

ANTIEPILEPTIC POTENTIAL OF METHANOLIC EXTRACTS OF *ALLIUM CEPA* BULBS (RED AND YELLOW VARIETY) IN ALBINO MICE**Maryam Manzoor**

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Abstract

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Objective: The study was aimed to evaluate the anticonvulsant potential of methanolic extracts of red and yellow onion bulbs.

Method: The anticonvulsant activity of methanolic extracts of red and yellow onion bulbs (200 and 400mg/kg B.W) was evaluated against seizures induced by pentylenetetrazole (60mg/kg B.W), strychnine (1.2 mg/kg B.W) and picrotoxin (6mg/kg B.W) in albino mice. **Results:** The preliminary phytochemical screening revealed the presence of flavonoids, alkaloids, glycosides, terpenoids and tannins in both varieties of onion bulbs. Acute

oral toxicity test indicated that LD50 of red and yellow onion bulb was more than 2000mg/kg B.W. The methanolic extracts of both varieties at the doses of 200 and 400mg/kg B.W significantly delayed the onset of seizures induced by pentylenetetrazole, strychnine and picrotoxin when compared with controlled groups. **Conclusion:** The results indicates that red and yellow onion, possess anticonvulsant potential since they significantly delayed the onset of seizures against pentylenetetrazole, strychnine and picrotoxin.

INTRODUCTION

The epilepsy is known to be the common neurological disease which effect populace regardless of their race, social backgrounds and age. It has been estimated that approximately 50 million individuals worldwide have epilepsy and most of the affected people belongs to poor class with very little or sometimes no access to medicals treatments (Meinardi *et al.*, 2001).

Epilepsy is the designation of an encephalon disorder characterized predominantly by recurrent and capricious interruptions of mundane encephalon function, called epileptic seizures. Epilepsy is not a singular disease entity but a variety of disorders reflecting underlying encephalon

dysfunction that may result from many different causes. Epilepsy occurs both in males and females without any statistically significant gender discrimination (Christensen *et al.*, 2005).

Many antiepileptic drugs are now available, which decrease the episodes and duration of seizures and improves patient health and quality of life. Despite the availability of many antiepileptic drugs (AED's), many patients are unable to get a complete relief from seizures and appearance of significant potential adverse effects ranging from aplastic anemia and hepatic failure leading ultimately to death results in loss of therapeutic effect of these AED's (Kaushik *et al.*, 2013). Many medicinal plants have been used as antiepileptic because of having some active ingredients with potential antiepileptic properties (Samleti *et al.*, 2012).

Onion, *Allium cepa* (family: Amaryllidaceae) has various varieties of onion each with their own unique flavor, ranging from mildly sweet to very strong i.e. red, yellow, white and green and has many therapeutic properties. The bulb of onion has anthelmintic, anti-inflammatory, antiseptic, antispasmodic, carminative, diuretic, expectorant, febrifuge, hypoglycemic, anxiolytic and hypotensive properties. (Kumar *et al.*, 2010).

In this study, the researchers opted to evaluate the anticonvulsant activity of methanolic extract of red onion and yellow onion bulbs. The extracts were tested to check the delay in the onset of seizure caused by Pentylentetrazole (PTZ), Strychnine (STN) and Picrotoxin (PCT) respectively.

Material and Methods

Plants

Red onion and yellow onions were purchased from local mart.

Chemicals

Pentylentetrazol (sigma), Strychnine (BDH chemicals), Picrotoxin(sigma), Diazepam (Valium by Roche), Methanol (Scientific Traders), Normal saline (ShazebPharma). Normal saline was used as control group and red and yellow onion methanolic extract was dissolved in normal saline prior to use.

Animals

Albino mice of both sexes weighing from 18 g to 30 g were used. Animals were obtained from the Animal house of the University of Veterinary and animal sciences, Lahore. Propylene cages were used to house the animals in special temperature and humidity controlled animal house and were acclimatized for at least one week before experiments with 12-12 hourly light dark cycle. Mice were acclimatized for seven days and standard diet along with water (ad libitum) was provided to all mice throughout study.

Plant extraction

Red onion and yellow onion bulbs were washed and chopped, then soaked in methanol for 7 days with occasional shaking. After 7 days the mixtures were filtered first from muslin cloth, then the filtrate was again filtered from whatmann filter paper no. 1. The filtrates were evaporated through rotary evaporator operating at temperature 45 °C. The semi-solid gummy material was obtained which were placed in china dishes and covered with aluminum foil. The extracts were placed at 2-8 °C until further use.

Phytochemical identification test

The phytochemical identification test was done for the presence of alkaloids, flavonoids, terpenoids, tannins, glycosides and Saponins (Jadon *et al.*, 2014).

Acute toxicity

The acute toxicity test was carried out for Red and yellow onion bulbs extract to investigate any possible toxicity. The albino female mice were given dose of 2000mg/kg. All the groups were evaluated for any reaction for the first 4h and then mortality was noticed for 14 days.

Anticonvulsant activity.

Pentylentetrazol induced seizures

Animals were divided into six groups, (n=5 mice of either sex in one group). Group I received vehicle (normal saline); group II was allotted for standard drug (diazepam 5 mg/kg); group III, and IV (200 and 400 mg/kg BW) received ROME at different dose levels; group V and VI (200 and 400 mg/kg BW) received YOME at different dose levels. All treatment and standard groups were statistically compared with vehicle groups. Vehicles, extracts were administered by oral route and standard drugs were administered by intra-peritoneal (*i.p.*) route. Mice were administered with extracts for seven days and on the experimental day, PTZ 60 mg/kg was injected intra-peritoneally to mice 60 min after vehicle or extracts and 30min after standard drug. Immediately after PTZ administration mice were observed for the onset of convulsions and mortality (Bhosle,2013; Basavaraj *et al.*, 2011;Umukoro 2013).

Strychnine induced seizures

Animals were divided into six groups, (n=5 mice of either sex in one group). Group I received vehicle (normal saline); group II was allotted for standard drug (diazepam 5 mg/kg); group III and IV (200 and 400 mg/kg BW) received ROME at different dose levels; group V and VI (200 and 400 mg/kg BW) received YOME at different dose levels. All treatment and standard groups were statistically compared with vehicle groups. Vehicles, extracts were administered by oral route and standard drugs were administered by intra-peritoneal (*i.p.*) route. Mice were administered extracts for seven days and on the experimental day, STN 1.2 mg/kg was injected intra-peritoneally to mice 60 min after vehicle or extracts and 30min after standard drug. Immediately after STN administration mice were observed for the onset of convulsions and mortality (Jeremiah *et al.*, 2015; Basavaraj *et al.*, 2011; Lahon *et al.*, 2016;Umukoro 2013).

Picrotoxin induced seizures

Animals were divided into six groups, (n=5 mice of either sex in one group). Group I received vehicle (normal saline); group II was allotted for standard drug (diazepam 5 mg/kg); group III, and IV (200 and 400 mg/kg BW) received ROME at different dose levels; group V and VI (200 and 400 mg/kg BW) received YOME at different dose levels.. All treatment and standard groups were statistically compared with vehicle groups. Vehicles, extracts are administered by oral route and standard drugs were administered by intra-peritoneal (*i.p.*) route. Mice were administered extracts for seven days and on the experimental day, PCT 6mg/kg was injected intra-peritoneally to mice 60 min after vehicle or extracts and 30min after standard drug. Immediately after PCT administration mice were observed for the onset of convulsions and mortality (Basavaraj *et al.*, 2011; Lahon *et al.*, 2016; Umukoro 2013)

Statistical analysis

The results obtained were expressed as mean ± SEM (Standard error of mean) of five animals. For statistical analysis, data was analyzed by 2 way ANOVA followed by Tukey’s test. Effects were considered to be significant at the P<0.05 level.

3. Results

3.1. Phytochemical Identification

Phytochemical identification of red onion and yellow onion bulbs indicated the presence of flavonoids, alkaloids, glycosides, terpenoids and tannins in all the extracts. However, saponins were absent in red onion, yellow onion. (Table 1).

Table 1: Photochemical identification of methanolic extract of Red and Yellow Onion Bulbs.

Treatments	Red ^a Onion	Yellow ^a Onion
Flavonoids	+	+

Alkaloids	+	+
Glycosides	+	+
Terpenoids	+	+
Tannins	+	+
Saponins	-	-

^a +, Present; -, Absent

3.2. Anticonvulsant activity

3.2.1. PTZ induced seizures

PTZ (60 mg/kg) induced seizures and mortality was observed in mice. The control group which was administered with normal saline produced seizures at 54 seconds (Table 2). Diazepam treated animal showed no seizures and absolute zero mortality. Red and yellow onion extracts at the doses of 200 and 400 mg/kg B.W significantly delayed the onset of seizures ($p < 0.0001$; Figure 1) with reduction in mortality up to 40% and 60% with red onion extract 200 and 400 mg/kg B.W, while only 20% with yellow onion extract 400mg/kg B.W. Green tea and black tea extracts at the doses of 200 and 400 mg/kg B.W also significantly delayed the onset of seizures ($p < 0.0001$; Figure 1) with reduction in mortality up to 40% and 60% with green tea extract 200 and 400mg/kg B.W, while 40% with black tea extract 400mg/kg B.W.

3.2.2. Strychnine induced seizures

Strychnine (1.2 mg/kg) induced seizures and mortality was observed in mice. The control groups, normal saline produced seizures at 120 seconds (Table 2). Diazepam treated animal showed no seizures and absolute zero mortality. Red onion extract at the doses of 200 and 400 mg/kg B.W and the yellow onion extract 400mg/kg B.W significantly delayed the latency of onset of seizures ($p < 0.0001$; Figure 2) with no reduction in mortality at any dose. The yellow onion extract at a dose of 200mg/kg did not delay the latency of onset of seizure significantly ($p > 0.05$; Figure 2) with no

reduction in mortality. and 400 mg/kg B.W significantly delayed the seizures ($p<0.05$ and $p<0.0001$; Figure 2) with no reduction in mortality rate.

3.2.3. Picrotoxin induced seizures

Picrotoxin (6mg/kg) induced seizures and mortality was observed in mice. The negative control group, normal saline, produced seizures at 292.2 seconds (Table 2). Diazepam treated animal showed no seizures and absolute zero mortality. Red onion extracts at the doses of 200 and 400mg/kg B.W significantly delayed the onset of seizures ($p<0.0001$; Figure 3) with 20% reduction in mortality at both doses. Yellow onion 200mg/kg B.W did not delay the onset of seizures significantly ($p<0.6694$; Figure 3) while yellow onion extract 400mg/kg B.W significantly delayed the onset of seizures ($p<0.0013$; Figure 3) with no reduction in mortality rate.

Table 2: Effect of methanolic extract of red and yellow onion bulbs on latency of seizures induced by a= pentylene tetrazole, b= picrotoxin and c= strychnine Treatment	Mean Onset of Clonus ± SEM in seconds(n=5)	No. Of Animals Convulsed	Percentage protection	Percentage mortality
Negative	(a) 54.1± 2.110	(a) 5/5	(a) 0%	(a) 100%

Control (Normal Saline 10ml/kg)	(b) 292.2± 3.441 (c) 47.84 ± 0.354	(b) 5/5 (c) 5/5	(b) 0% (c) 0%	(b) 100% (c) 100%
Positive Control (Diazepam 5mg/kg)	NIL	0/5	100%	0%
RACBE 200mg/kg	(a) 118.7± 1.320 (b) 326.4± 5.269 (c) 112.0± 0.353	(a) 3/5 (b) 5/5 (c) 5/5	(a) 40% (b) 20% (c) 0%	(a) 60% (b) 80% (c) 100%
RACBE 400mg/kg	(a) 143.6± 5.100 (b) 364.4± 5.741 (c) 142.78±0.245	(a) 2/5 (b) 5/5 (b) 5/5	(a) 60% (b) 20% (c) 0%	(a) 40% (b) 80% (c) 100%
YACBE 200mg/kg	(a) 83.8 ± 1.776 (b) 299 ± 4.207 (c) 55.38 ± 3.08	(a) 5/5 (b) 5/5 (c) 5/5	(a) 0% (b) 0% (c) 0%	(a) 100% (b) 100% (c) 100%
YACBE 400mg/kg	(a) 103.5 ± 1.910 (b) 317.2 ± 3.652 (c) 88.8 ± 0.470	(a) 1/5 (b) 5/5 (c) 5/5	(a) 80 % (b) 0% (c) 0%	(a) 20% (b) 100% (c) 100%

3.3. Acute oral toxicity

The results obtained indicated that red onion, yellow onion, green tea and black tea extract at oral doses up to 2000mg/kg did not produce any symptom of acute toxicity and none of the mice died during 14-day study period. All animals did not exhibit diarrhea, loss of appetite, change in skin color, sedation and mortality (Table 3).

Table 2: Acute oral toxicity parameters observed after administration of Red and Yellow Onion bulb extracts

Parameter	Response	
	Red Onion	Yellow Onion
Loss of Appetite	No change	No change
Diarrhea	No change	No change
Skin discoloration	No change	No change
Impaired water intake	No change	No change
Sedation	No change	No change
Death	Nil	Nil

Discussion

The preliminary phytochemical analysis of the plants helps to determine the active constituents in the plant which acts as therapeutic agents. (Wadood et al., 2013). It was reported earlier that phytochemical constituents of *Allium cepa* includes alkaloids, flavonoids, glycosides, tannins, terpenoids, resins and steroids, while saponins were absent in *Allium cepa* (Boukeria et al., 2016; Gazuwa et al., 2003). The preliminary phytochemical testing of methanol extracts of RACB and YACB in this study indicated the presence of flavonoids, alkaloids, glycosides, tannins and terpenoids, while it indicated the absence saponins, reducing sugars and alkaloids

Acute toxicity study is the screening of adverse effects which occurs after single dose administration via oral or dermal route given as a single dose or multiple doses in 24 hours or via inhalation route in 4 hours (OECD 401, 1987). The intraperitoneal lethal dose for onion bulb is 8700mg/kg in mice, while the oral lethal dose is 4090mg/kg in mice (Provital 2012). Clarke and Clarke define the safety as any substance having LD₅₀ more than 1000mg/kg. OECD measures the safety of a substance at LD₅₀ more than 3000 mg/kg. Methanol extract of *Allium cepa* bulb has the LD₅₀ of more than 4800mg/kg when used orally (Oyewusi et al., 2015). The LD₅₀ of methanol extracts of both RACB and YACB were found to be more than 2000mg/kg body weight

which was safe dose which did not show any hazardous effects and did not produce mortality in any mice. Absence of any adverse effect represents the safety of plant extract, and none of the adverse events were observed with both RACBE and YACBE at both 300 mg/kg body weight and 2000mg/kg body weight.

The following study exhibited a potent antiepileptic potential of red and yellow onion bulbs while the enhanced anticonvulsant activity of RACBE as compared to YACBE has been observed. The most probable reason behind which is the increased flavonoids and antioxidants content in RACBE than YACBE (Cheng et al., 2013).

The RACBE and YACBE has increased the latency of onset of convulsions in PTZ induced seizures significantly. The antiepileptic effect related to delay in onset of clonus was dose dependent i.e. maximum at increased doses while less at reduced dose. PTZ induced seizures involves alteration in GABAergic neurotransmission. PTZ inhibits GABA-A dependant receptors linked with chlorides channels which are dose dependant and independent of voltage. Earlier, it was suggested that PTZ act by binding to BZD binding site, but later on it was revealed that PTZ bind to PCT binding site but it differs in 6 position amino acids in TM2 (Huang et al., 2001). This explains that both RACBE and YACBE increase the inhibitory effect of GABA-A linked with chloride channels complex and reduce the potential of seizures and increase latency of onset of clonus. The high levels of antioxidants in RACBE and YACBE explains the more potent anticonvulsant activity of RACBE and YACBE in PTZ induced seizures in mice, the potential being more in red onion bulbs(Cheng et al., 2013).

STN is a receptor specified antagonist of glycine and inhibits glycine by competitive inhibition (Zhang et al., 1995).

Both RACBE and YACBE possess antioxidant potential which reduce the oxidative stress in brain as increased by strychnine. Certain alkaloids have been reported to provide protection against seizures induced by STN (Faggion et al., 2011). This might provide a link for effective delay in onset of clonus by RACBE and YACBE against STN to inhibit the glycine linked

neurotransmission because of presence of alkaloids which ultimately decreases the muscles contraction and seizures potential.

The effect produced was totally dose dependent which might be because of difference in antioxidant potential in extracts at different doses. The extracts were unable to provide protection to mice against mortality but they increased the delay in onset of clonus which was for short timed duration. The inability to provide protection to mice against mortality relates to insufficient dose of the extract. Increased doses might be able to provide protection against mortality.

PCT is a pro convulsing drug substance that directly inhibits the chloride channels; hence it is thought to produce the convulsions by altering GABAergic neurotransmission (Wannang et al., 2008). Development of Seizures because of PCT is slower than that of PTZ (Sefil et al., 2104). Flavanoids in red and yellow onion bulbs possess antioxidant as well as antiepileptic activity against PCT induced seizures and delay the onset of clonus (Slimistad et al., 2007; Fredotovic et al., 2017) RACBE and YACBE are rich in antioxidant which acts as a potent antioxidant and reduces the oxidative stress in brain, thus preventing the seizures generation (Jaiswal et al., 2013).

The results obtained from this study reveal that methanol extract of YACB and RACB possess certain bioactive metabolites which include Alkaloids, glycosides, flavonoids, tannins and terpenoids. Flavanoids are thought to be neuroprotective. Flavanoids also possess antioxidant property which strengthens the hypothesis of antiepileptic activity of RACBE and YACBE by binding to GABA-A with its linked chloride channels, glycine and NMDA receptors against PTZ, PCT and STN induced seizures. There exists a significant difference between delayed latency of onset of clonus produced by RACBE and YACBE which might be due to difference in antioxidants and flavonoids content in both (Olayeriju et al., 2015). The presence of high level of flavonoids makes Red and yellow onion bulbs extract as a good option to treat epilepsy.

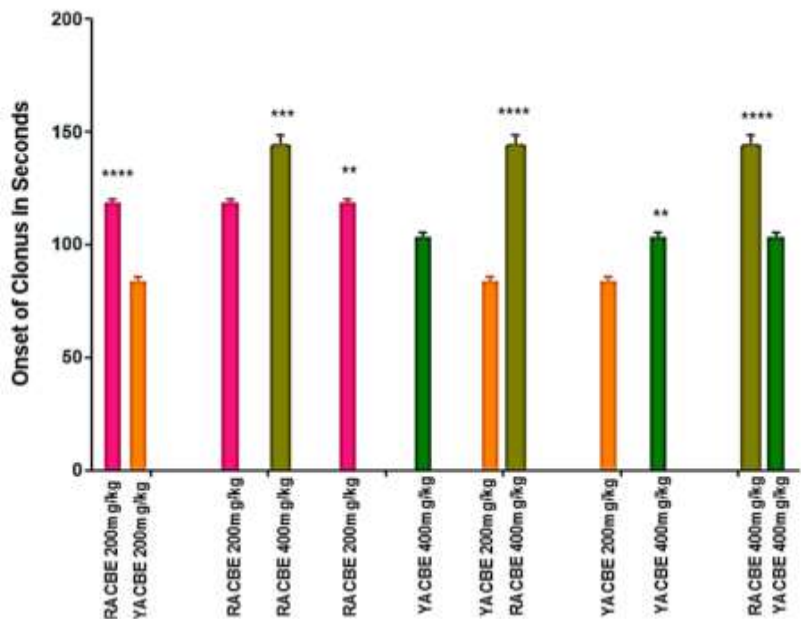


Figure 1: Effect of RACBE (200,400mg/kg P.O) & YACBE (200,400mg/kg P.O), Normal Saline (10ml/kg P.O) and Diazepam (5ml/kg I.P) on Onset of Clonus in PTZ Induced Seizures Model. . Data are presented as mean $\pm$ S.E.M, $\alpha=0.05$, $n=5$, using 2-Way ANOVA followed by Tukey's Test, ****= $P < 0.0001$, **= $P < 0.01$.

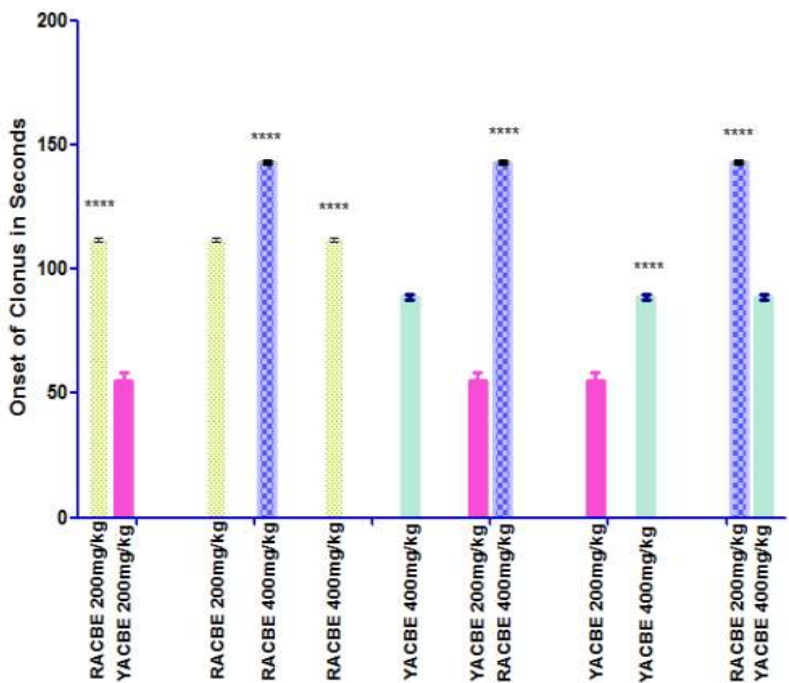


Figure 2 : Effect of RACBE (200,400mg/kg P.O) & YACBE (200,400mg/kg P.O), Normal Saline (10ml/kg P.O), and Diazepam (5mg/kg I.P) on onset of Clonus in STN Induced Seizures Model. Data are presented as mean $\pm$ S.E.M, $\alpha=0.05$, $n=5$, using 2-Way ANOVA followed by Tukey's Test, ****= $P<0.0001$

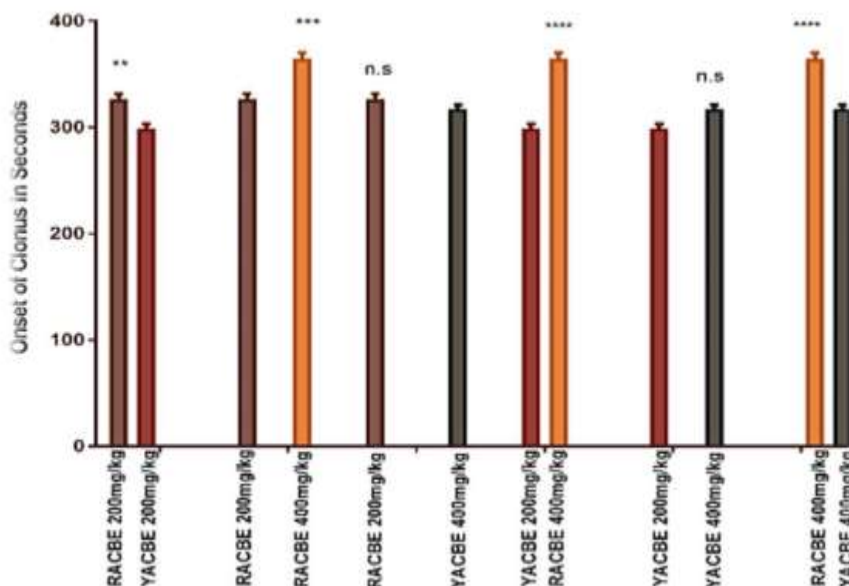


Figure3 : Effect of RACBE (200,400mg/kg P.O) & YACBE (200,400mg/kg P.O), Normal Saline (10ml/kg P.O), and Diazepam (5mg/kg I.P) on onset of Clonus in PCT Induced Seizures Model. Data are presented as mean $\pm$ S.E.M, $\alpha=0.05$, $n=5$, using 2-Way ANOVA followed by Tukey Test, ****= $P<0.01$

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