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A Research Article On Evaluation of *Rubus ellipticus* Smith. (Hisalu) Leaf Extract for Antiepileptic Potential in Animal Models

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ABSTRACT

Epilepsy is a common neurological disorder marked by recurrent seizures linked to aberrant neuronal activity in the brain. Epilepsy results from the development of a neuronal hyperexcitability due to contagious injury of various causes, which leads to intermittent focal or generalized seizures. Literature survey reveals that *Rubus ellipticus* Smith. exhibits various activities like Antidiabetic, Antioxidant, Nephroprotective, Antitumour, Wound healing, Anti-inflammatory, Analgesic, Antiepileptic etc . Beside these, the Phytochemicals of *Rubus ellipticus* Smith. includes Flavonoids, Tannins, Saponins, Steroids, Carbohydrate, Phenolic compounds, glycosides, Triterpenes, ascorbic acid and antioxidant .

The present study has been conducted to evaluate the leaf extract of *Rubus ellipticus* Smith. for antiepileptic activity in various animal models i.e., Picrotoxin-Induced convulsion model and Electrically Kindled model. In Picrotoxin-induced convulsion model, a single dose of Picrotoxin (7.5mg/kg) i.p was administered to rats and diazepam (12mg/kg) i.p was used as an standard. In Electrically kindled seizure model, two subconvulsive electric shocks per day for 6 days using auricular electrodes (21mA, 0.1 sec) were given to animals and phenytoin (50mg/kg) i.p was used as an standard. The animals were divided into 4 groups, six animals in each group. According to OECD TG-423 (Acute oral toxicity), the animals received leaf extract of *Rubus ellipticus* Smith. at dose levels of 200 mg/kg and 400mg/kg B.W.

At the end of treatment, Lipid Peroxidation, Superoxide dismutase, NO, Reduced glutathione were estimated to evaluate the role of oxidative stress in epilepsy. GABA and calcium level was also evaluated at the end of treatment period. Histopathological study of brain was also done as the study provide supportive evidence for biochemical analysis.

Results of the study concluded that the high dose level of methanolic leaf extract of *Rubus elllipticus* Smith. (400mg/kg B.W.) has shown a marked reduction in oxidative stress and calcium level in the brain of epileptic rats and caused significant improvement in neurotransmitters like GABA and antioxidant enzymes like GSH and SOD. The methanolic leaf extract of *Rubus ellipticus* Smith. at a dose level of 400 mg/kg has shown the most significant ($P<0.001$) effect on both the models, except for the NO level in Picrotoxin-induced model. However, the methanolic leaf extract of *Rubus ellipticus* Smith. at a dose level of 200 mg/kg shown significant ($P<0.01$) in NO level in electrically induced kindling model and less significant ($P<0.05$) in NO level in Picrotoxin-induced convulsion model

Keywords: Epilepsy, *Rubus ellipticus* Smith., Kindling, Picrotoxin, Oxidative stress.

INTRODUCTION

Epilepsy is a common neurological disorder marked by recurrent seizures linked to aberrant neuronal activity in the brain. Epilepsy results from the development of a neuronal hyperexcitability due to contagious injury of various causes, which leads to intermittent focal or generalized seizures. Epilepsy is the third leading contributor to the global burden of disease for neurological disorders [1]. Epilepsy is a disease that can occur in all mammalian species, Perhaps more frequently as brains have become more complex. The common causes of seizures are Brain malformations, Lack of oxygen during birth, Head trauma, Stroke.

Epileptic seizures are divided into two kinds i.e., Partial seizures and Generalized seizures. Partial seizures have a single, localized origin in the brain, but they can spread to a small or big area, or even the entire brain. Generalized seizures affect the entire brain, including the reticular system, resulting in aberrant electrical activity in both hemispheres and are characterized by a sudden loss of consciousness [2]. A seizure results when a sudden imbalance occurs between the excitatory (Glutamate) and inhibitory (GABA) neurotransmitters within the network of cortical neurons. The effect of most of antiepileptic agents is to enhance the response to GABA (gamma amino butyric acid), by facilitating the opening of GABA-activated chloride channels [3].

In a state of oxidative stress, free radicals trigger a cascade of events leading to epileptogenesis. During this latent, free of seizures period, a cascade of neurological changes takes place and finally leads to spontaneous recurrent seizures [4]. The Brain is very much susceptible to degeneration and oxidative stress because of its low antioxidant enzyme activity [5]. Presently, Nox2 is considered as the main source of ROS at initial stages of epileptogenesis [4]. The detection and recognition of EEG signal are the most important means to diagnose epilepsy [6]. MicroRNA-134 is a brain-enriched small non-coding RNA involved in controlling neuronal microstructure and brain excitability.

Rubus ellipticus Smith. belonging to family Rosaceae is commonly known as Yellow Himalayan raspberry, Golden Himalayan raspberry, Hisalu/Hinsar [7]. About 750 or more than species of Rubus distributed worldwide as well as some regions of India. Globally, it is found in Asian and African continents and its nearby islands, including Sri Lanka [8]. In India, it is found native to the states of Assam, Sikkim, Tamil Nadu, Kerala and Maharashtra. Hisalu is mostly found in summer season in both Kumaon and Garhwal regions of Uttarakhand [7]. The blooming season of the plant is from March to April, whereas the fruiting season is from April to May bearing golden yellow fruits. *Rubus ellipticus* Smith. exhibits various Properties like Antidiabetic, Antioxidant, Nephroprotective, Antitumour, Wound healing, Anti-inflammatory, Analgesic, Antiepileptic, Antiprotozoal, Antipyretic because they are rich in carbohydrates, vitamins, proteins and minerals. The fruit is edible, Possess Antidiabetic and Antioxidant properties. Fever, stomach problems, diarrhoea, and dysentery are treated with the juice of the leaf and root [9]. The active constituents present in Hisalu are Flavonoids, tannins, carbohydrates, steroids, phenols, glycosides, mineral salts, ascorbic acid and vitamin C. The Pentacyclic Triterpene acid "elliptic acid" from the leaves of *Rubus ellipticus* Smith. has been isolated. Some derivatives of Kaempferol, quercetin,

gallic acid, ellagic acid, caffeic acid, Ursolic acid, Acuminatic acid, methyl gallate and methyl brevifolincarboxylate is also reported.

MATERIALS AND METHODS

PLANT MATERIAL

The *Rubus ellipticus* Smith. leaves were collected from Bhimtal region, Uttarakhand and authenticated from BSI, Dehradun with Accessioned No. 869.

PREPARATION OF EXTRACT

The leaves of the plant *Rubus ellipticus* Smith. collected was rinsed with distilled water. The leaves of the plant were air dried under shade for 12 days. The dried leaves were milled to a fine powder. Then further extracted through soxhlet apparatus using methanol for about 8-9 hours. The extract was collected, filtered and dried using the electrical water bath. The extract was stored in well closed container at 5˚C in refrigerator [10]

QUALITATIVE AND QUANTITATIVE PHYTOCHEMICAL SCREENING

Detection of Flavonoids

Shinoda test: Small piece of magnesium ribbon were added to a extract followed by drops of concentrated HCL. Pink- tomato red colour indicates the presence of flavonoids.

Detection of Tannins

Lead acetate test: The Extract was dissolved in water and to that lead acetate solution was added, it provides white precipitate.

Detection of Saponin

Foam test: Shake the drug extract vigorously with water. Persistant foam observed.

Detection of Steroids

Salkowaski: 2ml chloroform and 2ml conc. Sulphuric acid was added to 2ml extract, after shaken gives a red chloroform film and greenish yellow fluorescence of acid layer [11].

Flavonoid Estimation

For total flavonoid determination, quercetin was used to make the standard calibration curve. Stock quercetin solution (1000ppm) was prepared. Then the serial dilutions using methanol (100-1000µg/ml) was prepared. Stock test solution (1000ppm) was prepared. 1ml of aliquot was withdrawn from test solution and each dilution of standard transferred into test tube. 4ml of distilled water followed by subsequent addition of 0.3ml of 5% $NaNO_2$ and 0.3ml of 10% $AlCl_3$ was added in each test tube. The Samples was incubated for 5 minutes at room temperature after which 2ml of 1M NaOH was added to the mixture until yellowish orange colour was appeared. Then finally volume was maked up to 10ml with distilled water. Absorbance was measured at 510nm by using colorimeter. The concentration of total flavonoids content in test sample expressed as mg quercetin equivalent [12].

Tannin Estimation

100µl of 10mg/ml extract was added to a clean test tube containing 7.5ml of distilled water. The folin-ciocalteu reagent (0.5 ml) was added to the mixture. 1 ml of a 35% solution of sodium carbonate was added to mixture. The mixture in the tube was transferred to a 10ml volumetric flask and the volume of the mixture was made up to 10ml by distilled water. The mixture was shaken and kept at room temperature for 30min in the dark. Gallic acid was used as a standard and reference standard solutions (20-100µg/ml) were prepared. The absorbance for the solutions was measured against a blank that was prepared in the same manner as the test solution without adding any extract. A UV-visible spectrophotometer was used to measure the absorbance at 725nm. Tannin content was expressed as mg gallic acid equivalence/gm of extract (mg GAE/g) extract [13].

Saponin Estimation

40ml of 20% ethanol was added to one gram of material. The mixture was incubated for 4 hours at 55◦C in a water bath with constant stirring. Filter paper, a vaccum pump, and a funnel were used to filter the mixture. Re-extraction of the residue was done using 20ml of 20% ethanol. A 200ml separating funnel was then filled with the concentrate after the volume of filtrate was decreased at 90◦C in a water bath. 20ml of diethyl ether were added and thoroughly stirred. The upper ether layer was discarded while the lower portion was collected. 20ml of butan-1-ol fraction (upper) were added, violently mixed, and then 5ml of 1% aqueous NaCl were added. The butan-1-ol fraction (upper) was taken and dried in the oven to a consistent weight. The residue were weighed and recorded as saponin content [14].

Steroidal Estimation

Sulphuric acid (4N; 2M H_2SO_4), iron (III) chloride (0.5%w/v) and potassium hexacyanoferrate (III) solution were added to 100µl of the extract respectively. For 30 minutes, the solution mixture was maintained in a water bath at 70±50◦C. Using a spectrophotometer, the absorbance was measured at a wavelength of 780nm. The result, which was taken from a standard curve was reported as cycloartenol equivalents (mg CA/g of dried material) [15].

EXPERIMENTAL ANIMALS

Male rats weighing 200-250gm respectively and 8-12 weeks old were used for experimental study. The rats were housed in standard propylene cage and provided with standard diet and water *ad libitum*. Animals were housed in groups of 4-5 per cages and kept under room temperature (24±2˚C) and relative humidity (50-70%) in a 12h light dark cycle. All procedures involving animals were conducted in accordance with CPCSEA of government of India, and guidelines was followed and prior permission was taken from the Institutional Animal Ethical Committee (273/PO/Re/S/2000/CPCSEA).

ACUTE TOXICITY STUDY

Animals were selected randomly for acute oral toxicity study. Suspension of crude extract were orally administered to animals by using oral gavage at dose 5, 50, 300, 2000 mg/kg body weight as per acute toxic class method (OECD TG-423). LD_{50} value were calculated and safe dose range were selected for the main study [16].

SELECTION OF DOSES

The dose of Phenytoin (50mg/kg) i.p was selected on the basis of previous study of anticonvulsant activity of stem bark of *Pongamia pinnata* in rats [17]. The dose of Diazepam (12mg/kg) i.p and Picrotoxin (7.5mg/kg) i.p were selected on the basis of previous study done on Evaluation of antiepileptic activity of aqueous and methanolic stem bark extract *Psychotria camptotus* Verdc. of in rats [18].

EXPERIMENTAL DESIGN

Male rats were divided into 4 groups, six animals in each group.

ELECTRICALLY KINDLED SEIZURE MODEL

Kindling is a phenomenon in which repeated treatment (e.g once daily) with a subconvulsant electrical or chemical stimulus results in the development of overt convulsions. Two subconvulsive electric shocks per day for 6 days using auricular electrodes (21mA, 0.1 sec) were received by animals until all the animals exhibited clonic convulsions [19]. 12 shocks were required to induce convulsions in each animal. The severity of seizures was graded as follows :

Grade 0: Normal

Grade I: Facial movements

Grade II: Head nodding

Grade III: Raising forelimb and mild forelimb clonus

Grade IV: Marked rearing with oral movements and forelimb clonus

Grade V: Repeated falling on back and clonic seizures

On next day, the rats were randomly separated into four groups six animals in each.

Group 1 (Control group): The animals received Normal saline (1ml/kg) i.p

Group 2 (Standard group): Kindled animals received Phenytoin (50mg/kg) i.p

Group 3 (Treatment group 1): Kindled animals received lower dose of methanolic extract of leaves of *Rubus ellipticus* Smith. i.p.

Group 4 (Treatment group 2): Kindled animals received higher dose of methanolic extract of leaves of *Rubus ellipticus* Smith. i.p.

After 45min of administration of vehicle and extract and 30min after administration of phenytoin the kindled animals received kindling stimulus. The seizure grades of rats which received the extract and phenytoin was compared with vehicle.

PICROTOXIN (PTX) INDUCED CONVULSION MODEL

Male rats were divided into 4 groups six animals each. Diazepam (12 mg/kg) i.p used as a reference standard.

Group 1 (Control group): The animals received Normal saline p.o

Group 2 (Standard group): Animals received Diazepam (12 mg/kg) i.p

Group 3 (Treatment group 1): Animals received lower dose of methanolic extract of leaves of *Rubus ellipticus* Smith. p.o for 14 days

Group 4 (Treatment group 2): Animals received higher dose of methanolic extract of leaves of *Rubus ellipticus* Smith. p.o for 14 days

On 14th day, Picrotoxin (7.5mg/kg) i.p was administered to all the groups 45min after the vehicle and extract, 30min after the standard and then behavioural parameters were noted during the next 30min i.e., clonic seizures, tonic seizures, time of onset of seizures and time of death.

METHOD FOR ESTIMATION OF OXIDATIVE PARAMETERS

The animals were sacrificed by cervical dislocation under pentobarbitone anaesthesia. Brain was isolated and washed with ice cold normal saline solution, minced into small pieces. Tissue was homogenized in phosphate buffer (pH 7.4) with the help of homogenizer. Then homogenate was centrifuged at 3000 rpm for 10 min. The supernatant were used for estimation of LPO, SOD, GSH levels.

Estimation of Lipid Peroxidation (LPO)

2ml homogenate were mixed with 2ml TCA (20%) and after cooling for 15min centrifugation was done and the 2ml supernatant was taken out and mixed with 2ml TBA. The mixture was kept in boiling water bath for 10 min, cooled and the absorbance of the supernatant was measured at 535mm using spectrophotometer. Blank consists of 1.5ml distilled water and 1.5ml TCA (20%) and 3ml TBA. The content of Malondialdehyde (MDA) expressed as nmol/ml, was calculated using the formula [20].

$Y = 0.0113x - 1.0061$

Estimation of Superoxide Dismutase (SOD)

0.5 ml homogenate was diluted with 0.5 ml distilled water, 0.25 ml ethanol and 0.15 ml chloroform was added, Shaked and centrifuged at 2000rpm for 10 min and the supernatant was separated. Then 0.5 ml carbonate buffer, 0.5 ml EDTA, 0.4 ml epinephrine was added to 0.5 ml supernatant. Blank consists of 1.5ml Distilled water, 0.38 ml ethanol, 0.15 ml chloroform, 3.6 ml carbonate buffer, 1.2 ml EDTA was added to 1.2 ml supernatant**.** The initial absorbance at zero minute was checked and taken for 3 min with 30 sec, interval at 480 nm using spectrophotometer. The SOD level expressed as EU/ dl, was calculated using the formula [21].

$SOD = -0.008x - 0.487$

$$SOD = \frac{0.25x}{x} \times 50$$

X = final absorbance – initial absorbance

Estimation of Reduced Glutathione (GSH)

1ml Homogenate was mixed with 1ml TCA (10%), Cooled for 10 minutes and centrifuged at 2000rpm. The supernatant was separated and mixed with 4ml DTNB and 1.5ml phosphate buffer. Mixed well and kept at room temperature for 5 min. Absorbance was measured at 412nm. The blank consists of 1ml distilled water, 1 ml TCA (10%), 4 ml DTNB and 1.5 ml phosphate buffer was added to 0.5 ml supernatant. The amount of glutathione was expressed as mg of GSH/g of tissue [22].

$Y = 0.006x + 0.144$

Y = absorbance of test sample

X = concentration of reduced glutathione in test sample

Estimation of Nitrate

Griess reagent (1% Sulfanilamide in 5% H_3PO_4 and 0.1% Napthylethylenediamine dihydrochloride, in a ratio 1:1). 2ml homogenate was obtained and Centrifuged at 3000 rpm for 5min. Samples were stored overnight in a freezer. The day of experiment 750µl Aliquot was mixed with 750 µl Griess reagent, Protected from light and maintained at room temperature for 15min. The concentration of nitrate in the sample was determined spectrophotometrically at 540nm against blank. The Equation obtained from standard curve of nitrate is Y=0.005X-0.012; X=Y+0.012/0.005 and expressed as µ mol/L [23].

METHOD FOR ESTIMATION OF BIOCHEMICAL PARAMETERS

Estimation of GABA

Brain homogenate was placed in bottle containing 8ml of ice-cold absolute alcohol and kept for 1hr at 0˚C. Centrifuged for 10min at 16000 rpm and the supernatant was collected in petri dish. The precipitate was washed three times with 3-5ml of 75% alcohol and washes were combined with supernatant. The contents were evaporated to dryness on water bath at 70-90˚C under air. 1ml water and 2ml chloroform were added to the dried mass and centrifuged at 2000 rpm. Upper phase containing GABA was separated and 10µl of it was applied as a spot on whatman filter paper. The mobile phase contained n-butanol (50ml), acetic acid (12ml) and water (60ml). The chamber was saturated with mobile phase for 30min. The paper chromatogram was developed with ascending technique. The paper was dried using hot air and 0.5% ninhydrin solution in 95% ethanol was spreaded. Then the paper was dried for 1h at 90˚C. Blue coloured spot developed on paper was cutted and heated with 2ml ninhydrin solution on water bath for 5min. 5ml water was added to the solution and kept for 1h. Supernatant was evacuated and measured the absorbance at 570nm. The stock solution of GABA as standard ,

1mg/ml was prepared in 0.01N HCL. Serial dilutions were prepared of concentrations 1ng/10µl to 1000ng/10µl. To obtain standard concentration curve for GABA same procedure was followed replacing brain homogenate with standard GABA solutions [24].

Estimation of Calcium

The amount of calcium present in the sample will be measured Photometrically between 540-600nm. Normal range = 8.5- 10.2mg/dl [25]. It is calculated using the formula:

$$\text{Calcium(mg/dl)} = \left(\frac{\text{absorbance of test}}{\text{absorbance of standard}}\right) \text{x conc. of standard (mg/dl)}$$

Assay Procedure

Pipette into tubes marked	**Blank**	**Standard**	**Test**
Working reagent	1000µl	1000µl	1000µl
Distilled water	10µl		
Standard		10µl	
Test			10µl

HISTOPATHOLOGY OF BRAIN

At the end of dosing period one animal from each group was euthanized using pentobarbitone. Brain was isolated, cutted into small pieces and washed three times with phosphate buffer saline (PBS) and kept in 10% formalin solution for 24 hours. Dehydration and clearing of the tissue was done using increasing concentration of alcohol and kept in xylene and alcohol for 2 hours. Tissue section was set in paraffin for 1 hour and then stained with Haematoxylin and Eosin (H/E) and Gomerialdhyde-fuchsin (GAF), a beta cell specific staining. Stained sections were evaluated quantitively (morphometric) and qualitatively (morphological).

STATISTICAL ANALYSIS

The statistical analysis of data was done using graph pad 9.0 software. All values were presented as mean ± SEM. Various comparisons were done between groups ANOVA followed by Dunnett multiple comparison. P value <0.001 was considered as most significant data. P value <0.01 was considered as significant data and P value <0.05 was considered as less significant data.

RESULTS AND DISCUSSION

The Antiepileptic Potential were carried out on the leaves of the plant *Rubus ellipticus* Smith. in Experimental animal models. According to the literature survey, the leaves of *Rubus ellipticus* Smith. were used for curing epilepsy. The different doses (200mg/kg and 400mg/kg) of *Rubus ellipticus* Smith. were used to evaluate the antiepileptic Potential. Brain tissue homogenate was used for estimating different biochemical Parameters to

evaluate the effect of these treatments and standard preparation in epileptic rats. Results obtained by this study has been summarized below.

Table 1. Percentage yield of Methanolic leaf extract of *Rubus ellipticus* Smith.

S.No.	Plant Extract	%Yield
1.	MetOH Extract of Leaves of *Rubus ellipticus* Smith.	26.29%

Table 2. Qualitative Phytochemical Screening of Methanolic leaf extract of *Rubus ellipticus* Smith.

Test Performed	Methanolic extract
Test for Tannins	
Lead acetate test	+
Ferric chloride test	+
Test for Flavonoids	
Shinoda test	+
Sodium hydroxide + extract shows yellow colouration, which decolourises after addition of acid	+
Test for steroid	
Salkowski test	+
Test for saponin	
Foam test	+

Table 3. Flavonoid Content of Methanolic leaf extract of *Rubus ellipticus* Smith.

S.No.	Concentration of extract (µg/ml)	Flavonoid content (mg of quercetin equivalent/g dry material)
1.	Leaves (1000µg/ml)	43.6±1.76

Values are mean ± S.E.M, n= 3

Table 4. Tannin Content of Methanolic leaf extract of *Rubus ellipticus* Smith.

S.No.	Concentration of extract (µg/ml)	Tannin content (mg of gallic acid equivalent/g dry material)
1.	Leaves (1000µg/ml)	22.1±0.17

Values are mean ± S.E.M, n= 3

Table 5. Saponin Content of Methanolic leaf extract of *Rubus ellipticus* Smith.

S.No.	Plant Extract	% Saponin Content
1.	MetOH Extract of Leaves of *Rubus ellipticus* Smith.	3±0.05

Values are mean ± S.E.M, n= 3

Table 6. Steroidal Content of Methanolic leaf extract of *Rubus ellipticus* Smith.

S.No.	Concentration of extract (µg/ml)	Steroidal content (mg of cycloartenol equivalent/g dry material)
1.	Leaves (1000µg/ml)	35.5±0.550

Values are mean ± S.E.M, n= 3

Table 7. Development of seizures severity upon twice daily stimulation by auricular electrodes in rats.

Stimulus no.	Seizures score
1	0.58±0.10
2	0.91±0.05
3	1.12±0.10
4	1.33±0.09
5	1.45±0.10
6	2.33±0.13
7	2.29±0.09
8	2.37±0.10
9	2.41±0.10
10	2.87±0.12
11	3.54±0.10
12	4.45±0.10

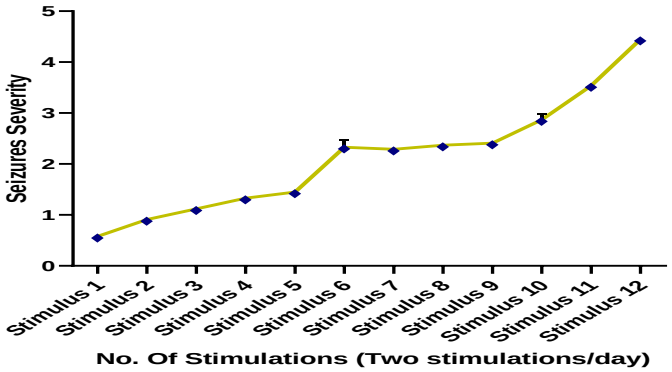


Figure 1. Development of seizures severity upon twice daily stimulation by auricular electrodes in rats

Table 8. Effect of *Rubus ellipticus* Smith. Leaf extract on seizure intensity in fully kindled rats

Groups	**Seizure score after treatment**
Positive control	4.80±0.16
Standard	0.30±0.21***
REMLE LD (200mg/kg)	3.45±0.10***
REMLE HD (400mg/kg)	2.25±0.11***

All values are expressed as mean ± S.E.M, N= 6 in each group, One-way ANOVA followed by dunnett multiple comparison test. P values : *P<0.05, **P<0.01, ***P<0.001 when results of day 14 were compared with positive control group.

REMLE LD: *Rubus ellipticus* Methanolic Leaf Extract Low dose, REMLE HD: *Rubus ellipticus* Methanolic Leaf Extract High dose.

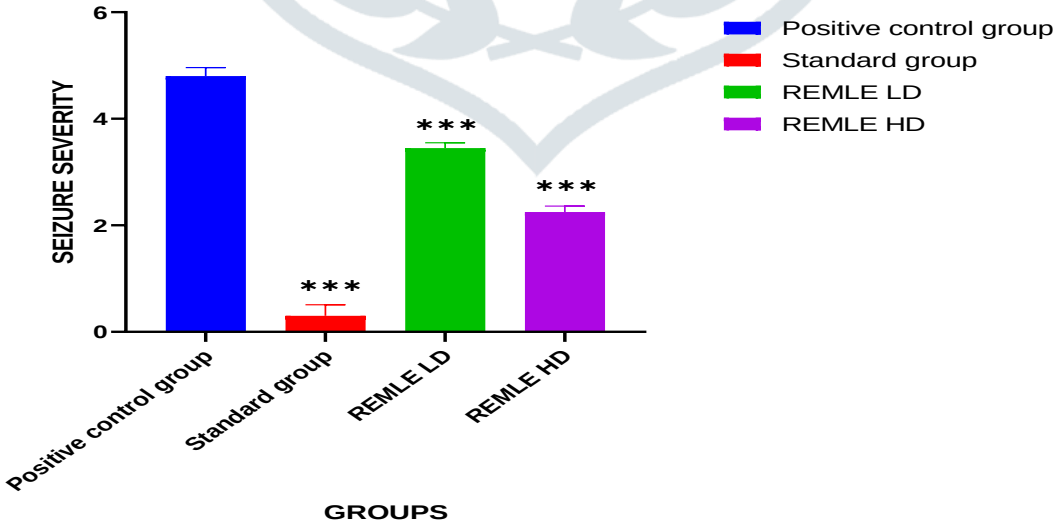


Figure 2. Effect of *Rubus ellipticus* Smith. leaf extract on seizure intensity in fully kindled rats

Table 9. Effect of *Rubus ellipticus* Smith. Leaf extract on Picrotoxin-induced convulsion model

GROUPS	ONSET OF CLONIC CONVULSIONS (min)	DURATION OF CONVULSIONS (min)	MORTALITY (%)
Positive control	1.45±0.21	10.53±1.86	100%
Standard	28.2±0.35***	1.25±0.06***	0%
REMLE LD (200mg/kg p.o)	19.06±0.19**	4.11±0.11**	38.53%
REMLE HD (400mg/kg p.o)	20.22±0.16***	3.15±0.20***	0%

All values are expressed as mean ± S.E.M, N= 6 in each group, One-way ANOVA followed by dunnett multiple comparison test. P values : *P<0.05, **P<0.01, ***P<0.001 when results of day 14 were compared with positive control group.

REMLE LD: *Rubus ellipticus* Methanolic Leaf Extract Low dose, REMLE HD: *Rubus ellipticus* Methanolic Leaf Extract High dose.

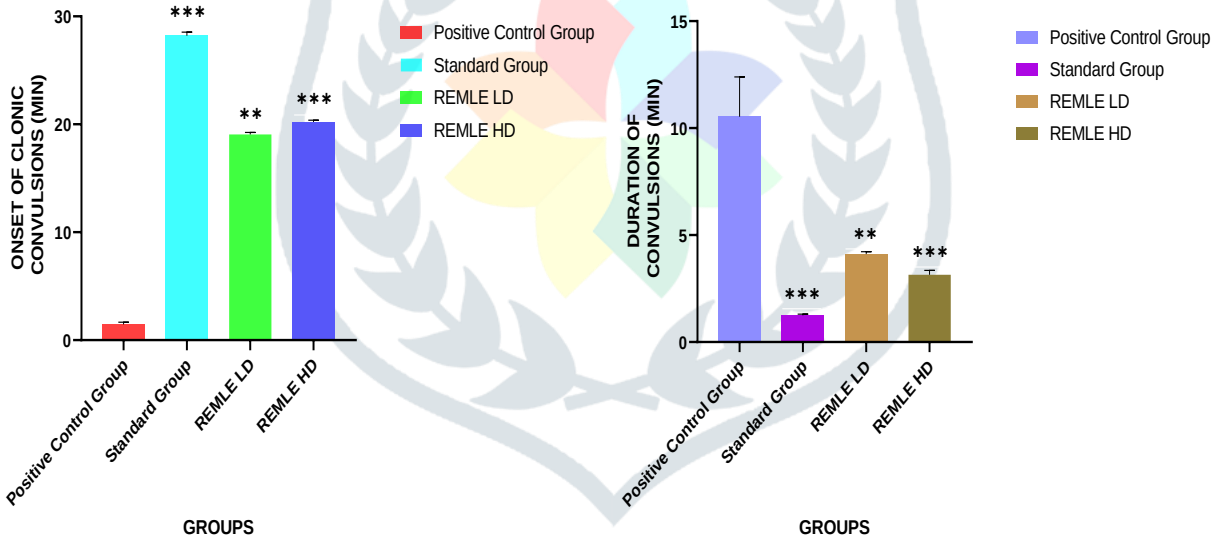


Figure 3. Effect of Rubus ellipticus Smith. leaf extract on onset and duration of clonic convulsions

Table 10. Effect of *Rubus ellipticus* Smith. Leaf extract on LPO, Nitrate, GSH AND SOD level in Electrically kindled seizure model.

GROUPS	LPO (nmol/ml)	Nitrate (µmol/litre)	GSH (mg of GSH/g of tissue)	SOD (EU/dl)
Positive control	103.78±0.63	111.33±1.12	76.55±0.69	45.51±1.82
Standard	90.72±0.45***	54.32±1.04***	131.64±1.19***	83.08±2.20***

REMLE LD (200mg/kg i.p)	98.68±0.33**	94.34±0.32**	92.42±0.34**	57.42±0.27**
REMLE HD (400mg/kg i.p)	95.02±0.31***	80.58±0.48***	109.27±0.30***	71.35±0.37***

All values are expressed as mean ± S.E.M, N= 6 in each group, One-way ANOVA followed by dunnett multiple comparison test. P values : *P<0.05, **P<0.01, ***P<0.001 when results of day 14 were compared with positive control group.

REMLE LD: *Rubus ellipticus* Methanolic Leaf Extract Low dose, REMLE HD: *Rubus ellipticus* Methanolic Leaf Extract High dose.

Table 11. Effect of *Rubus ellipticus* Smith. Leaf extract on LPO, Nitrate, GSH AND SOD level in Picrotoxin induced convulsion model.

GROUPS	LPO (nmol/ml)	Nitrate (µmol/litre)	GSH (mg of GSH/g of tissue)	SOD (EU/dl)
Positive control	98.53±0.34	140.73±0.59	67.04±1.01	43.41±0.47
Standard	78.22±0.28***	41.68±0.38***	118.76±0.73***	90.90±0.44***
REMLE LD (200mg/kg p.o)	84.67±1.47**	81.46±2.24*	83.33±1.52**	58.34±1.65**
REMLE HD (400mg/kg p.o)	69.50±2.29***	43.12±2.23**	106.37±2.26***	76.11±1.90***

All values are expressed as mean ± S.E.M, N= 6 in each group, One-way ANOVA followed by dunnett multiple comparison test. P values : *P<0.05, **P<0.01, ***P<0.001 when results of day 14 were compared with positive control group.

REMLE LD: *Rubus ellipticus* Methanolic Leaf Extract Low dose, REMLE HD: *Rubus ellipticus* Methanolic Leaf Extract High dose.

Table 12. Effect of *Rubus ellipticus* Smith. Leaf extract on GABA and Calcium level in Electrically kindled seizure model.

GROUPS	GABA (ng/g of tissue)	CALCIUM (mg/dl)
Positive control	30.69±1.15	14.61±0.57
Standard	51.15±1.46***	6.87±1.05***
REMLE LD (200mg/kg i.p)	39.11±0.41**	11.96±0.34*
REMLE HD (400mg/kg i.p)	47.66±0.24***	9.18±0.19***

All values are expressed as mean ± S.E.M, N= 6 in each group, One-way ANOVA followed by dunnett multiple comparison test. P values : *P<0.05, **P<0.01, ***P<0.001 when results of day 14 were compared with positive control group.

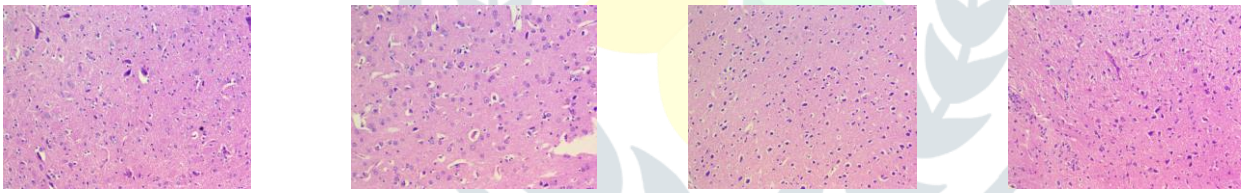
REMLE LD: *Rubus ellipticus* Methanolic Leaf Extract Low dose, REMLE HD: *Rubus ellipticus* Methanolic Leaf Extract High dose.

Table 13. Effect of *Rubus ellipticus* Smith. Leaf extract on GABA and Calcium level in Picrotoxin-induced model.

GROUPS	GABA (ng/g of tissue)	CALCIUM (mg/dl)
Positive control	25.45±0.32	17.09±0.31
Standard	55.52±0.36***	7.38±0.29***
REMLE LD (200mg/kg p.o)	44.25±1.09**	11.36±0.10**
REMLE HD (400mg/kg p.o)	50.97±0.30***	10.06±0.17***

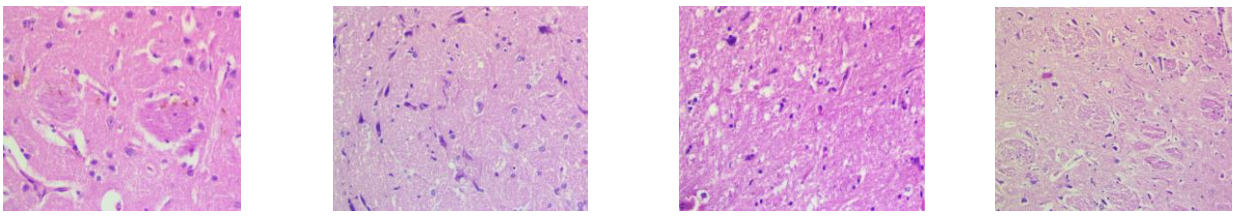
All values are expressed as mean ± S.E.M, N= 6 in each group, One-way ANOVA followed by dunnett multiple comparison test. P values : *P<0.05, **P<0.01, ***P<0.001 when results of day 14 were compared with positive control group.

REMLE LD: *Rubus ellipticus* Methanolic Leaf Extract Low dose, REMLE HD: *Rubus ellipticus* Methanolic Leaf Extract High dose.



(a) Positive control group **(b) Standard group** **(c) Treatment group (200mg/kg)** **(d) Treatment group (400mg/kg)**

Figure 4. Effect of Methanolic leaf extract of *Rubus ellipticus* Smith. on Histopathology of brain in Electrically kindled seizure model



(a) Positive control group **(b) Standard group** **(c) Treatment group (200mg/kg)** **(d) Treatment group (400mg/kg)**

Figure 5. Effect of Methanolic leaf extract of *Rubus ellipticus* Smith. on Histopathology of brain in Picrotoxin-induced convulsion model

CONCLUSION

The Present study Possess anticonvulsant activity and protected against the Picrotoxin-induced convulsion and electrically kindled seizures in rats. The study clearly indicated that methanolic leaf extract of *Rubus ellipticus* Smith. has a beneficial effect on the various components of epilepsy like oxidative stress, antioxidant system, GABA, calcium level in both models. The study has been carried out at two dose levels (200mg/kg and 400mg/kg B.W.) of methanolic leaf extract of *Rubus ellipticus* Smith. The high dose level of leaf extract (400mg/kg B.W.) has shown a marked reduction in oxidative stress and calcium level in the brain of epileptic rats and caused significant improvement in neurotransmitters like GABA and antioxidant enzymes like GSH and SOD. On comparing the effects on two models, it is evident that the methanolic leaf extract of *Rubus ellipticus* Smith. at a dose level of 400 mg/kg has shown the most significant ($P<0.001$) effect on both the models, except for the NO level in Picrotoxin-induced model. However, the methanolic leaf extract of *Rubus ellipticus* Smith. at a dose level of 200 mg/kg shown significant ($P<0.01$) in NO level in electrically kindled seizure model and less significant ($P<0.05$) in NO level in Picrotoxin-induced convulsion model.

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