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Investigation of Anticonvulsants Activity of Leave Extract of Lawsonia Inermis in Wistar Rats

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Epilepsy, Phytochemical, Medications, Antioxidants

ABSTRACT:

Due to the unpleasant effects and limited effectiveness of antiepileptic medications, epilepsy—one of the most prevalent severe neurological disorders—is a leading cause of morbidity and mortality. People often look to medicinal plants as a possible gold mine for novel chemicals with healing properties. A. Rich's L. inermis Traditional Ethiopian medicine makes use of a herb called vatke to cure epilepsy. Nevertheless, there has been no scientific study conducted on its anticonvulsant properties. Hence, the purpose of this work was to assess the anticonvulsant activity of L.inermis (A. Rich.) Vatke's solvent fractions and 80% methanol root bark extract in mice. Both the pentylenetetrazole and maximal electroshock-induced seizure tests showed a substantial anticonvulsant effect ($p < 0.001$) when administered at higher doses of ME400 and BF400 compared to the control. Nonetheless, in the PTZ-induced seizure test, the chloroform fraction exhibited a considerable anticonvulsant effect ($p < 0.001$), whereas the aqueous fraction exhibited the lowest anticonvulsant activity in both seizure-induced tests. Leaf extract from L.inermis (A. Rich.) was found to include tannins, alkaloids, saponins, flavonoids, phenols, steroids, and terpenoids, according to phytochemical screening.

1. Introduction

The World Health Organization, also known as the WHO, and the entities that collaborate with it have determined that the epidemic of epilepsy remains a significant public health concern. Hyperexcitability and an imbalance between excitation and inhibition are two of the defining characteristics of the disorder known as epilepsy, which ultimately leads to seizures1. Epilepsy is one of the most prominent neurological illnesses that impact people all over the world, as stated by the World Health Organization (WHO). Epilepsy affects around fifty million people all over the world, making it one of the most widespread neurological conditions. The neurological illness known as epilepsy is marked by recurrent seizures that are brought on by a sudden increase in the electrical activity of the brain. Epilepsy is a condition that affects the brain. The explanation for this phenomenon is that neuronal discharges that are abnormal or hyperexcitability of neurons that are synchronised, respectively, account for this phenomenon. It is essential to keep in mind that the frequency of epileptic seizures varies substantially from one individual to the next. There are a number of different reasons that can lead to epilepsy, which is a neurological illness. There is a possibility that epileptic seizures could cause damage to the brain or other regions of the body for that matter. Abnormal neuronal activity is the root cause of these seizures, which are marked by jerky or shaking motions in the body. These seizures are characterised by abnormal movements in the body. It is difficult to get at good estimates of the incidence and prevalence of epilepsy because it is a complex undertaking to identify persons who may have epilepsy. This makes it difficult to arrive at correct numbers. It is estimated that between 362,000 and 415,000 persons in England are affected with epilepsy. This numbers falls somewhere in the middle of the range. In addition, there would be an increase in the number of individuals, estimated to be between 5 and 30 percent, which would amount to an extra 124,500

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people, who have been diagnosed with epilepsy but for whom the diagnosis is incorrect. There are around fifty cases of epilepsy for every one hundred thousand individuals in the United Kingdom each year, and the prevalence of active epilepsy is estimated to be between five and 10 cases for every one thousand persons in the country. When it comes to people who have active epilepsy, two-thirds of them have their epilepsy under control, which is considered to be satisfactory. One further potential method is to undergo surgical operations. Not only can optimal management improve health outcomes, but it also has the ability to assist in minimising additional ramifications, which are typically negative, on the activities of persons when it comes to their social lives, educational pursuits, and career opportunities. It is possible for even a single seizure to bring about changes in the development of the brain, which can then lead to changes in both cognitive and behavioural patterns. The presence of epileptic episodes is linked to the presence of unfavourable clinical characteristics.2. The patients' lives are significantly damaged as a result of these seizures, particularly those patients who endure repeated recurrences of the seizures. This is especially true for patients who have frequent seizures. Patients who suffer from epileptic seizures are more likely to encounter mental, behavioural, and neurological disorders as a consequence of themselves experiencing seizures. The manifestation of seizures can take place in a variety of different regions of the brain, and the degree of effectiveness is dependent on the characteristic area, the types of seizures, and the location of the brain in which abnormal neuronal activity is taking place. Seizures can be caused by a number of different factors.a. 1. Epileptic patients are subjected to social stigma and prejudice; the misconceptions and negative attitudes that society has towards this illness may prevent epileptic persons from receiving treatment and leading a life that is filled with self-assurance to the fullest extent possible. The International League Against Epilepsy (ILAE) revised previous classifications by utilising terminology and concepts that are suitable for the modern era in their most recent classification of epileptic seizures and epilepsies (epilepsy syndromes), which was published in 2010. Berg et al. (2010), Berg and Millichap (2013), and Muro and Connolly (2014) are some of the authors who contributed to this revision. There are three different forms of seizures: generalised seizures, focal seizures (which were traditionally more generally referred to as partial seizures), and epileptic spasms. Also known as epileptic seizures. Individuals who are affected by focal seizures have neural networks that are confined to a specific region of one hemisphere of the brain. Generalised seizures begin with neuronal networks that are distributed bilaterally. This is the starting point for the condition. The focal nature of a seizure may be present in the beginning, but it may eventually become generalised. There are two possible locations where a seizure can originate: the brain or the tissues that are positioned below the cortex. Through the utilisation of a detailed history, the outcomes of an electroencephalogram (EEG), and many other additional information, a physician is able to classify the type of seizure or epilepsy in a significant number of instances. Following this, a suitable diagnostic diagnosis and treatment plan are produced for the patient. The basic classifications that are included under the umbrella term of generalised seizures include absence seizures, generalised tonic-clonic seizures (GTC), myoclonic seizures, and atonic seizures.

2. Material and Methods

Extraction process

The leaves of the L.inermis plant were let to dry for two days in a shaded area at room temperature. After that, they were dried at temperatures ranging from 40 to 50 °C for three to four hours. The result was a coarse powder that was subsequently crushed. Using ethanol and Pertroleum ether, the Soxhlet extraction method was employed to get extracts from 980 grammes of L.inermis powder (EELI, PELI, EEPI and PEPI).

The parts of a soxhlet extractor are as follows: a thimble, a round-bottom flask, a syphon tube, a distillation channel, an expansion adapter, a condenser, plumbing for the intake and departure of cooling water, and a heat source. A porous "thimble" constructed of robust filter paper or cellulose is utilised in conjunction with the thimble chamber of the Soxhlet device to house the sample powder. The extraction solvent is heated in a round-bottomed flask using a heating mantle or other suitable heating source. It is the solvent that determines the temperature at which extraction takes place. The solvent at the base of the flask evaporates into the condenser as the temperature rises, and then it drips

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back into the sample thimble. The procedure is complete when the liquid reaches the syphon arm, since it is drained back into the bottom flask through the syphon tube, which contains a clear solution. One possible advantage of this method is that, instead of running the sample through the heated solvent numerous times, it only needs to be run through once. Thermolabile compounds should not be treated using this procedure due to the possibility of degradation caused by high temperatures. By reusing heat from the distillation flask, this process maintains a reasonably high extraction temperature. Filtering the extract is unnecessary, and the transfer equilibrium can be preserved by continuously adding new solvent to the solid matrix. The solvent was evaporated at low pressure in a Rotaevaporator to obtain a semisolid bulk once the extraction procedure was completed.

Phytochemical screening

There is a lot of potential medicinal use for phytochemicals that exhibit biological action. The following procedures were used to determine the secondary plant elements present in the ethanolic and petroleum ether extracts of the leaves of L.inermis:

Test for the presence of Reducing Sugars

The mixture was heated in a water bath for five minutes after adding 5 ml of a 1:1 ratio of Fehling's solution I (A) and Fehling's solution I1 (B) to 2 ml of the extract. Free reducing sugars were detected by the presence of a brick-red precipitate.

Test for the presence of Anthraquinones

10 millilitres of benzene was mixed with half a gramme of the extract, then filtered. 5 millilitres of a 10% ammonia solution was then added to the filtrate. Anthraquinone was detected when the ammonical (lower) phase took on a pink, red, or violet hue after shaking the mixture.

Test for the presence of Saponins

In a test tube, 10 millilitres of distilled water was added to dissolve half a gramme of the extract. The tube was then sealed with a cork, agitated violently for 30 seconds, and left to stand for 45 minutes. Saponins were detected by the presence of foaming, which remains when heated.

Test for the presence of FIavonoid

A few drops of a 10% ferric chloride solution were added to some of the dissolved extract. If the nucleus was phenolic, it would be green or blue in colour.

Test for the presence of Steroids and terpenes

After dissolving 0.5g of the extract in 2 ml of acetic anhydride, the mixture was chilled thoroughly on ice. A cautious addition of sulphuric acid followed. A steroidal nucleus was detected as the colour changed from violet to blue to green.

Test for the presence of Tannins

A small amount of 10% ferric chloride was added to a solution that included 0.5 g of the extract in 5 ml of water. When tannins are present, a precipitate that is blue-black, green, or blue-green in colour indicates their existence.

Test for the presence of Alkaloids

Mixing 0.5g of ethanol extract with Sml of 1% aqueous hydrochloric acid on a steam-bath produced a filtrate. Adding a few drops of Mayer's reagent to 1 ml of the filtrate and Dragendorff's reagent to another lml of filtrate completed the process. If the extracts show signs of turbidity or precipitation when treated with either of these reagents, it means that alkaloids are present.

Test for the presence of Resins

Before allowing the components to separate, 10 ml of petroleum ether extract was put to a test tube along with the same volume of copper acetate solution. The mixture was then agitated violently. The presence of resin is indicated by a green hue.

Test for the presence of Aldehydes and Ketones

Two millilitres of 95% ethanol and three millilitres of 2,4-dinitrophenylhydrazine reagent were added to thirty milligrammes of CA extract. After that, it was left alone for fifteen minutes. A good result for ketones and aldehydes is the presence of precipitate.

Test for the presence of Phenols

15 milligrammes of CA extracts were dissolved in half a millilitre of methylene chloride, and three to five drops of ferric chloride solution, which was made in the

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same solvent, were added. The mixture was then mixed with a drop of pyridine. If there were phenols, it would turn the mixture into a red wine.

3. Determination of Acute Toxicity

The process for categorising drugs according to their acute toxicity was done in line with the OECD-423 standards for category IV chemicals. Three male and one female albino rat were randomly selected from the bigger group for this study. Despite being told to starve for four hours, the animals were permitted to drink as much water as they wanted. Oral administration of rutin powder extracts was carried out at a dose no more than 2,000 mg/kg. We tracked the mortality rate for three days. A toxic dose was defined as one that caused mortality in at least two-thirds or three-quarters of the animals tested. Conversely, if only one rat out of three died, the same dosage was given again to make sure it had an adverse effect. Subsequently, the procedure was repeated, this time with an increased dosage. This measure was taken if the risk of death was not detected.

Experimental Design

There were a minimum of six rats each group in the eleven groups that were created. Each experiment utilised its own set of animals.

Table 1 Experimental design- PTZ induced epilepsy

S. No	Group Name	Treatment	Dose and Route
I	Normal control	Saline	1ml, Ip
II	Disease control	PTZ	60mg Ip.
III	Standard	Diazepam+ PTZ	10mg, Ip+60mg, IP
IV	Low Dose-EELI	EELI+PTZ	200mg, Po +60mg, Ip.
V	High Dose-EELI	EELI+PTZ	400mg Po+60mg Ip.
VI	Low Dose-PELI	PELI+PTZ	200mg, po+60mg Ip.
VII	High Dose-PELI	PELI+PTZ	400mg po+60mg Ip.
VIII	Low Dose-EEPI	EEPI+PTZ	200mg, po+60mg, Ip.
IX	High Dose-EEPI	EEPI+PTZ	400mg PO+60mg, Ip.
X	Low Dose-PEPI	PEPI+PTZ	200mg, PO+60mg Ip.
XI	High Dose-PEPI	PEPI+PTZ	400mg p.o+60mg,Ip.

Strychnine (STR) induced seizures

A frequently administered convulsant medication, strychnine causes tonic-clonic seizures characterised by a prominent tonic phase. When comparing this to seizures caused by other convulsants, there are two main differences: first, the tonic phase is more prominent, particularly in the extension of the hindlimbs; and second, antiepileptic medicines are unable to alleviate strychnine-induced seizures. Since strychnine's action mechanism is well-established, this model has a number of advantages. Strychnine blocks the action of chloride ionophore at glycine receptors, which are like GABAa receptors in a supramolecularcomplex. The primary inhibitory transmitter in the brainstem and spinal cord of higher animals was found to be glycine. An inhibitory postsynaptic potential's amplitude can be decreased by strychnine because it blocks chloride conductance caused by glycine.

Thirty minutes following drug delivery, rats were intraperitoneally injected with STR at a dose of 3.5 mg/kg [26]. We tracked how many convulsions occurred, how long they lasted, and when they started. We documented the percentage of death and protection at 10 minutes in addition to the convulsion signs and severity using the following scale.

Maximal Electroshock (MES)-Induced Seizure

The ECT unit (Ugo Basile, Italy) was used to administer a sinus wave stimulus (current intensity-70 mA, duration 0.2 s) as described in other research. Tonic hind limb extension (THLE) was monitored in the animals.

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Table 2 Experimental design- STR induced epilepsy

S. No	Group Name	Treatment	Dose and Route
I	Normal control	Saline	1ml, ip
II	Disease control	STR	3.5 mg,i.p.
III	Standard	Diazepam+ STR	10mg, i.p +3.5 mg,i.p.
IV	Low Dose-EELI	EELI+ STR	200mg, p.o+3.5 mg,i.p.
V	High Dose-EELI	EELI+ STR	400mg p.o+3.5 mg,i.p.
VI	Low Dose-PELI	PELI+ STR	200mg, p.o+3.5 mg,i.p.
VII	High Dose-PELI	PELI+ STR	400mg p.o+3.5 mg,i.p.
VIII	Low Dose-EEPI	EEPI + STR	200mg, p.o+3.5 mg,i.p.
IX	High Dose-EEPI	EEPI+ STR	400mg p.o+3.5 mg,i.p.
X	Low Dose-PEPI	PEPI	200mg, p.o+3.5 mg,i.p.
XI	High Dose-PEPI	PEPI	400mg p.o+3.5 mg,i.p.

Table 3 Experimental design- MES induced epilepsy

S. No	Group Name	Treatment	Dose and Route
I	Normal control	Saline	1ml, i.p
II	Disease control	MES	70 mA, duration 0.2 s
III	Standard	Diazepam+MES	10mg, i.p + 70 mA, duration 0.2 s
IV	Low Dose-EELI	EELI+ MES	200mg, p.o+ 70 mA, duration 0.2 s
V	High Dose-EELI	EELI+ MES	400mg p.o+70 mA, duration 0.2 s
VI	Low Dose-PELI	PELI+ MES	200mg, p.o+70 mA, duration 0.2 s
VII	High Dose-PELI	PELI+ MES	400mg p.o+70 mA, duration 0.2 s
VIII	Low Dose-EEPI	EEPI+ MES	200mg, p.o+70 mA, duration 0.2 s
IX	High Dose-EEPI	EEPI+ MES	400mg p.o+70 mA, duration 0.2 s
X	Low Dose-PEPI	PEPI+ MES	200mg, p.o+70 mA, duration 0.2 s
XI	High Dose-PEPI	PEPI+ MES	400mg p.o+70 mA, duration 0.2 s

One trial passive avoidance task

Following the procedure, a passive avoidance apparatus was used to assess memory retention deficits. The device had a guillotine door that joined two independent rooms. There was a light in one room and darkness in the other.

Both rooms had steel grids laid up on the floor for the purpose of administering electric shocks. In a nutshell, the light chamber was utilised for each animal during the acquisition trial. The initial latency (IL) to enter the dark chamber was recorded after a 60 s habituation interval, and the guillotine door separating the light and dark chamber was opened. Experiments were not conducted on animals with an initial delay period greater than 60 s.

Elevated plus maze

There is a quadrangular centre to the elevated plus maze, which is made up of a cross with two open arms and two closed arms. Perched above the ground, the labyrinth lies. Methods for evaluating learning and memory were based on that of Sharma and Kulkarni (1992). In the first trial, called the initial latency test, the animals were observed as they moved from one open arm to the other, with their backs to the central platform. The time it took for them to reach either of the enclosed arms was then recorded.

Biochemical Parameters

The animal parts that had been frozen in liquid nitrogen were thawed and then processed for the determination of glutathione (GSH, µg/g tissue), catalase (U/mg protein), and thiobarbituric acid reactive substances

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(TBARSs, nmol/g tissue) as a measure of malondialdehyde (MDA, nmol/g tissue). In a pH 7.4 phosphate buffer, a 10% homogenate of the tissues was made. Thiobarbituric acid reactive substances (TBARS) and reduced glutathione (GSH) assays were conducted using an aliquot. A centrifuge was used to spin the homogenate at 7,000 rpm for 30 minutes at 4°C.

Estimation of protein

The Bradford method (1976) was used to determine the protein content of the tissues. By adding 100 µl of homogenate to 900µl of phosphate buffer, the supernatant was diluted ten times. In a test tube, 10µl of the diluted supernatant was collected. The absorbance was measured at 595 nm in a double beam spectrophotometer against a blank after adding 50.0 µL of 0.1M NaOH and 1ml of Bradford reagent, vortexing the mixture, and letting it sit for 10 minutes.

Estimation of thio barbituric acid reactive substances (TBARS)

The methodology outlined was used for the estimation of TBARS. The premise of the test is that acetic acid separates the tissue's lipids and proteins. Sodium dodecyl sulphate dissolves the protein in the reaction mixture.

Estimation of reduced glutathione (GSH)

Using the described method, GSH was estimated. At a pH of 8.0, the reaction between an aliphatic thiol molecule and 5, 5'-dithio bis (2-nitrobenzoic acid) yields one mole of p-nitrothiophenol anion for every mole of thiol. This anion can be used to assess the concentration of thiol at 412 nm because of its colour, which is yellow.

Procedure

The glutathione standard was pipetted into separate reaction tubes together with 50.0 µl of each sample, and different aliquot amounts ranging from 1 to 10 nmol were used. Using 0.3M phosphate buffer (pH 8.0) to get the reaction mixture volume up to 3.0 ml, 0.6 mM dithio-bis (2-nitrobenzoic acid) synthesised in 1.0% trisodium citrate was added to generate colour. Within 10 metres, a double beam spectrophotometer measured the absorbance of the resulting yellow colour at 412 nm..

Calculations

The concentration was plotted against the absorbance measurements of the glutathione standard to create a linear standard curve. It was from this standard curve that the GSH concentration in the sample was extrapolated.

Catalase

The method of was used to estimate catalase. Fundamentals of Testing Hydrogen peroxide's absorption peak increases monotonically with decreasing UV wavelength in the visible spectrum. Decreased extinction at 240 nm is a direct indicator of H2O2 degradation. Catalase activity is measured by the difference in extinction per unit time.

Reagents

Phosphate buffer (50.0 mM at pH 7).

H_2O_2 (30.0 mM).

Estimation of Acetylcholinesterase activity in rat hippocampus

This approach was used to quantify acetylcholinesterase (AChE), a cholinergic marker, in the hippocampus of rats. Subanesthetic ether was used to decapitate the animals. The hippocampus was extracted, rinsed with cold saline, and then preserved in liquid nitrogen. Within 48 hours, the biochemical examination was carried out.

4. Result and discussion

Lawsonia inermis

Phytochemical Screening

Table 4: Phytochemical screening of extracts

	LI	
Metabolite	**Ethanolic extract**	**Petrolium Ether extract**
Phenols	+	+
Alkaloids	+	-
Tannins	+	+
Lipids	-	-
Amino acids	-	-
Saponins	-	-
Flavonoids	+	+
Resins	+	+
Steroids/Terpenes	-	-

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Acute Toxicity Study

Acute oral toxicity experiments conducted in accordance with OECD standards 423 demonstrated that EELI and PELI were not harmful. No symptoms of lethargy, poisoning, or abnormal behaviour were observed in animals that were administered 2000 mg/kg of EELI and PELI. The LD50 for EELI and PELI is greater than 2,000 mg/kg, according to the GHS classification system (OECD, 2001). The ED50 of EELI and PELI was found to be 200 mg/kg, and 400 mg/kg was also selected for the experiment, because the ED50 is often defined as 10% of the LD50. Table 2 and Table 3 provide the outcomes of the rat studies.

Table 5: Acute toxicity study of EELI and PELI at a dose of 2000 mg/kg

Parameters	1st hour	2nd hour	3rd hour	4th hour
Piloerection	-	-	-	-
Edema	-	-	-	-
Urine stains	-	-	-	-
Alopecia	-	-	-	-
Loss of writhing reflex	-	-	-	-
Circling	-	-	-	-
Nasal sniffing	+	+	+	+
Lacrimation	-	-	-	-
Seizures	-	-	-	-
Righting reflex	+	+	+	+
Grip strength	+	+	+	+
Eye closure at touch	+	+	+	+
Rearing	+	+	+	+
Straub tail	-	-	-	-

+=Present/affected, -=Absent/not affected

Table 6: Acute oral administration of EELI and PELI at a dose of 2000 mg/kg

Parameters observed	Day													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Piloerection	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Edema	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Urine stains	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Alopecia	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Loss of writhing reflex	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Circling	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Nasal sniffing	+	+	+	+	-	-	-	-	-	-	-	-	-	-
Lacrimation	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Seizures	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Righting reflex	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Grip strength	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Eye closure at touch	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Rearing	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Straub tail	-	-	-	-	+	+	+	+	+	+	+	+	+	+

+=Present/affected, -=Absent/not affected

Figure 1 shows that the groups treated with MES, PTZ, and STR had a significantly reduced retention delay in the open field (P 0.001). In a dose-dependent manner, the retention latency in the open field was considerably increased in the EELI and PELI treatment groups compared to the disease control groups, and these effects were preserved when compared to the standard drug Diazepam.

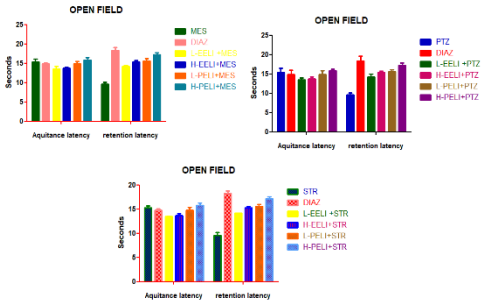

Figure 1. The impact of pharmaceuticals on open fields.

Data (Mean ± SEM) for each group was generated using six rats and subsequently analysed using ANOVA and Tukey's post hoc test. αp < 0.001, in comparison to the control group without disease; ap < 0.001, ; bp < 0.01 and ;cp < 0.05 in comparison to the group given Diazepam.

The retention delay in the Elevated Plus Maze was dramatically reduced (P < 0.001) in the groups that were treated with MES, PTZ, and STR, as illustrated in figure 2. When compared to the disease control group and the conventional drug Diazepam, the retention latency in the Elevated Plus Maze was considerably increased in the EELI and PELI treatment groups, and this impact was dose dependent.

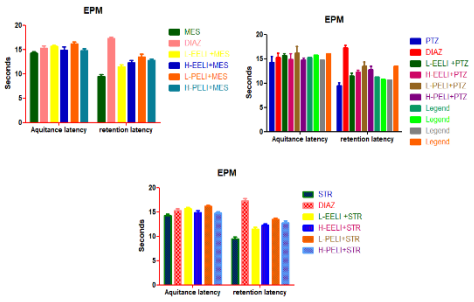

Figure 2. Effect of drugs on EPM

Data (Mean ± SEM) for each group was generated using six rats and subsequently analysed using ANOVA and Tukey's post hoc test. αp < 0.001, in comparison to the control group without disease; ap < 0.001, ; bp < 0.01 and ;cp < 0.05 in comparison to the group given Diazepam. The retention delay in passive avoidance

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was dramatically reduced (P < 0.001) in the groups treated with MES, PTZ, and STR, as illustrated in figure 3. Retention latency in Passive Avoidance was considerably increased (dose dependently) in the EELI and PELI treatment groups compared to the illness control groups, and these effects were restored when compared to the conventional medication diazepam.

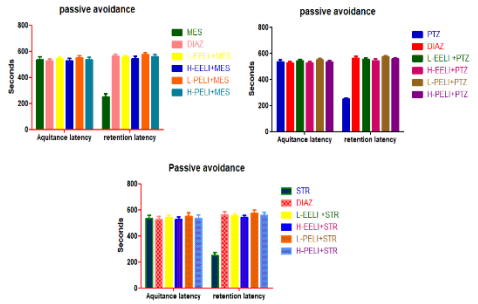

Figure 3. Effect of drugs on Passive Avoidance

Data (Mean ± SEM) for each group was generated using six rats and subsequently analysed using ANOVA and Tukey's post hoc test. αp < 0.001, in comparison to the control group without disease; ap < 0.001, ; bp < 0.01 and ;cp < 0.05 in comparison to the group given Diazepam.

The figure 3 shows that the MES, PTZ, and STR treated group experienced clonic seizures much more quickly than the standard group (P < 0.001). When compared to the illness control group, the EELI and PELI therapy groups considerably postponed clonic seizures, and both groups regained their properties to a greater extent than the control group.

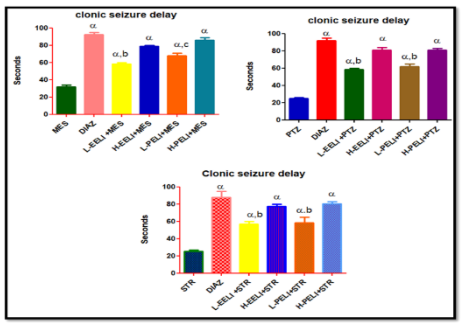

Figure 4. Effect of drugs on clonic seizure delay

Data (Mean ± SEM) for each group was generated using six rats and subsequently analysed using ANOVA and Tukey's post hoc test. αp < 0.001, in comparison to the control group without disease; ap < 0.001, ; bp < 0.01 and ;cp < 0.05 in comparison to the group given Diazepam.

The length of clonic seizures was considerably longer in the groups treated with MES, PTZ, and STR compared to the standard group (P < 0.001), as illustrated in figure 4. In contrast to the illness control group, the EELI and PELI therapy groups considerably reduced the duration of clonic seizures, and these groups also restored their properties to a level comparable to the standard group.

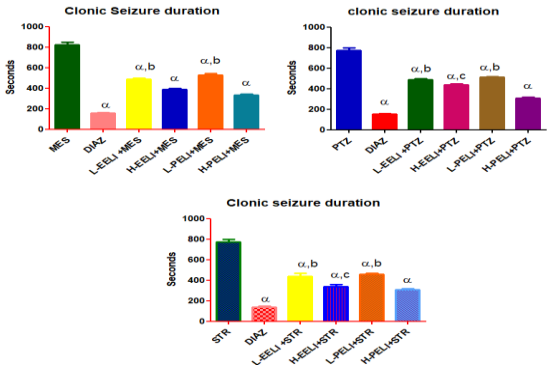

Figure 5. Effect of drugs on clonic seizure duration

Six rats were used to generate data (Mean ± SEM) for each group, which was then analyzed using ANOVA and Tukey's post hoc test. $^{\alpha}p < 0.001$, relative to the disease control group; $^{a}p < 0.001$, ; $^{b}p < 0.01$and ;$^{c}p < 0.05$ relative to Diazepam group. As shown in the figure 5 the tonic seizure duration significantly increased P < 0.001 in the MES, PTZ and STR treated group in comparison to standard group where as In the extracts of EELI and PELI treatment groups tonic seizure duration was decreased significantly in comparison to disease control group and in accordance with the standard group these groups restored their property significantly.

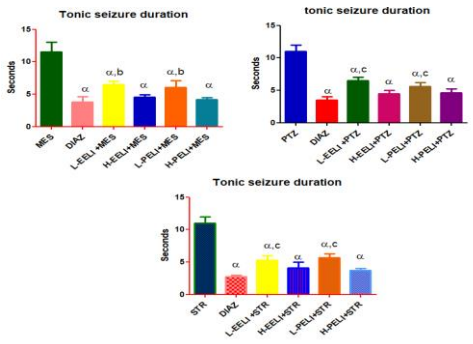

Figure 6. Effect of drugs on tonic seizure duration

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Data (Mean ± SEM) for each group was generated using six rats and subsequently analysed using ANOVA and Tukey's post hoc test. αp < 0.001, in comparison to the control group without disease; ap < 0.001, ; bp < 0.01 and ;cp < 0.05 in comparison to the group given Diazepam.

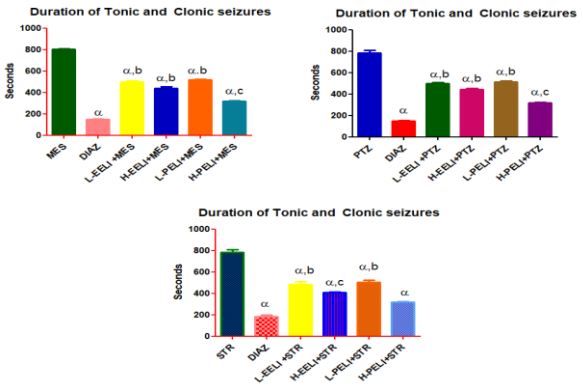

Figure 7. Effect of drugs on duration of tonic and clonic seizures

Six rats were used to generate data (Mean ± SEM) for each group, which was then analyzed using ANOVA and Tukey's post hoc test. $^{\alpha}p < 0.001$, relative to the disease control group; $^{a}p < 0.001$, ; $^{b}p < 0.01$and ;$^{c}p < 0.05$ relative to Diazepam group. As shown in the figure 8.a-e the neurotransmitters GABA, Seratonin, Dopamine, Ach levels were significantly increased P < 0.001 in the MES, PTZ and STR treated group where as the Glutamate and Noradrenalin levels were significantly decreased P < 0.001 in the MES, PTZ and STR treated group which is evidenced for the induction of Epilepsy. In the extracts EELI and PELI treatment groups the GABA, Seratonin, Dopamine, Ach levels were significantly decreased and the Glutamate and Noradrenalin levels were dose dependently increased significantly.

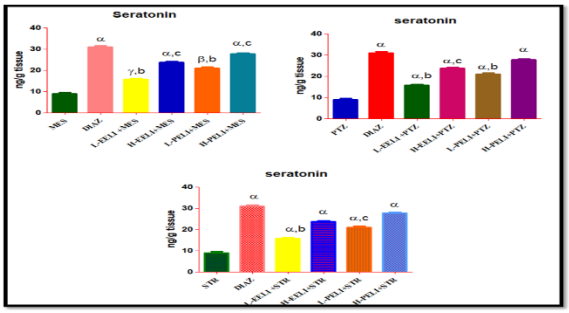

Figure 8.a. Effect of drugs on Seratonin

Six rats were used to generate data (Mean ± SEM) for each group, which was then analyzed using ANOVA and Tukey's post hoc test. $^{\alpha}p < 0.001$, relative to the disease control group; $^{a}p < 0.001$; $^{b}p < 0.01$and ;$^{c}p < 0.05$ relative to Diazepam group.

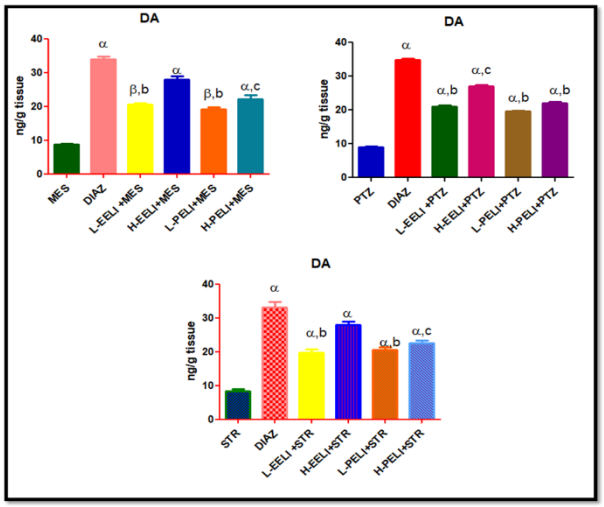

Figure 8.b. Effect of drugs on Dopamine

Six rats were used to generate data (Mean ± SEM) for each group, which was then analyzed using ANOVA and Tukey's post hoc test. $^{\alpha}p < 0.001$, relative to the disease control group; $^{a}p < 0.001$, ; $^{b}p < 0.01$and ;$^{c}p < 0.05$ relative to Diazepam group.

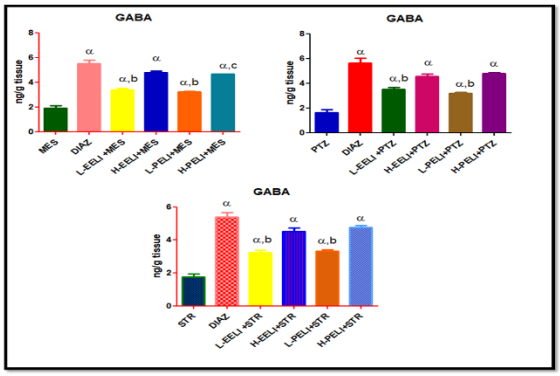

Figure 8.c. Effect of drugs on GABA

Six rats were used to generate data (Mean ± SEM) for each group, which was then analyzed using ANOVA and Tukey's post hoc test. $^{\alpha}p < 0.001$, relative to the disease control group; $^{a}p < 0.001$, ; $^{b}p < 0.01$and ;$^{c}p < 0.05$ relative to Diazepam group.

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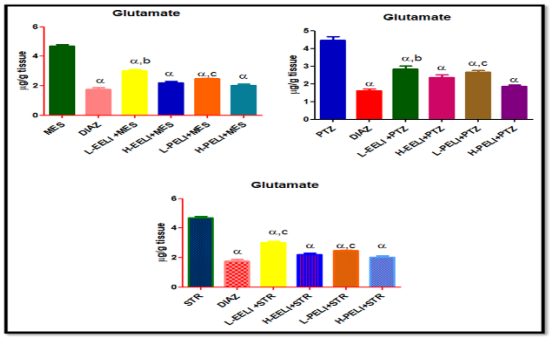

Figure. 8.d Effect of drugs on glutamate

Data (Mean ± SEM) for each group was generated using six rats and subsequently analysed using ANOVA and Tukey's post hoc test. * p < 0.001, in comparison to the control group without illness; * ap < 0.001, in comparison to the group given Diazepam; * bp < 0.01 and * cp < 0.05.

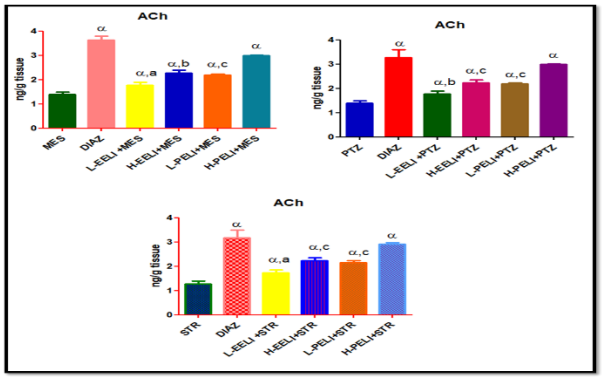

Figure 8.e Effect of drugs on Acetyl Choline

Data (Mean ± SEM) for each group was generated using six rats and subsequently analysed using ANOVA and Tukey's post hoc test. * p < 0.001, in comparison to the control group without illness; * ap < 0.001, in comparison to the group given Diazepam; * bp < 0.01 and * cp < 0.05.

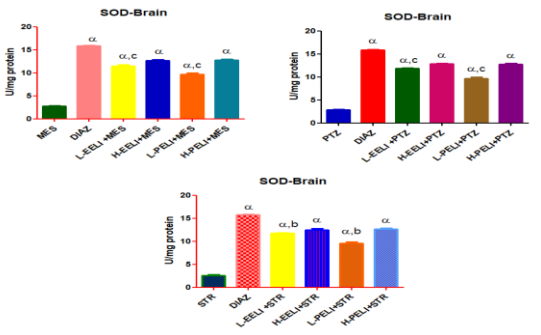

Figure 9. Effect of drugs on SOD-brain

Figure 9 shows that compared to the standard group, the MES, PTZ, and STR treated group had significantly lower brain SOD levels (P < 0.001). Brain SOD levels in the EELI and PELI therapy groups were much higher than in the disease control group, and these groups also showed a marked improvement in contrast to the control group's original state, as was the case with the standard group.

Data (Mean ± SEM) for each group was generated using six rats and subsequently analysed using ANOVA and Tukey's post hoc test. αp < 0.001, in comparison to the control group without disease; ap < 0.001, ; bp < 0.01 and ;cp < 0.05 in comparison to the group given Diazepam.

The figure shows that the brain GSH levels in the groups treated with MES, PTZ, and STR are considerably lower (P < 0.001) compared to the control group. Brain GSH levels in the EELI and PELI therapy groups were much higher than in the illness control group, and these groups also showed a marked improvement in contrast to the control group's original state.

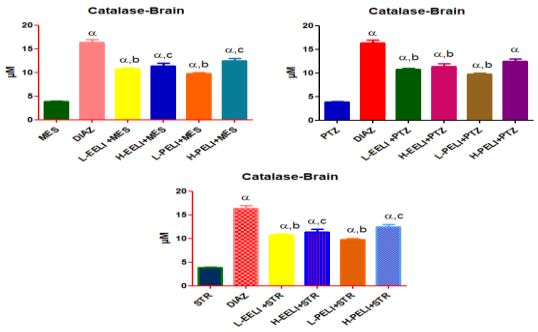

Figure 10. Effect of drugs on Catalase brain

Data (Mean ± SEM) for each group was generated using six rats and subsequently analysed using ANOVA and Tukey's post hoc test. αp < 0.001, in comparison to the control group without disease; ap < 0.001, ; bp < 0.01 and ;cp < 0.05 in comparison to the group given Diazepam.

Figure 10 shows that compared to the standard group, the MES, PTZ, and STR treated group had significantly lower brain MDA levels (P < 0.001). The brain MDA level was considerably higher in the EELI and PELI therapy groups compared to the disease control group,

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and these groups also showed a marked improvement in comparison to the standard group.

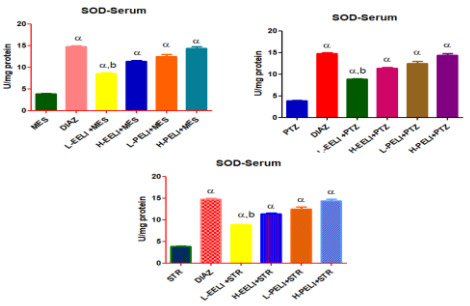

Figure 11. Effect of drugs on Serum SOD

Six rats were used to generate data (Mean ± SEM) for each group, which was then analyzed using ANOVA and Tukey's post hoc test. $^{\alpha}p < 0.001$, relative to the disease control group; $^{a}p < 0.001$, ; $^{b}p < 0.01$and ;$^{c}p < 0.05$ relative to Diazepam group.

The Serum catalase levels in the groups treated with MES, PTZ, and STR were considerably lower ($P < 0.001$) compared to the control group, as illustrated in figure 11. When compared to the disease control group and the standard group, the EELI and PELI treatment groups' serum catalase levels were much higher, and these groups also showed a marked improvement in their condition.

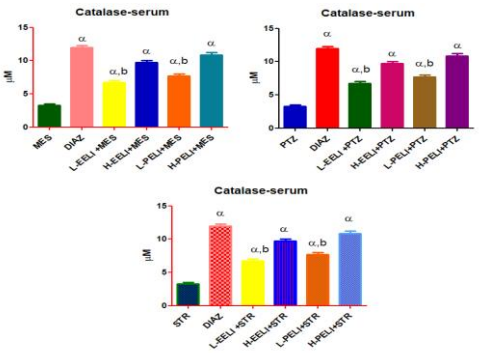

Figure.12. Effect of drugs on serum catalase

Data (Mean ± SEM) for each group was generated using six rats and subsequently analysed using ANOVA and Tukey's post hoc test. $\alpha p < 0.001$, in comparison to the control group without disease; $ap < 0.001$, ; $bp < 0.01$ and ;$cp < 0.05$ in comparison to the group given Diazepam.

Figure 12 shows that compared to the standard group, the MES, PTZ, and STR treated group had significantly lower serum GSH levels ($P < 0.001$). Compared to the illness control group, the EELI and PELI treatment groups' serum GSH levels were much higher, and these groups also showed a marked improvement in terms of restoring their properties, according to the standard group.

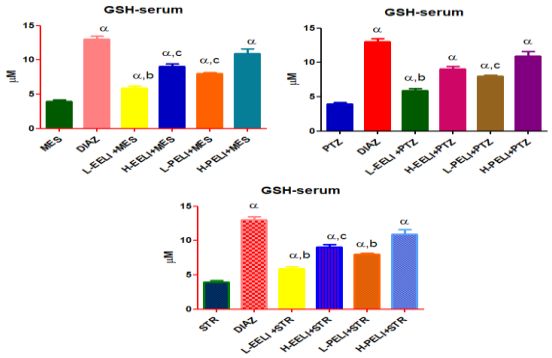

Figure 13. Effect of drugs on Serum GSH

Six rats were used to provide data (Mean ± SEM) for each group, which was then analysed using ANOVA and Tukey's post hoc test. $\alpha p < 0.001$ when compared to the disease-free control group; $ap < 0.001$, ; $bp < 0.01$ and ;$cp < 0.05$ when compared to the group that was administered Diazepam.

The control group had significantly higher serum MDA levels ($P < 0.001$) compared to the groups treated with MES, PTZ, and STR, as shown in figure 16. Both the EELI and PELI treatment groups had significant improvement as compared to the standard group, and their blood MDA levels were significantly higher than those in the disease control group.

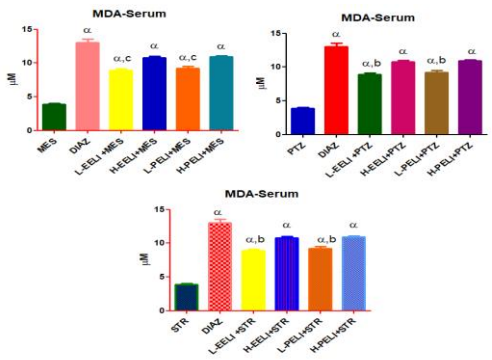

Figure 14. Effect of drugs on Serum MDA

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Data (Mean ± SEM) for each group was generated using six rats and subsequently analysed using ANOVA and Tukey's post hoc test. * $p < 0.001$, in comparison to the control group without illness; * $ap < 0.001$, in comparison to the group given Diazepam; * $bp < 0.01$ and * $cp < 0.05$.

Histopathological study of brain cortex of EEFP treated rats

Results from H&E x 400 staining showed that all treatment groups had cortical histological abnormalities.

A: The group that was treated with scopolamine shows neurodegenerative alterations (arrow), neurophagia, and gliosis.

In group B, there may be mild neurodegeneration (arrow) and neurophagia; in groups C and D, there is substantial neurodegeneration and neurophagia, even at modest doses of EELI and PELI (arrow).

Parts E and F When administered at high doses, EELI and PELI cause mild neuronal degeneration and significant perivascular edoema (arrow).

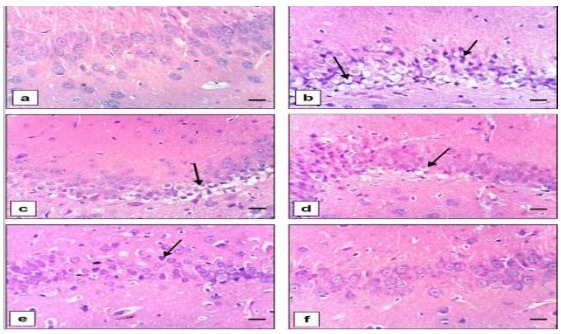

Figure 15 Histopathological changes in the brain cortex of EELI and PELI-treated rats.

5. Discussion

The results of the study suggest that both Lawsonia inermis and its derivatives contain abundant amounts of natural antioxidants that may have beneficial health effects. Among the several plant extracts tested, the ethanol and pertroleum ether samples showed the most potent antioxidant activity against a range of free radicals. Furthermore, rats treated with PTZ, STR, and MES showed strong neuroprotective effects against free radical toxicity when exposed to the ethanol and pertroleum ether extract of Lawsonia inermis, and these effects were dose-dependent. To further understand how Lawsonia inermis affects the induced behavioural pattern in rats, we employed the elevated plus maze (EPM) test, open field, and passive avoidance. Lawsonia inermis ethanol and petroleum ether extracts, when given orally to rats, improved their immunity against epilepsy.

The antioxidant and neuroprotective effects of Lawsonia inermis are the focus of this groundbreaking study, which is the first of its kind. According to the results, the antioxidant and neuroprotective properties of Lawsonia inermis ethanolic extracts are significantly higher than those of petroleum ether extracts. One probable conclusion from this study is that Lawsonia inermis contains components with strong antioxidant and neuroprotective properties. Neuroprotection is a consequence of the components' actions, which are linked to GABA activation and glutamate inhibition. To discover the active compounds included within these extracts and to learn how this extract exerts its neuroprotective benefits, additional research is needed.

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