# Pharmacological evaluation of Momordica Charantia leaves extract for Antioxidant and Anticonvulsant activity

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**Abstract:** Epilepsy is a central nervous system (neurological) disorder characterized by a bizarre feelings, sensations, and behaviors. Muscle spasms, convulsions, and loss of consciousness occasionally from epileptic seizures. Neuronal dependent on neurotransmitters in the central nervous system. Epilepsy is a chronic neurological disease in developing countries. The access to appropriate care for the management of this disease being difficult in our countries, a large number of patients resort to the use of medicinal plants. Bitter gourd (Momordica charantia L.) known also as bitter apple or bitter melon or balsam pear, is a tropical vine belonging to the order Cucurbitales, family Cucubitaceae and genus Momordica. The plant is cultivated as medicinal as well as vegetable crop widely in India, China and South East Asia. Even though whole plant is palatable in nature, bitter gourd is mainly grown for its fruit part. To contribute to the scientific knowledge of this plant and to promote the development of phytomedicines, we evaluated antioxidant and anticonvulsant properties of Momordica Charantia leaves extract.

**Key-words:** Antioxidant activity, Anticonvulsant activity, Momordica Charantia, haematological parameters

**Introduction:** Humanity is facing different kinds of diseases (microbial, parasitic, viral, cardiovascular, etc.) and the management of health issues is proving to be a real social problem, especially in developing countries [1]. According to estimated research, about

80% of African populations use traditional medicines to meet their primary health care needs. Thus, health care largely depends on medicinal plants and the local knowledge associated with them. In traditional African environments, the use of plants against various diseases has been increasingly popular in recent years [2]. This enthusiasm can be explained by cultural reasons, the decline in purchasing power, the high cost of conventional drugs and distrust of so-called modern synthetic products. The search for new treatments accessible and available to the population is necessary and urgent. Medical plants are an ideal alternative (the best source) to chemical or special drugs that are too expensive to manufacture or buy for developing countries [3]. This is how several plants are used not only for health care in traditional medicine but also for the search for new active molecules following one of the greatest challenges of global public health which is only the resistance of agent's pathogens to antimicrobials [4]. It is therefore important to carry out ethnobotanical studies to identify the local uses of plant species. Nowadays, epilepsy is better understood and so-called conventional medicine offers effective treatments [5]. It is a chronic neurological disease marked by synchronous and paroxysmal discharges of neurons. It is characterized by recurrent spontaneous seizures that can become complicated in status mal. From the neurological point of view, an imbalance is noted between excitatory neurons with glutamate or acetylcholine and inhibitory neurons with ɤ-amino butyric acid [6]. The WHO estimates that around 50 million people suffer from epilepsy worldwide, 80% of whom live in developing countries18. Still in these countries, about 90% of people with the disease do not receive appropriate treatment, which explains why they remain marginalized and have a lower quality of life than other chronic patients [7]. However, contemporary medicine shows its limits: side effects, compliance difficulties and even resistance to treatment. For these reasons, some patients turn to complementary medicine, traditionnal medicine. Epilepsy, one of the oldest recognized neurological disorders, affects over 50 million people worldwide, with 75% of patients in low-income countries lacking adequate treatment [8]. Despite the availability of more than 20 anticonvulsant drugs, about one-third of patients continue to experience drug-resistant seizures, increasing the risk of premature mortality, injury, psychosocial complications, and reduced quality of life [9]. Furthermore, the

toxicity and side effects of current treatments underscore the need for safer, more effective alternatives.

Bitter gourd (Momordica charantia L.) known also as bitter apple or bitter melon or balsam pear, is a tropical vine belonging to the order Cucurbitales, family Cucubitaceae and genus Momordica. The plant is cultivated as medicinal as well as vegetable crop widely in India, China and South East Asia. Even though whole plant is palatable in nature, bitter gourd is mainly grown for its fruit part. Fruits, flowers and young shoots are used as flavouring agents in various Asian dishes. Fruits are cooked with other vegetables especially in soups for the slight bitter taste. However, in Indian cuisines, fruits are mainly used after blanching or par boiling or soaking in salt water to reduce bitterness. Fruits can also be canned or pickled or dehydrated in addition to cooking or deep frying. It is considered widely as a folk lore medicine against diabetes amongst the indigenous population of Asia, South America, India and East Africa. Apart from fruits, the roots, leaves and vines are used as a suppressant for tooth ache, diarrhoea and furuncle. Various products of bitter gourd like bitter gourd tea, which is known as gohyah or herbal tea made from dried slices of bitter gourd, is gaining popularity as herbal medicine The aim of the present study was to evaluate antioxidant activities of extract of plant with emphasis on psychosis and cognition which may lead us to the discovery of a promising drug for the treatment of anxiety, depression, insomnia, epilepsy.

## Material And Methods

### Antioxidant activity

**DPPH free radical scavenging activity:** The free radical scavenging activities of the plant extracts on the stable radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) will be estimated. 2.0 ml of a methanol solution of the sample (extract/control) at different concentration (50–250µg/ml) will be mixed with 3.0 ml of a DPPH methanol solution (20 µg/ml). After 30 min reaction period at room temperature in dark place the absorbance was measured at 517 nm against methanol as blank by UV spectrophotometer.

Inhibition of free radical DPPH in percent (I %) was calculated as follows:

$I\% = (1 - A\ sample/A\ blank) \times 100$

where A blank is the absorbance of the control reaction (containing all reagents except the test material). Extract concentration providing 50% inhibition (IC50) will be calculated from the graph plotted inhibition percentage against extract concentration.

**Nitric oxide (NO) scavenging activity:** Nitric oxide (NO) scavenging activity will be measured spectrophotometrically. Sodium nitroprusside in aqueous solution at physiological pH spontaneously generate nitric oxide, which interacts with oxygen to produce nitrite ions will be determined by the use of Griess reagents. A volume of 2 ml of 10 mM sodium nitroprusside and 0.5 ml of phosphate buffer saline (pH 7.4) will be mixed with 0.5 ml of plant extract and ascorbic acid individually at various concentrations (50–250 μg/ml). The reaction mixture will be incubated at 25 °C for 150 min. After 150 min, 0.5 ml of incubation solution will be withdrawn and mixed with 1 ml of sulfanilic acid reagent (0.33 in 20% glacial acetic acid) and will be allowed to stand for 5 min at room temperature for completing diazotization. Then 1 ml of 0.1% w/v napthylethylenediamine dihydrochloride will be added, mixed well and the mixture will be incubated at room temperature for 30 min. The absorbance will be taken at 540 nm. The amount of nitric oxide (NO·) radical inhibited by the extract will be calculated using the following equation:

$I\% = (1 - A\ sample/A\ blank) \times 100$

Where, A blank is the absorbance of the control reaction (containing all reagents except the test material).

**Anticonvulsant Activity:** 6 Hz Seizure Test was carried out as per the previously described procedure. In brief, mice were pretreated with extracts (doses ranging from 50 to 800 mg/kg, p.o.) for 3 days, the dose selection was done based on acute toxicity study, 100 mg/kg dose was selected and based on the response observed at 100 mg/kg, further dose levels were selected to establish dose-response correlation. Hence, in the process of establishing the dose-response correlation minimum dose was started with 100 mg/mg and saturation was observed after 200 mg/kg. On Day-3, 1 h after the last dose, tetracaine

ophthalmic solution (0.5% tetracaine hydrochloride) was applied, 30 min later the corneal electrodes were wetted with saline and electrical stimulation was given for 3 s (200 μs-duration, 32-mA mono polar rectangular pulses at 6 Hz) to all animal, using a constant current device ECT Unit 5780 (Ugo Basile, Comerio, Italy). In general, after the electrical stimulation, a stunned posture will be seen along with rearing and repeated movements that lasted from 60 to 120 s in control animals. After the seizures, animals will be resumed to normal exploratory behavior. The end point in this model is defined as protection against the seizures, the animals which gain their normal exploratory behavior within 10 s of stimulation are considered as protected

$$\%\text{Protection} = 100 - \left[\left(\frac{\text{Number of animal showed seizures}}{\text{Total number of animals used}}\right) \times 100\right]$$

**Effect of plant extract on haematological parameters in mice:** The blood samples were placed in a well labeled sample bottles containing ethylene diamine Tetraacetic acid (EDTA). The sample bottle was shaken gently to mix the blood with EDTA to prevent clotting and the following tests were conducted.

**Effect of the extract on packed cell volume (PCV):** The PCV was determined using microhematocrit centrifuge (Hawksley, England). A micro capillary tube sealed with plastacine at one end was nearly filled with blood sample and centrifuged in the equipment at 10,000 rpm /min for 5 minutes. After centrifugation, the PCV was read using a Hawksley microhematocrit reader.

**Effect of the extract on total white blood cell (WBC) count:** 0.02 ml of blood was diluted in 0.38 ml of white blood cell diluting fluid (Turk's solution) to make a 1:20 dilution of the blood. The diluted sample was loaded in a Neubauer Counting Chamber and all the cells on the four corner square (1mm2) areas were counted using a light microscope of x10 objective. The number of cells counted for each blood sample was multiplied by 50 (fifty) to obtain the total WBC count per micro liter of blood.

**Effect of the extract on differential WBC count:** The blood sample was shaken gently and a drop of blood was placed on a clean grease-free slide. A thin smear of blood was

made on the slide using a cover slip. The smear was allowed to air dry and was stained using Leishman stain. The stained slides were later examined under oil immersion x100 objective, using a light microscope. Result for each type of white blood cell was expressed as a percentage of the total count converted to the absolute value per micro liter of blood.

**Effect of plant extract on brain weight:** The brain has isolated in for their gross examinations and means weights were noted for treated and control groups.

**Result And Discussion**

**Anti-oxidant Activity:** Analysis of the free radical scavenging activities of the selected plants extracts revealed a concentration dependent free radical scavenging activity resulting from reduction of DPPH, NO radical to non-radical form. The scavenging activity of Ascorbic acid, a known antioxidant used as positive control, was however higher.

**Sample code for Momordica Charantia leaves extract**

PEMC: Petroleum ether extract of Momordica Charantia leaves

EAMC: Ethyl acetate extract of Momordica Charantia leaves

EOMC: Ethanol extract of Momordica Charantia leaves

AQMC: Aqueous extract of Momordica Charantia leaves

**DPPH radical scavenging model** DPPH radical is considered to be a model for a lipophilic radical. A chain in lipophilic radicals was initiated by the lipid autoxidation. DPPH is a stable free radical at room temperature and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The reduction capacity of DPPH was determined by the decrease in its absorbance at 517nm, which is induced by antioxidant. Positive DPPH test suggests that the samples were free radical scavengers. The scavenging effect of l-Ascorbic acid, and plant extracts increased gradually with increase in concentration.

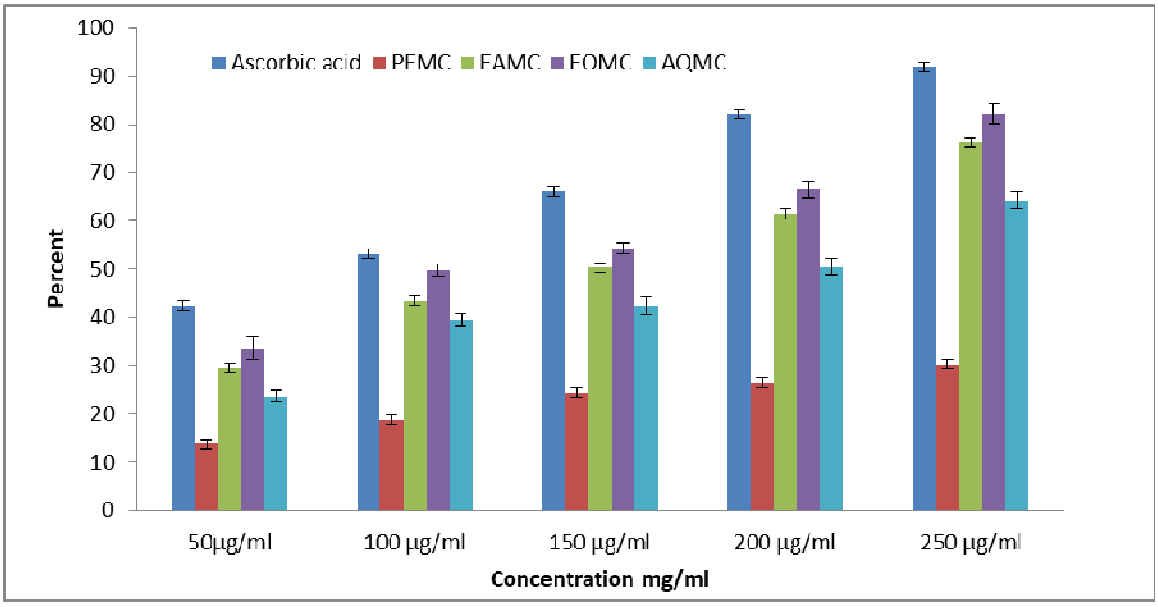



**Figure 1: Effect of Momordica Charantia leaves extracts on DPPH radical scavenging model**

Momordica Charantia leaves extracts showed dose dependent inhibitory effect on on DPPH radical. Ethanol and ethyl acetate extract of Momordica Charantia leaves were found more effective than aqueous and Petroleum ether extract. Ethanol extract (EOMC) significantly inhibit DPPH radical in comparison to standard ascorbic acid.

**Nitric oxide radical scavenging activity:** Nitric oxide plays an important role in various types of inflammatory processes in the body. In the present study Momordica Charantia leaves extracts checked for its inhibitory effect on Nitric oxide production. Nitric oxide radical generated for sodium nitroprusside at physiological pH was found to be inhibited by the extracts. The ethanol extract of Momordica Charantia at varied concentrations showed remarkable inhibitory effect of nitric oxide radical scavenging activity.

Momordica Charantia leaves extracts showed dose dependent inhibitory effect on on Nitric oxide radical. Ethanol and ethyl acetate extract of Momordica Charantia leaves were found more effective than aqueous and Petroleum ether extract. Ethanol extract (EOMC) significantly inhibits Nitric oxide radical in comparison to standard ascorbic acid.

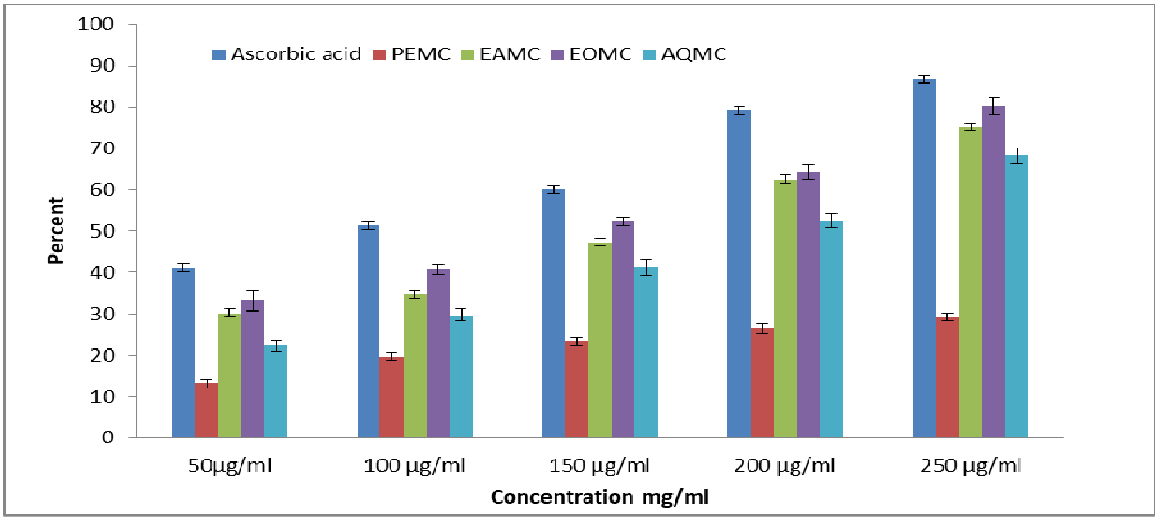



**Figure 2: Results of Momordica Charantia extracts on Nitric oxide radical scavenging model**

**Anticonvulsant Activity:** The 6 Hz seizure test is reported to involve a minimal, clonic phase, followed by stereotyped and automatistic behaviors which mimic the partial or limbic epilepsy in humans. At present, the 6 Hz seizure test is preferred for early identification of anticonvulsant activity of new compounds which are effective against therapy-resistant epilepsy. 6 Hz seizure model is known as very sensitive and useful model, which is known to facilitate in identifying a very mild agent also against drug-resistant epilepsies. Hence, this model is considered to be highly useful in identifying a new anticonvulsant drug, and also it is useful in avoiding false negatives. Therefore, all extracts of Momordica charantia leaves extracts evaluated against 6 Hz seizure test.

The result of Anticonvulsant Activity using 6 Hz seizure test of Momordica charantia leaves extracts showed that the ethanol extract of Momordica charantia leaves (EOMC) at 200 and 400 mg/ kg and standard drug has alleviated 6-Hz-induced seizures significantly and dose dependently. This effect was dose dependent and slightly observed at the low dose (200 mg /kg) of ethyl acetate extracts of Momordica charantia leaves (EAMC). In this test, ethanol extract (200, 400 mg/kg) has showed promising anticonvulsant effect.

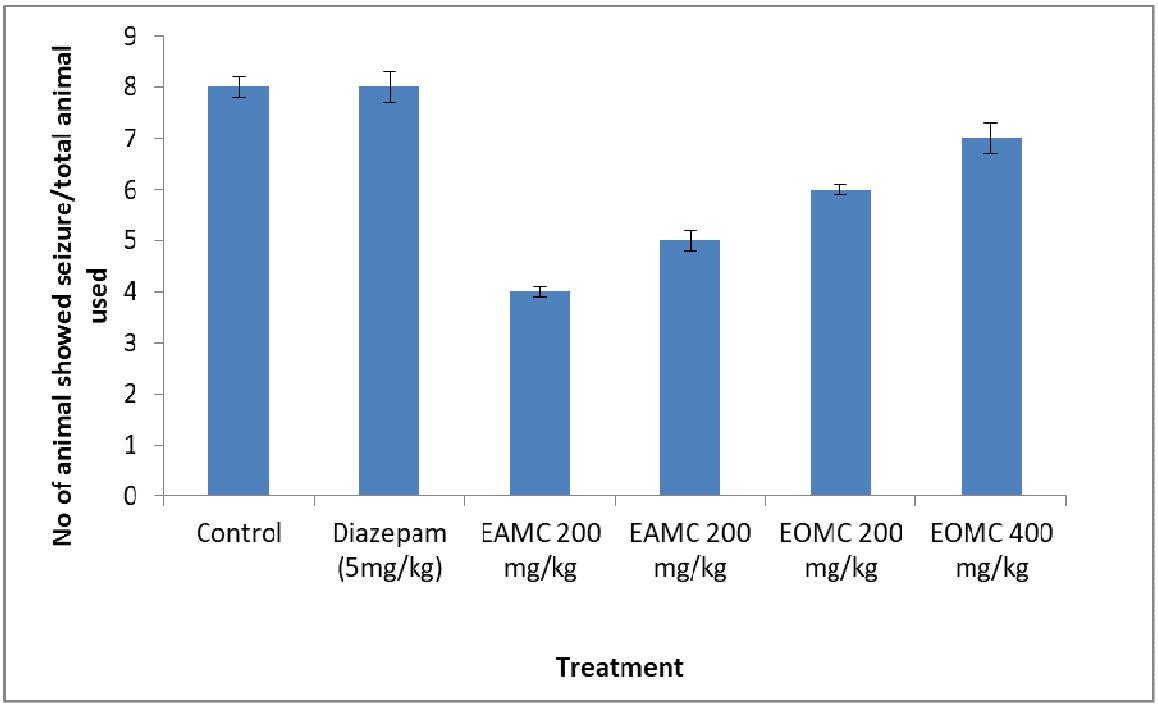



**Figure 3: Effect of Momordica Charantia leaves extracts on 6-Hz induced seizures in mice**

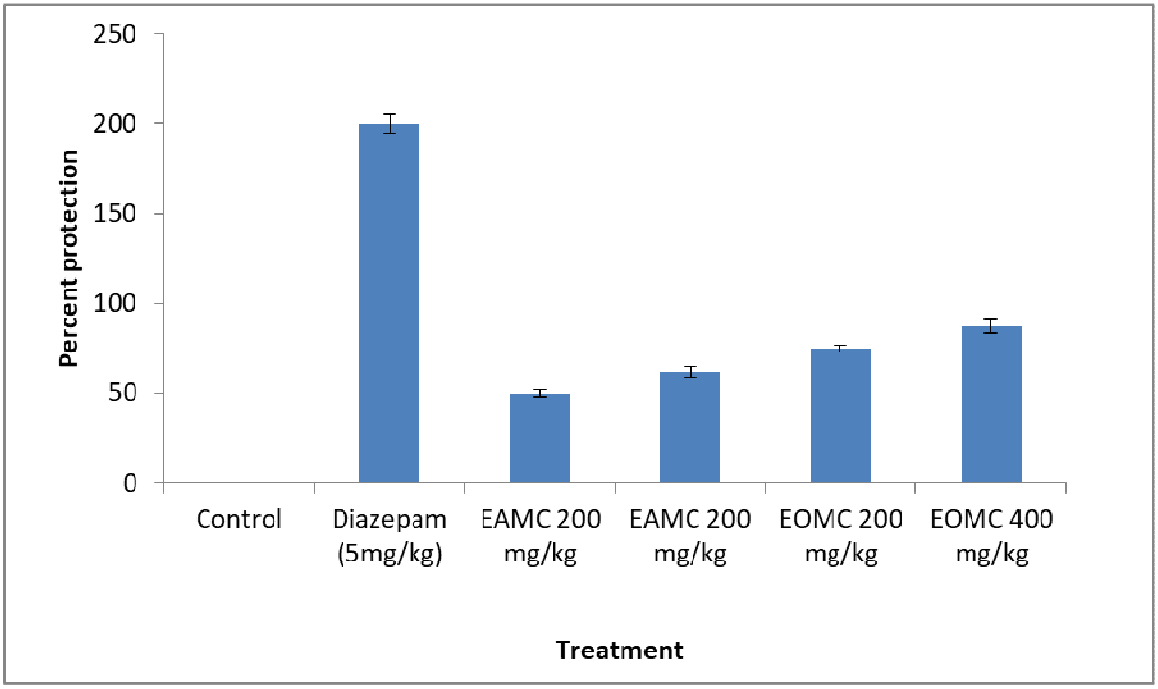



**Figure 4: Effect of Momordica Charantia leaves extracts on 6-Hz induced seizures in mice**

**Effect of plant extract on haematological parameters in mice:** The blood samples were placed in a well labeled sample bottles containing ethylene diamine Tetraacetic acid (EDTA). The sample bottle was shaken gently to mix the blood with EDTA to prevent clotting and the following tests were conducted. The effect on hematological parameters by Momordica charantia leaves extracts cited in Table 5.36. The haematological parameters like mean haemoglobin content, WBC, RBC, and differential cell counts were not significantly different in Momordica charantia leaves extract treated group in comparison to normal control group.

**Effect of the extract on packed cell volume (PCV):** The PCV was determined using microhematocrit centrifuge (Hawksley, England). A micro capillary tube sealed with plastacine at one end was nearly filled with blood sample and centrifuged in the equipment at 10,000 rpm /min for 5 minutes. After centrifugation, the PCV was read using a Hawksley microhematocrit reader.

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**Effect of the extract on differential WBC count:** The blood sample was shaken gently and a drop of blood was placed on a clean grease-free slide. A thin smear of blood was made on the slide using a cover slip. The smear was allowed to airdry and was stained using Leishman stain. The stained slides were later examined under oil immersion x200 objective, using a light microscope. Result for each type of white blood cell was expressed as a percentage of the total count converted to the absolute value per micro liter of blood.

**Table 1: Effect of Momordica Charantia leaves extracts on on haematological parameters in mice**

| Treatment | RBC/cu mm*$10^4$ | WBC/cu mm | Hb gm/dl |
|---|---|---|---|
| Solvent (5ml/kg) | 6.5 ± 0.25 | 4334 ± 114 | 9.18 ± 0.47 |
| Diazepam (4mg/kg) | 7.2 ± 0.37 | 3214 ± 138 | 10.23 ± 0.68 |
| EAMC 200 mg/kg | 6.5 ± 1.01 | 4495 ± 128 | 9.3± 0.83 |
| EAMC 400 mg/kg | 6.8 ± 0.92 | 4514 ± 149* | 9.8 ± 0.91* |
| EOMC 200 mg/kg | 6. 6 ± 1.21 | 4404 ± 158 | 9.4 ± 0.46 |
| EOMC 400 mg/kg | 7.5 ± 1.09* | 4581 ± 186** | 10.1 ± 0.72** |

Values are mean ± SEM (n=6); *P <0.05, **P <0.01 compared to respective control group

**Effect of plant extract on brain weight:** The brain isolated in various groups did not reveal any abnormalities in their gross examinations and difference in their mean weights both in treated and control groups.

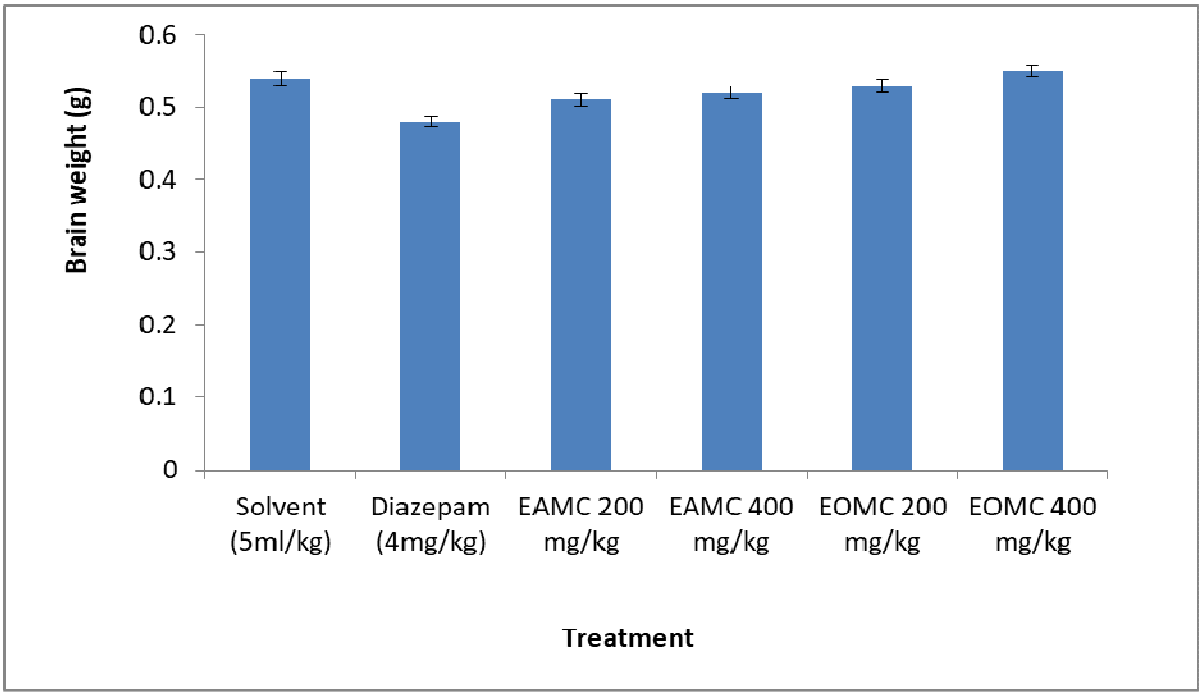



**Figure 5: Effect of Momordica Charantia leaves extracts on brain weight**

Mice treated with Momordica charantia leaves extract showed signs of sedation and analgesia, suggesting possible central depressant and analgesic effects in Irwin's test. Irwin's test is quite simple test can provide relevant information about a potential therapeutic indication, a specific mechanism of action, or a specific physiological function. For example, the presence of sedation in this test could be suggestive of a possible anxiolytic, antipsychotic, or anticonvulsant activity. Data from this test can also be used to assess the safety pharmacology of investigational drug agents. In the first 24 h after administration, the plant extract caused no mortality and appeared to cause no apparent toxicity even at a relatively high dose. The haematological parameters like mean haemoglobin content, WBC, RBC, and differential cell counts were not significantly different in Rhinacanthus nasutus leaves and Momordica charantia leaves extract treated group in comparison to normal control group. Momordica charantia leaves extract has significant CNS depressant, anticonvulsant, and analgesic effects and is deemed to be relatively safe.

**Conclusion**

Analysis of the free radical scavenging activities of the selected plants extracts revealed a concentration dependent free radical scavenging activity resulting from reduction of DPPH, NO radical to non-radical form. The scavenging activity of Ascorbic acid, a known antioxidant used as positive control, was however higher. DPPH radical is considered to be a model for a lipophilic radical. A chain in lipophilic radicals was initiated by the lipid autoxidation. DPPH is a stable free radical at room temperature and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The reduction capacity of DPPH was determined by the decrease in its absorbance at 517nm, which is induced by antioxidant. Positive DPPH test suggests that the samples were free radical scavengers. The scavenging effect of l-Ascorbic acid, and plant extracts increased gradually with increase in concentration. Momordica Charantia leaves extracts showed dose dependent inhibitory effect on on DPPH radical. Ethanol and ethyl acetate extract of Momordica Charantia leaves were found more effective than aqueous and Petroleum ether extract. Ethanol extract (EOMC) significantly inhibits DPPH radical in comparison to standard ascorbic acid. Nitric oxide plays an important role in various types of inflammatory processes in the body. In the present study Momordica Charantia leaves

extracts checked for its inhibitory effect on Nitric oxide production. Nitric oxide radical generated for sodium nitroprusside at physiological pH was found to be inhibited by the extracts. The ethanol extract of Rhinacanthus Nasutus at varied concentrations showed remarkable inhibitory effect of nitric oxide radical scavenging activity compared to other extract. Results revealed that all the tested extract showed the percentage of inhibition in a dose dependent manner. The ethanol extract of Momordica Charantia at varied concentrations showed remarkable inhibitory effect of nitric oxide radical scavenging activity.

The result of Anticonvulsant Activity using 6 Hz seizure test of Momordica charantia leaves extracts showed that the ethanol extract of Momordica charantia leaves (EOMC) at 200 and 400 mg/ kg and standard drug has alleviated 6-Hz-induced seizures significantly and dose dependently. This effect was dose dependent and slightly observed at the low dose (200 mg /kg) of ethyl acetate extracts of Momordica charantia leaves (EAMC). In this test, ethanol extract (200, 400 mg/kg) has showed promising anticonvulsant effect.

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