance between excitatory and inhibitory neurotransmitters leading to hyperexcitable neuronal discharges. For the management of epilepsy a good number of antiepileptic drugs (AEDs) available but far from adequate. Hence, research is needed to find AEDs of natural origin with no side effects. In this context, present study was aimed at phytochemical characterization and antiepileptic effects of aqueous extract of *Ganoderma lucidum* (GAE) using different experimental models *in vivo*. In our previous work, we found higher antioxidative effects of its ethanolic extract using *in vitro* methods (10). It is supposed that aqueous extract of the mushrooms contain phenolic compounds and terpenoids which may have antioxidant and anticonvulsant potential.

MATERIALS AND METHODS

Sampling and extraction

Ganoderma lucidum fruiting bodies were collected in June-August (monsoon), 2012 from sub-tropical deciduous forests of Madhya Pradesh (Central India). *Ganoderma* was isolated from living *Mimusops elengii* tree parasitically causing basal stem rot in it. The specimens were photographed, soft brush cleaned, taken to Laboratory of Microbial Technology and Plant Pathology, Department of Botany, Dr. H. S. Gour University Sagar, M. P. for identification and authentication. A specimen voucher MTPP11/45 of the sample was deposited in herbarium of the department. The fruiting bodies were dried in an oven at 40 ^{0}C for 8 hours. The dried fruiting bodies were crushed to powder by using REMI electronic blender. About 50g powder of the mushroom was taken in 500ml of distilled water in soxhelet extraction unit for extraction at 100 ^{0}C for 16-18 hours. The *Ganoderma* aqueous extract (**GAE**) was then rotary evaporated at 60 ^{0}C, kept in a dessicator to dry and stored at 4 ^{0}C for further use.

Chemicals

GABA, Sodium Valproate, Phenytoin and Pentylene tetrazole were purchased from Sigma-Aldrich Co (Mumbai). All other unlabeled chemicals and reagents were of analytical grade.

Preliminary Phytochemical Analysis

Phytochemical tests were carried out on the rotavaporised extract using standard procedures as described by Harborne (19).

Fourier Transform Infra Red (FTIR) spectroscopy studies

FTIR spectroscopy of the extract was tested using high sensitive FTIR Spectrometer (Shimadzu 2191, Japan). Briefly one part of sample was mixed with 99% of dried potassium bromide powder (KBr). This mixture was subjected to FTIR spectrum analysis in the frequency range of 400 to cm^{-1}4000.

Liquid Chromatography-Mass Spectrometric (LCMS) studies

Phytochemical characterization was further done by separating the components of GEE and TEE by HPLC module (Agilent Technologies) with Autosampler G1329B injection volume 5 ul,

Quaternary Pump G1311C flow rate 0.5ml/min, Solvent A H2O with 0.1% formic acid: Solvent ACN (85:15) for a runtime of 35 minutes and Thermostat Column Compartment G1316A with a C18 reversed phase column. The column eluate was passed into the Electro Spray Ionisation (ESI) interface operating in negative and positive modes of Agilent Technologies MS QQQ Mass Spectrometer. The voltage on the ion spray interface was 4000 V and the fragmentor voltage of the orifice was set at 130V-150V. Selected (M+H+) or (M-H-) ions were analyzed by collision induced dissociation using nitrogen as the collision gas. MS2 scan type in both negative and positive polarities, scan time 500, analyzing the mass in the range of 100-1600 m/z were recorded.

Experimental animals

Wistar Albino rats of either sex (weighing 100-150 g) were obtained from Department of Research and Defence Organisation (DRDO), Gwalior. The animals were maintained in a well-ventilated room, fed on standard pellet feed and water *ad libitum*. All studies on animals were approved by Institutional Animal Ethics Committee (IAEC).

Acute Toxicity Study

Toxic effects of the extracts were analysed using the method of Lorke (25). The method consisted of two phases. In the first phase, three groups of three rats each were injected with GAE at doses of 10, 100 and 1000 mg/kg body weight IP and observed for signs of toxicity and death within 24 h. In the second phase, four groups of one rat each were treated with four more specific doses of the extract based on the result of the phase first. Finally, two doses of the extract were chosen on the basis of acute toxicity studies for anticonvulsant activity.

Anticonvulsant activity

Maximal electroshock-induced convulsion in rats

The method described by Swinyard and Kupferberg (26) and modified by Sayyah et al. (27) was further modified and used in this study. Briefly, 24 wistar albino rats were randomly divided into four groups containing 6 rats. The first group (control) received normal

saline 10 ml/kg body weight IP, second group (standard) was injected with 20 mg/kg of the standard drug phenytoin, third (GAE250) and fourth (GAE500) groups were treated with 250 and 500 mg/kg of GAE intraperitonealy. Thirty minutes later, maximal electroshock was administered to induce seizure in the rats in all groups using an electroconvulsometer with corneal electrodes placed on the upper eyelid of the rats. The shock duration, frequency, current were set and maintained at 1.5 s, 150 pulse/sec and 50 mA, respectively. Seizures were manifested as tonic hind limb extension (THLE) and duration of whole episode (28). The ability to prevent this feature or reduce the duration of the convulsion was considered as an indication of anticonvulsant activity (29).

Pentylene tetrazole (PTZ) induced convulsion in rats

In this study, animals (N=24) were divided in four groups of six animals each. Grouping was done as: first group as control received normal saline (NS) and PTZ (80 mg/kg i.p.), second group as positive control (PC) received NS + Valproic acid (30 mg/kg i.p.), third group (GAE250) received NS + GAE (250 mg/kg i.p.), and fourth group (GAE500) received NS + GAE (500 mg/kg) intraperitoneally. Pentylenetetrazole (PTZ 80mg/kg) was administered to all groups 30 minutes after receiving treatments to induce clonic convulsions. For a period of 30 minutes post-PTZ administration animals were observed for onset of convulsion and duration of convulsion. Incidence and mortalitiy % due to PTZ were also recorded.

Gama amino butyric acid (GABA) estimation

Shortly after the observation animals were sacrificed, and brains removed and submerged in ice-cold artificial cerebrospinal fluid. The brain tissue (1mg) was washed with saline to remove blood, blots to dry and submerged in 10 ml methanol. Subsequently, homogenized for 2 minutes and centrifuged at 10000 rpm for 15 minutes. 1 ml of the supernatant of the brain homogenate was mixed with 1 ml of water and centrifuged at 12000 rpm for 10 minutes. Afterwards 0.7 ml of the supernatant was added to a volumetric flask containing 0.6 ml of borax buffer (pH 8). The mixture was heated on

water bath at 800 C for 10 minutes. Final volume of the flask was adjusted to 5 ml with methanol. 5 ul of the membrane filtered solution was injected in C18 column (HPLC Alliance Waters, Millford USA; separation module 2965 coupled to Waters 2998 Photodiode array detector DAD Milford, MA USA; Waters Spherisorb reversed phase-C18 analytical column (250 mm × 4.6 mm i.d., 5 µm; ODS2) and eluted with methanol and water (62:38 v/v), flow rate 1 ml/min. The chromatograms were plotted at 330 nm. HPLC system control and data processing was performed by Empower software (Build 2154, Waters). The retention time of the sample was compared with that of the standard GABA. GABA in brain was quantified in terms of ng/mg brain tissue by plotting a standard curve of GABA (24, 25).

Statistical Analysis

The data were subjected to one-way analysis of variance (ANOVA) followed by *post hoc* Duncan's multiple comparison test and Students t-test wherever applicable (SPSS 16.0 version). Comparison where $P<0.05$ was considered as statistically significant. Standard curves were plotted in MS Excel 2007 for the matter of convenience.

RESULTS

Preliminary Phytochemical analysis and yield percentage

The extraction carried out by soxhelet unit result a yield of 62 per cent of the dry powder of GAE. Soxheletation was done at higher temperatures so as to extract maximum number of the secondary metabolites present in the mushroom. Aqueous extract of the mushroom have shown the presence of various secondary metabolites expected in good concentrations as indicated by the yield percentages presented in Table 1.

FTIR studies

FTIR spectroscopy is currently used to investigate the vibrations of molecules and polar bonds between different atoms on the basis of wave number of bands. The absorption intensity can be used for calculating the relative concentration. In the present study,

FTIR spectra of GAE are shown in Fig. 1. The typical signal pattern expected for phenolic compounds and several bands in other regions are present.

Signals acquired from FTIR (Fig. 1) were prominent and intense at the wave numbers (1/cm) 1350-1470 (variable; bending) corresponding to alkanes, 1500 and 1600 (weak; strecth) corresponding to aromatic rings, 1640-1680 (weak, medium; stretch) corresponding to alkenes, 1670-1760 (strong; stretch) indicating the presence of aldehydes, ketones, carboxylic acids and esters, 2500-3000 (broad; stretch) corresponding to carboxylic acids, 2850-2960 (strong; stretch) as alkanes and 3200-3600 (broad; stretch) corresponding to alcohols and phenols. Therefore, from these signals it can be interpreted that both the extracts contain polysachharides, flavonoids and other phenolic compounds in essential thus partially supporting the preliminary phytochemical analysis.

Mass spectrometric characterization of GAE

The extract was analyzed by LC-MS which provided sufficient data for tentative component identification. The molecular mass information of components and formation of adducts were obtained. Their molecular weights ranged from 200 Da upto 1000 Da which clued that compounds were mainly flavonoids, phenolic compounds and terpenoids. Compounds which have been tentatively identified on the basis of m/z values, formation of adducts, neutral losses and protonated molecular masses are presented in Figure 2 (A,B,C,D,E,F).

Anticonvulsant activity

MES induced convulsions

All the control animals after delivering electroshock to them exhibited seizures, loss of righting reflex with tonic forelimb and hindlimb extension, flexor, clonus and stupor phases of the convulsion. The duration of THLE and whole convulsive episode including flexor, clonus and stupor are presented in Figure 3 and 4. Lower doses of GAE showed insignificant reduction and at higher concentrations were found much more effective ($p<0.001$) against THLE and convulsion induced by MES when compared with control. At 500 mg/kg dose

GAE nearly abolished tonic hind limb extension in rats as that of positive control (PC), Phenytoin sodium. No mortality was observed in any of the rats delivered MES. However, incidence of seizures was found to vary in different groups of MES animals (Table 2).

PTZ induced convulsions

The results showed that i. p. administration of GAE led to a significant delay ($p<0.001$) in the onset of PTZ induced myoclonic absence seizures. At lower and higher doses both the mushroom extracts have shown significant reduction in the duration of the seizures Figure 5 and 6. Mortality per cent was reduced by PC and GAE500. In PC and GAE500 groups, 50% of animals showed incidence of seizures. Lower dose treatments of mushroom extracts and Control showed 100 % incidence each (Table 2).

HPLC determination of GABA

HPLC provided a convenient method for the determination of GABA content in brain tissue by plotting area units (AU) versus concentration of standard GABA (fig. 7). In MES assay, none of the groups showed any significant variation in GABA concentrations. Results presented in (table 2) have shown increase in the GABA levels (ng/mg wet tissue) of treated animals only in PTZ assay. GAE at higher doses showed significant increases in GABA level when compared with control (0.064 ng/mg).

DISCUSSION

The powdered fruiting bodies of mushroom were extracted with ethanol in soxhelet apparatus unit in order to obtain higher yields and maximum number of bioactive principles accumulated by the mushroom. Consequently, percentage yields were higher and phytochemical characterization was able to provide thorough information regarding the identification of bioactive compounds. For the last two-three decades, hyphenated instrumentation like LC-MS, GC-MS, LC-NMR have been implemented on a large scale to get clear insights in the identification of secondary metabolites from natural sources. Preliminary phytochemical analysis in present study was

further supported by FTIR and LCMS in the identification of compounds present in aqueous extracts of *Ganoderma* mushrooms. The compounds reported here have been tentatively identified on the basis of FTIR signals, m/z ratios, formation of product ions and adducts, and molecular mass of the compounds in particular by matching them with the previously reported compounds, Mass Bank Database, Phenol explorer and Dictionary of natural products. Although available literature reports the presence of huge array of compounds (polysachharides and terpenoids in particular) in *Ganoderma* species (26, 27, 28), there is indeed deficient work indicating flavonoids and other phenolic compounds in *Ganoderma.* We report here identification of flavonoids, phenolic compounds, and terpenoids as well on the basis of FTIR and LC-MS characterization. Triterpenoids and steroids have been reported to possess anticonvulsant activity (29). Thus, our study is assumed a primary step in characterizing the compounds present in the GAE extract.

Acute toxicity study (ATS) showed that lower (250 mg/kg) and higher (500 mg/kg) concentrations of GAE did not produce toxic or lethal effects; hence no mortality was seen during ATS.

The present study demonstrates that aqueous extract of mushroom have its effects against acute seizures triggered by GABA receptor antagonist (PTZ) and glutaminergic excitation inducers (maximal electro shock, MES) and, lipid peroxides produced in seizure induced tissue. Chemical seizure model induced by PTZ represents a valid and widely accepted model for generalized myoclonic and absence seizures. PTZ may cause seizures by inhibiting chloride channel associated with $GABA_A$ receptors (30, 31). Contrarily, MES test is considered as a characteristic physical model for generalized tonic-clonic seizures (32).

In this study, GAE had protective effects in above mentioned models of epilepsy. Phenytoin is one of the drugs effective at blocking seizures induced by MES. It is established that drugs like phenytoin display high affinity in blocking MES-induced seizures due to their greatest affinity at blocking voltage-sensitive sodium channels. It is

possible that GAE also showed similar effects by modulating the voltage gated sodium channels. While GAE showed its dose dependent protection against THLE and duration of the MES induced seizure, at higher dose GAE presented much pronounced effect against THLE than those of vigabatrin (2000mg/kg), an established AED (33). However, protection determined against THLE could not be explained on the basis of GABA levels of brain tissue in MES model. Following high dose PTZ (80 mg/kg) induced myoclonic and petit mal (absence) seizure types in all the animals. The PTZ model has been known to lead the discovery of the anticonvulsant drug valproic acid. It is also known that AEDs like valproic acid have the propensity to block PTZ-induced seizures due to their action on $GABA_A$ receptors or block thalamic T-type calcium ion channels. GAE extract significantly delayed the time for the onset of seizure and appeared to be more effective against PTZ-induced seizures. GAE at both doses prolonged the latency to onset and reduced the duration of the seizure. *G. lucidum* extracts reported in earlier studies inhibited sympathetic nerve activity in anaesthetized animals (34), elevated pain threshold, prolonged death time induced by caffeine, and relaxed skeletal muscle in mice (35). Furthermore, it has been shown to improve the insomnia severity scores in patients with neurasthenia (36). GAE produced the latency to onset and reduction of the duration in a dose dependent manner. The critical role of GABAergic stimulation of brain cells has been well established. Enhanced levels of endogenous GABA during epileptic absence seizures acts as a defence mechanism to terminate ongoing seizure activity. Valproic acid is known to exert its anticonvulsant effect by interacting with voltage dependent NA+ channels in addition to the GABA mediated inhibition (37,38). Hence, the protective effects of the mushroom GAE and higher dose in particular could be explained on the basis of elevated endogenous GABA concentration which abolished seizures. It is notable from the results that in MES none of the deaths were observed, while in PTZ higher doses of GAE protected more than 50% animals from epileptic mortality. Both mushroom extracts being efficacious in MES and PTZ models reflect

potential pharmaceutical applications for grand mal (THLE) and petit mal (absence) seizures.

The anticonvulsant efficacy of the test mushroom could also be explained on the basis of presence of phytochemicals like polysaccharides, terpenoids and flavonoids and their derivatives revealed by mass spectrometry. Extensive work on anticonvulsant potential of these bioactive compounds has been carried out (39). Nevertheless, flavonoids have been mostly linked to the anticonvulsant effects (40, 41). Recently, Jager and Saaby (42) have reviewed the protective role of flavonoids like apigenin, quercetin, kaempferol, rutin, naringenin, hesperidin, epicatechin and their derivatives in various central nervous system (CNS) disorders. As per the review, the flavonoids present in food and medicinal plants are consumed by humans; upon absorption aglycones and conjugates of flavonoids pass through the blood brain barrier. Furthermore, certain classes of flavonoids and their glycosides bind to benzodiazepine sites on the $GABA_A$ receptor thus producing anticonvulsant and central depressant effects (43, 44, 45). As per our results, GAE500 in particular is likely to abolish convulsions due to the binding of some bioactive flavonoids to benzodiazepine sites or elevating GABA concentrations thereby inhibiting hyperexcitation of neuronal discharges. Present state of knowledge of chemical constituents do not allow to attribute with certainity its anticonvulsant effect of any of the compounds identified herein the study. It is worthy to isolate the bioactive principles which are responsible for these activities, if this study is assumed to be a preliminary step.

CONCLUSIONS

It is clear from the study that test mushroom extracts showed protective effects against PTZ and MES seizure models which are widely used to determine the anticonvulsant activity in vivo. Though GAE have the anticonvulsant potential at lower doses but higher produced much pronounced effects against both types of convulsions. Polysaccharides, terpenoids, and flavoinoids were identified by

advanced techniques which could be possibly responsible for the activities. It is supposed to be a novel study in that *Ganoderma* has never been demonstrated to have anticonvulsant activity though a huge number of bioactive compounds have been isolated and identified in mushroom extracts. Furthermore, *Ganoderma* has been characterized for the first time and a number of flavonoids could be identified tentatively along with a thoroughly studied biological activity. However, more extensive studies are suggested to evaluate the precise mechanism of the bioactive compounds responsible for the anticonvulsant activity.

REFERENCES

1. Sanmee R, Dell B, Lumyong P, Izumori K, Lumyong S. Nutritive value of popular wild edible mushroom from northern Thailand. *Food Chem*. 2003; 82: 527–532.
2. Mattila P, Konko K, Eurola M, Pihlava JM, Astola J, Vahteristo L. Journal of Agricultural and Food Chemistry, 2001; 49.
3. Wagay JA, Javed J, Vyas D. Antihyperglycemic and insulin enhancing potential of *Morchella esculenta*. Research Journal of Agricultural sciences 2011;2 (3) 482-487.
4. Sheikh IA, Vyas D, Ganaie MA, Dehariya K, Singh V. HPLC determination of phenolics and free radical scavenging activity of ethanolic extracts of two polypore mushrooms. Int J Phar Pha Sci. 2014; 6(2): 679-684.
5. Dehariya P, Vyas D. Diversity of Mushrooms in Patheriya forest of Sagar Region. II Elixir Applied Botany 2013; 63: 18401-18405.
6. Dehariya P, Chaubey A, Vyas D. *Pleurotus sajor-caju*: an alternative source for bioconversion of wastes. *Mush Res*. 2010;19: 90-93
7. Wasser SP, Weis AL. Medicinal Mushrooms. *Ganoderma lucidum*, (Curtis: Fr.), P. Karst; Nevo, E., Eds. Peledfus Publ House: Haifa Israel 1997; 39.

8. Chang ST, Buswell J. *Ganoderma lucidum* (Curt.: Fr.) P. Karst. (Aphyllophoromycetideae)-a mushrooming medicinal mushroom. Int. J. Med. Mushrooms 1999; 1 (2): 139–146.
9. McKenna DJ, Jones K, Hughes K. Reishi Botanical Medicines. The Desk reference for Major Herbal Supplements, 2nd Ed.; The Haworth Herbal Press: New York London Oxford 2002; 825–855.
10. Jong SC, Birmingham JM. Medicinal benefits of the mushroom *Ganoderma*. Adv Appl Microbiol 1992; 37: 101–134. Translated.
11. Gao Y, Zhou S, Chen G, Dai X, Ye J. A phase I=II study of a *Ganoderma lucidum* (Curt. Fr.) P. Karst. Extract (ganopoly) in patients with advanced cancer. Int J Med Mushrooms. 2002;4(3): 207–214.
12. Wasser SP. Medicinal mushrooms as a source of antitumor and immunomodulating polysaccharides. Appl Microbiol Biotechnol. 2002; 60: 258–274.
13. Mau JL, Lin HC, Chen CC. Antioxidant properties of several medicinal mushrooms. J Agric Food Chem. 2002; 50: 6072-6077.
14. Lin JM, Lin CC, Chen MF, Ujiie T, Takada A. Radical scavenger and antihepatotoxic activity of *Ganoderma formosanum, Ganoderma lucidum* and *Ganoderma neo- japonicum.* Journal of Ethnopharmacology. 1995; 47: 33-41.
15. Jia J, Zhang X, Hua YS, Wua Y, Wang OZ, Li NN, Guo QC, Dong XC. Evaluation of in vivo antioxidant activities of *Ganoderma lucidum* polysaccharides in STZ-diabetic rats. Food Chemistry. 2009; 115: 32–36.
16. Ping CX, Yan C, Bing LS, Guo CY, Yun LJ, Ping LL. Free radical scavenging of *Ganoderma lucidum* polysaccharides and its effect on antioxidant enzymes and immunity activities in cervical carcinoma rats. Carbohydrate Polymers. 2009; *77*: 389–393.

17. Liu W, Wang H, Pang X, Yao W, Gao X. Characterization and antioxidant activity of two low-molecular-weight polysaccharides purified from the fruiting bodies of *Ganoderma lucidum*. Int J Biol Macromol. 2010; 46:51–457.
18. Kozarski M, Klaus A, Niksic M, Jakovljevic D, Helsper JPFG, Griensven LJLDV. Antioxidative and immunomodulating activities of polysaccharide extracts of the medicinal mushrooms *Agaricus bisporus, Agaricus brasiliensis, Ganoderma lucidum* and *Phellinus linteus*. Food Chemistry. 2011; 129: 1667–1675.
19. Harbone JB. Phytochemical Methods. Chapman and Hall. Ltd., London. 1973; 49-188.
20. Lorke D. A new approach to acute toxicity testing. Archives of Toxicology. 1983; 54:275– 287.
21. Swinyard EA, Kupferberg HJ. Antiepileptic drugs: detection, quantification and evaluation. Federation Proceedings. 1985; 44(3).
22. Sayyah M, Saroukhari G, Peirovi A, Kamalinejad M. Analgesic and anti-inflammatory activity of the leaf essential oil of *Lauraus nobilis* Linn. Phytother Res. 2002; 17:733– 736.
23. Swinyard EA. Laboratory evaluation of antiepileptic drugs: Review of laboratory methods. Epilepsia. 1969; 10:107–119.
24. Khushawar MY, Rajper AD. Liquid chromatographic determination of γ-aminobutyric acid in cerebrospinal fluid using 2-hydroxynaphthaldehyde as derivatizing reagent. J Chromatogr B 2003; 788: 413-418.
25. Nayak P, Chatterjee AK. Dietary protein restriction causes modification in aluminum- induced alteration in glutamate and GABA system of rat. Neurosci. 2003; 4: 1-8.
26. Muhsin TM, Al-Duboon AH, Khalaf KT. Bioactive compounds from a polypore fungus ganoderma applanatum (per s. ex Wallr.) Pat. Jordan Journal of Biological sciences. 2011; 4: 205-212.
27. Min BS, Gao JJ, Nakamura N, Hattori M. Triterpenes from the spores of Ganoderma lucidum and their Cytoxicity against

Meth-A and LLc Tumour cells. Chem Pharm Bull. 2000; 48(7):1026-1033.

28. Nishitoba T, Sato H, Sakamura S. New terpenoids, Ganolucidic Acid D, Ganoderic cid l, Lucidone C and Lucidenic Acid G, from the fungus Ganoderma lucidum. Agric Biol Chem. 1986; 50(3): 809-811.
29. Barua CC, Begum SA, Barua AG, Borah RS, Lahkar M. Anxiolytic and anticonvulsant activity of methanol extract of leaves of *Alternanthera brasiliana* (L.) Kuntze (Amaranthaceae) in laboratory animals. Indian J Exp Biol. 2013; 51(6): 450-457.
30. Almeida RN, Navarro DS, Assis TA, Medeiros A, Thomas G. Antidepressant effect of an ethanolic extract of the leaves of *Cissampelos sympodialis* in rats and mice. *J Ethnopharmacol.* 1998;*63:* 247-252
31. Ngo Bum E, Schmutz M, Meyer C, Rakotonirina A, Bopelet M, Portet C, Jeker A, Rakotonirina SV, Olpe HR, Herrling P. Anticonvulsant properties of the methanolic extract of *Cyperus articulatus* (Cyperaceae). *J Ethnopharmacol.* 2001;*76*: 145-150)
32. Loscher W, Schmidt D. Which animal models should be used in the search for new antiepileptic drugs? A Proposal based on experimental and clinical Considerations. Epilepsy research. 1988; 2:145-181.
33. Loscher W, Schmidt D. Strategies in antiepileptic drug development: is rational drug design superior to random screening and structural variation? *Epilepsy Res.* 1994; 17:95-134.
34. Lee SY, Rhee HM. Cardiovascular effects of mycelium extract of Ganoderma lucidum: inhibition of sympathetic outflow as a mechanism of its hypotensive action. Chem Pharm Bull 1990; 38: 1359–64.
35. Kasahara Y, Hikino H. Validation of the oriental medicine. Par 122. Central actions of adenosine, a nucleotide of Ganoderma lucidum. Phytother Res. 1987; 1: 173–6.

36. Tang W, Gao Y, Chen G, Gao H, Dai X, Ye J, et al. A Randomized, double-blind and placebo-controlled study of a Ganoderma lucidum polysaccharide extract (ganopoly) in neurasthenia. J Med Food 2005; 8: 53–8.
37. McLean MJ. In vitro electrophysiological evidence predicting anticonvulsant efficacy of memantine and flunarizine. POL J Pharmacol. 1987; 39: 513
38. Pauwels PJ, Leysen JI, Janssen PAJ. Ca*+and Na+ channels involved in neuronal cell death: Protection by flunarizine. Life Sci. 1991; 48: 1881-1893.
39. Librowski T, Czarnecki R, Mendyk A, Jastrzebska M. Influence of new monoterpenes homologues of GABA on the central nervous system activity in mice. Polish J Pharmacol. 2000; 52:317-321.
40. Rasilingam D, Duraisamy S and Subramanian R 2009 anticonvulsant activity of bioflavonoid gossypin. Bangladesh J Pharmacol. 4:51-54.
41. Rajnarayan K., Sripal RM, Chailuvadi MR, Krishna DR. Bioflavanoids classification, pharmacological, biochemical effect and therapeutic potential. Indian J Pharmacol. 2001; 32: 2-16.
42. Jager AK, Saaby L. Flavonoids and the CNS. Molecules 2011; 16: 1471-1485.
43. Marder M, Paladini AC. GABAA-receptor ligands of flavonoid structure. Curr Top Med Chem. 2002; 2: 853–867.
44. Marder M, Viola H, Wasowski C, Fernández S, Medina JH, Paladini AC. 6-Methylapigenin and hesperidin: new valeriana flavonoids with activity on the CNS. Pharmacol. Biochem. Behav. 2003; 75: 737–745.
45. Johnson GAR. GABAA receptor channel pharmacology. Curr Pharm Res. 2005; 11: 1867– 1885.

TABLES AND FIGURES

Table 1: Yield percentage and Phytochemistry of the GAE

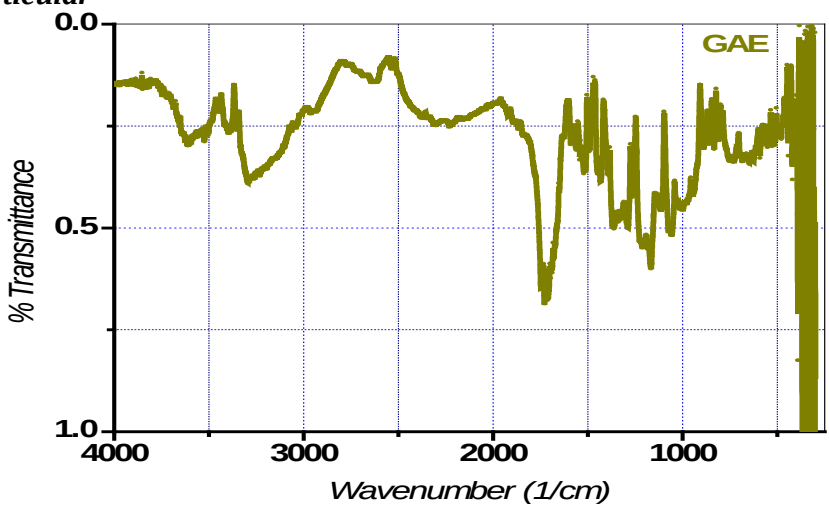
Extract	Yield percentage	Polysaccharides	Alkaloids	Steroids	Terpenoids	Flavonoids	Saponins	Anthocyanins	Phenolic acids
GAE	62%	+	-	-	+	+	-	-	+

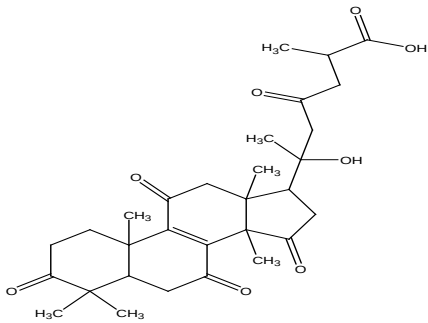
+ present; -- absent; GAE *Ganoderma* aqueous extract

Table 2: Seizure Incidence, Mortality and GABA content of animals with different treatments.

PTZ Groups	Incidence	Mortality (%)	GABA content	MES Groups	Incidence	Mortality (%)	GABA content
NS	0	0	0.254	NS	0	0	0.254
NC	6/6	66.6	0.064	NC	6/6	0	0.040
PC	3/6	16.6	0.181	PC	6/6	0	2.1
GAE250	6/6	50.0	0.126	GAE250	6/6	0	1.4
GAE500	3/6	16.6	0.172	GAE500	6/6	0	0.091

Fig. 1: FTIR showing the presence of carbonyl compounds in particular





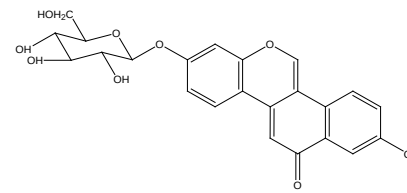
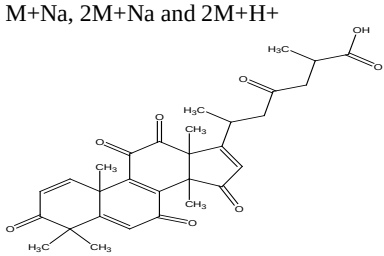
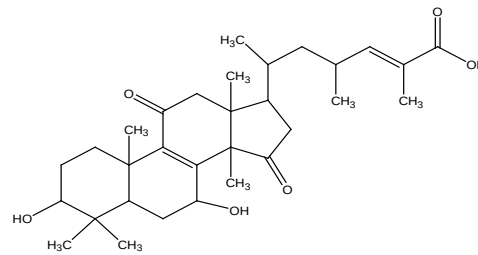


Fig. 2: LCMS characterization of the extract. A,B,C,D,E and F are compounds identified in the aqueous extract GAE on the basis of molecular ion peaks M+H+, M+Na, 2M+Na and 2M+H+

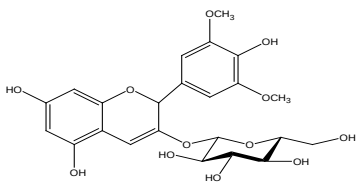
(A) 12-Hydroxy-3, 7, 11, 15, 23-pentaoxolanost-8-en-26-oic acid. m/z = 528.20



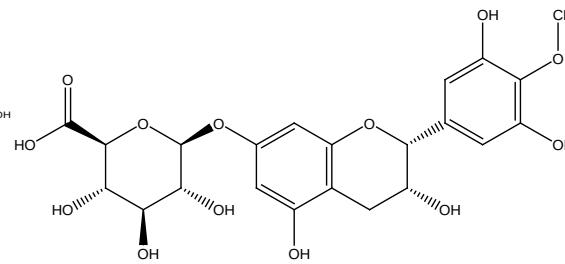
(B) 7, 4'-Dihydroxyisoflavone 7-O-glucoside; m/z=416.2



(C) 3, 7, 11, 12, 15, 23-Hexaoxolanost-8-en-26-oic acid; m/z=526.20

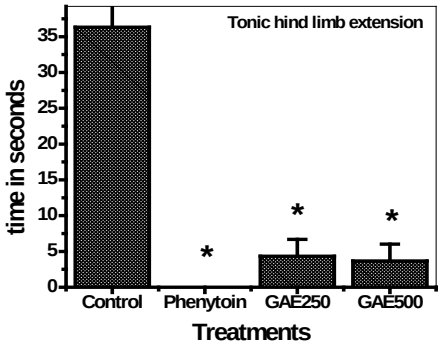


(D) 3, 7, 15-Trihydroxy-11, 23-dioxolanosta-8, 20 (22)-dien-26-oic acid; m/z = 512.20

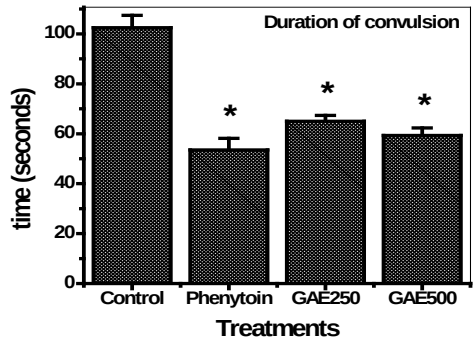


(E) Malvidin 3-O-glucoside ($C_{23}H_{25}O_{12}$); m/z = 493.20

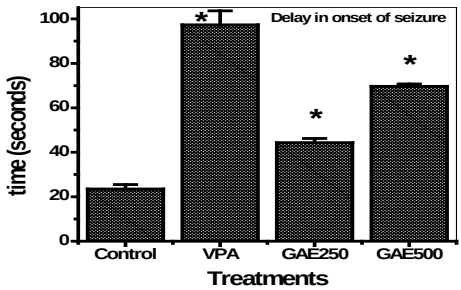
(F) 4'-O-Methyl-(-)-epigallocatechin 7-O-glucuronide (C22H23O13); m/z = 496.20



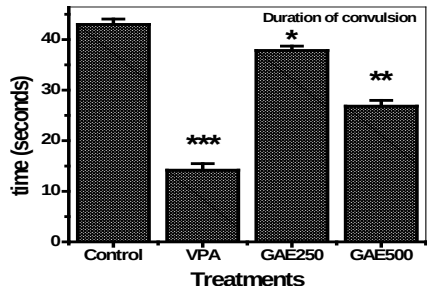
Values are mean±s.d.; *$p<0.001$*
Fig. 3: Protective effect of GAE on MES induced tonic hind limb seizures in rats.



Values are mean±s.d.; *$p<0.0001$*
Fig. 4: Protective effect of GAE on MES induced duration of convulsion in rats.



Values are mean±s.d.; *$p<0.001$*
Fig. 5: Protective effect of GAE as delay in onset on PTZ induced seizures in rats.



Values are mean±s.d.;
*$p<0.05$,**$p<0.01$,***$p<0.001$*
Fig. 6: Protective effect of GAE as duration of convulsion on PTZ induced seizures in rats.

Fig. 7: GABA standard curve