

# Dose Related Anxiolytic Effects of Methylalcholic Extract of Piper Betel Linn in NMR Mice

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## ABSTRACT

**Aim:** To evaluate the anxiolytic effect of the oral administrated doses 15mg/kg, 30mg/kg and 60mg/kg of piper betel linn in mice.

**Methodology:** This work was done at Hamdard University Karachi from 2008-2009. To observe the effects of these doses, two important tests light/dark box activity test and elevated plus maze test were carried out.

**Results:** All doses of piper betel linn presented a significant anxiolytic-like effect comparable to saline. But in comparison with the well-known anxiolytic drug diazepam, relief of anxiety at all these doses were lesser or same as diazepam.

**Conclusion:** This might be due to the presence of chavibetol and glutamic acid constituents in leaves (study in progress). Further studies should focus on understanding the mechanism of action of these constituents from this plant.

**Key words:** Methylalcholic extract, Piper Betel Linn, Light-dark choice test, elevated plus maze, Anxiolytic

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## INTRODUCTION

The Piper betel Linn is belonging to the Piperaceae family, which includes pepper and kava. Malaysia is a parent country of this plant but now these days different countries like India, Srilanka, Bangladesh, Burma and Nepal are also rising these plants for different purposes.<sup>1</sup> In different languages and religions name of piper betel is different, for example word Saptasira was used in Vedic and Tambool, Nagvelleri, and Nagani names were in Sanskrit.<sup>2,3</sup> Betel leaf has been used and cultivated from early time because many scientists have discovered hints of leaves in the holy caves of Asia (North West) in between 5500-7000BC, but more earlier its systematic and organized agriculture came to be practiced.<sup>6,7</sup>

A number of investigations were made on Betel vines plants. Various biological active compounds were isolated from leaves and stems, their amounts depends on climate, nature and season<sup>3,5</sup>. Some workers found that plants leaves were used in earlier time for different types of diseases, discomfort and conditions for example, whole leaves or juice of it was useful for relieving discomfort of digestive tract, and holding arouses sexual desires etc.<sup>1,4</sup> Some chemists have been isolated important essential oil and other compounds from the leaves of betel and mostly they were chavibetol, chavicol, and hydroxychavicol derivatives. These oils isolated were helpful in treating respiratory and other infectious diseases and conditions.<sup>8-14</sup> some workers believed that the presence of these essential oils, mainly phenols and terpenes are responsible for odour and smell of leaves.

More than thousand years ago, stalks and leave of betel vine were used for different types of illnesses in Ayurveda, traditional Indian medicine and many folk medicine in South Asia<sup>14,15</sup>. Sometime they used these to cure digestive and throat problems of children.

A number of biological, biochemical and pharmacological studies on piper betel vine showed and

authenticated the traditional uses, experimental line studies and pharmacological activity<sup>15-20</sup>. Razak and Co-workers (2003), Gulhane and associates (2015) and other scientists worked on various aspects of piper betel vine like respiratory diseases, anxiety, depression, fertility, cancer, inflammation and cardiovascular diseases. Preclinical tests have shown that betel leaf possess power or potency against bacteria, cariogenic effects, fungi, larva, anxiety, protozoa, filaria, allergy, diabetes, ulcer, infertility, cardiac problems, hyperlipidaemia, platelet aggregation, and immunomodulatory effects.<sup>21-25</sup> Beside these activities piper betel also has effects on CNS and anti-inflammatory activities in the traditional uses of medicine which have also been proved with experimental animals.<sup>26</sup>

## MATERIALS AND METHODS

**Plant extraction:** Piper Betel Linn was cultivated at home, specie was identified from Botany department, Karachi University. Fresh leaves were taken, cleaned in water and meshed them with the help of mortar and pestle. 1 kg of the material was soaked for 48 h in 1.5 L methanol and a comprehensive extraction was achieved and that was evaporated to dryness. The dried extract was dissolved in distilled water before each experimental session.

**Experimental animals:** NMR mice (30-35g) of either sex used in this consideration were collected from the Laboratory Animal Centre of Pharmacy Faculty of Hamdard University from 2008-2009 The animals were kept in well ventilated hygienic cages maintained understand environmental conditions (23-35°C, 12 h/12 h light/dark cycle) and were fed with standard rodent diet and water ad libium. NMR Mice were habituated for 14 days before the initiation of the experiment.

**Experimental design:** NMR mice (n=30) were classified into five classes, each class consist of six mice, one group or class was treated with normal saline (1ml/kg), 2<sup>nd</sup> was reference or diazepam treated group (1 mg/kg) and remaining three groups were test groups, doses of piper Betel Linn leaves extract (15, 30, and 60 mg/kg). All treatments of five groups were given orally. After one hour

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treatments of saline, diazepam and test doses, light/dark box activity<sup>37</sup> and elevated plus maze<sup>28</sup> for each mouse were performed for 5 minutes. During the test period, number of event and total time spent in the light box and open arm of elevated plus maze were monitored.

**Statistical analysis:** One- way analysis of variance (ANOVA) and post hoc (Newman-Keul) tests were used for analysis of data. The graphs were drawn by using Microsoft excel software.

**RESULTS**

Figures 1A, 1B and 2A and 2B showed the anxiolytic effects of methanolic extract at doses of 15 mg/kg, 30mg/kg and 60mg/kg on number of events and time spent in light/dark activity box and open arm of elevated plus maze experiments. Results illustrated statistical significant (P<0.01) effects of these doses of methanolic extract on novel anxiety of mice. Comparison of effects among these doses showed that doses of methanolic extract between 15-30mg/kg produced dose dependent effect but it was lesser at the highest dose of 60mg/kg than 15 and 30mg/kg doses. Comparison of these three doses of methanolic extract (15mg/kg, 30mg/kg and 60mg/kg) with diazepam (1mg/kg) as reference dose showed that only dose of 30mg/kg of methanolic extract has approximately same effect as diazepam exert but this anxiolytic effect is lesser at the dose of 15mg/kg and 60mg/kg than diazepam (1mg/m1).

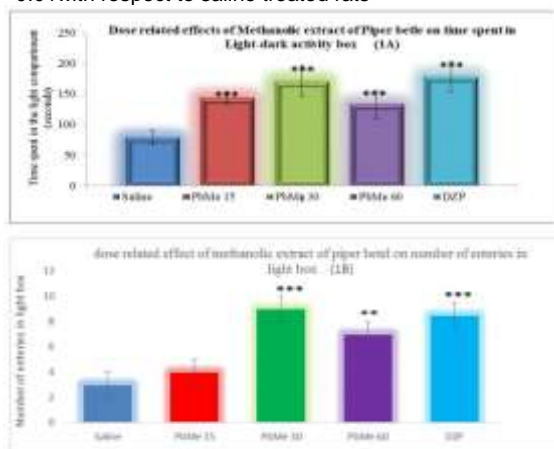
**DISCUSSION**

The anxiolytic activity of piper betel leaves extracts evaluated in mice this study. This animal model, especially with mouse, has been considered to be one of the most widely validated tests for determining of sedative and anxiolytic substances.<sup>28</sup> Present study explained that treatment of these doses, 15mg/kg, 30mg/kg and 60mg/kg showed antianxiety effects. Previous studies have also reported same findings that doses of ethanolic extract between 200-400mg/kg concentration increased the number of entries and time duration in light box of light-dark activity box and Elevated plus-maze.<sup>26</sup> They also described that betel leaves decreased the ability to enter in dark box of light/dark activity box. Our results also showed that effects of all doses are not similar to the results of standard or reference anxiolytic drug diazepam. same results of comparison of piper betel extract with diazepam was reported by Gulhane and co-workers (2015). They explained that diazepam shows anxiety releasing behaviour and its mechanism of action through GABA as anxiolytic drug is established but in case of piper betel, mode of action and mechanism has not been recognized realistically. The results of present study require further research work on piper betel. It is necessary to understand its anxiolytic action, doses and strength of doses should be increased.

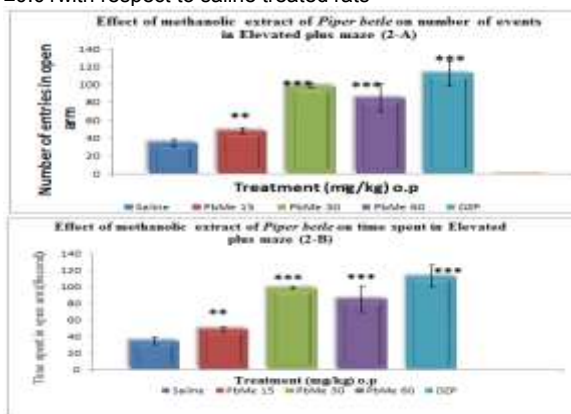
In light of review it is suggested that this effects may be due to presence of active compounds, volatile oil tannis, chavicals and most important constituents' alkaloid and arakene<sup>12</sup>. Some workers explained that Piper betel has Antioxidative properties that may directly affect the immunomodulatory functions and neurological activities

which may describe the anxiolytic activity of piper betel Linn.<sup>29</sup> The review studies on alcoholic extract of piper betel determined that this extract may accelerate the release of the neurotransmitter, acetylcholine, and cause activation of the postsynaptic receptor and decrease the secretion of neurotransmitters produced by dopaminergic and noradrenergic cells.<sup>30,31</sup> The interaction among these transmissions may influence on behaviour of mice and reduce the novel anxiety of the mice. Thus, betel chewing affect parasympathetic, GABAergic and sympathetic functions indicating its effects on central and autonomic nervous systems.<sup>32</sup> Some workers found antiplatelet and anti-oxidative effects of Piper betel extracts that may indirectly be related to anti-anxiety effect of methanolic extract of piper betel.<sup>21,33</sup> Some scientist suggested that the aqueous components of inflorescence Piper betel extracts are possible (ROS) reactive oxygen, species hunters and may inhibit platelet accumulation possibly via this effect.<sup>21,34,35,36</sup>

**Fig. 1:** Anxiolytic effects of piper betel at different doses(15,30 and 60mg/Kg).. Result are Significant statistically at the level of p\*\*\* ≤0.01with respect to saline treated rats



**Fig.2:** Anxiolytic effects of piper betel at different doses(15,30 and 60mg/Kg). Results are significant statistically at the level of p\*\*\* ≤0.01with respect to saline treated rats



**CONCLUSION**

Authentic studies in the past have stated unequivocally that betel leaf and its functioning compounds exhibit anxiolytic

effects of different histological origins. Since to its abundance, affordable cost and safety in consumption. Betel leaf remains a species with large potential and countless possibilities for further investigation.

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