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Concomitant administration of ethanol leaf extract of *Thymus vulgaris* on Diazepam– induced Sedation and Hypnosis in Wister Rat.

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Abstract
Thymes, belong to relatives of the oregano genus Origanum. They have medicinal, culinary and ornamental uses. The species are mostly cultivated and used for culinary purposes is Thymus vulgaris. Diazepam is commonly used in clinical setting in treatment and management of several conditions such as convulsion, insomnia, anxiety disorder and sleep disorder. This study wass aimed evaluating the effect of Thymus vulgaris, on diazepam– induced Sedation and Hypnosis in Wister Rat. A total of thirty (24) wister rats of 120–210 g of either sex were divided into four groups of six mice per group. Rats in all group received diazepam (4 mg/Kg). Group 2, 3 and 4 received concurrent dose of Thymus vulgaris (100, 200 and 400 mg/Kg) intraperitoneally. After 2 minutes of administration of the drugs sedative and hypnotic study was carried out. There was no significant (P<0.05) effect on sedation period by the extract when compared to group administered only diazepam. There was also significant (P<0.05), increase in sleep latency and sleep duration at 200 and 400 mg/kg when compared to the diazepam group. Result from the study showed that thymus vulgaris, a regular part of African spice during cooking, has synergistic effect on CNS acting drugs. This may necessitate the need for medical consideration, to patient who are under prescription for CNS related conditions.

Keyword: *diazepam, Thymus vulgaris, rats, sedative, hypnotic*

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I. Introduction

Benzodiazepines (BZDs), usually act as positive allosteric modulators on the gamma amino butyric acid (GABA)-A receptor [1,2]. GABA is the most common neurotransmitter in the central nervous system found in high concentrations in the cortex and limbic system. GABA is inhibitory in nature, thus reduces the possible excitability of neurons. GABA result into calming effect on the brain [3]. The GABA receptors are designated A, B, and C. Diazepam is mainly used to treat anxiety, panic attacks, insomnia, and symptoms, of acute alcohol withdrawal [4]. It is also used as a premedication for inducing sedation, anxiolysis, or amnesia before certain medical procedures [4,5]. Diazepam, is the preferred drug for treating benzodiazepine dependence because of it long half-life allowing better dose reduction. Benzodiazepines have a relatively high safety in overdose [6,7]. Adverse effects of diazepam include confusion (especially pronounced in higher doses), anterograde amnesia and sedation [8].

Approximately 150 species of *Thymus,* are abundantly found mainly in Asia, Africa, and North America. Recently, its range has been widely been extended to the Iberian Peninsula, with most of the species being endemic [9]. *Thymus vulgaris* L. is a medicinal plant of Lamiaceae family [9,10]. In traditional medicine, some *Thymus* specie are used for their antiseptic, antivirotic, antihelminthic, expectorant, antispasmodic, antimicrobial, antifungal, antioxidative, carminative, sedative, and diaphoretic effects. They are usually given by infusion, or are used externally in baths to cure skin disease and rheumatic [11]. Thyme contains high concentrations of phenols, including carvacrol, thymol, 1,8-cineole, borneol, q-cymene, linalool, a-pinene,

and camphor. Carvacrol and thymol are the main phenolic components that are primarily responsible for its antioxidative activity [12,13].

Active ingredients, of *T. vulgaris* are useful in the treatment of convulsions, respiratory diseases, smooth muscle spasm and bloating [14]. Meanwhile, *T. vulgaris* has an antispasmodic action on guinea pig ileum by decreasing the amplitudes of the muscle contractions during peristalsis by affecting the anticholinergic and serotonergic pathways [15].The aim of this study is to evaluate the effect of Thymus vulgaris on Diazepam - Induced Sedation and Hypnosis in Wister Rat.

II. Material and Method

Plant collection

Fresh leaves of *Thymus vulgaris,* were collected from its natural habitat from nearby Karu village, Nasarawa State, Nigeria. The plant was authenticated from Department of Botany, Bingham University, Nasarawa State, Nigeria.

Plant extraction

The leaves were shadow dried for two weeks. The dried plant material was further reduced into small pieces and pulverized. The powdered material was macerated in 70% ethanol. The liquid filtrates were concentrated and evaporated to dryness at 40°C *in vacuum* using rotary evaporator. The ethanol extract was stored at -4°C until used.

Animals

Male and female wister rats were obtained from Bingham University, Animal House. They were maintained on standard animal pellets and given water ad libitum. Permission and approval for animal studies were obtained from the College of Health Sciences Animal Ethics Committee of Bingham University.

Hind-limb parting test

Evaluation was done according to previously described method by simeon et al [16]. A total of thirty (24) wister rats of 120–210 g of either sex were divided into four groups of six mice per group. Rats in all group received diazepam (4 mg/Kg), while group 2, 3 and 4 received a concurrent dose of caffeine (100, 200 and 400 mg/Kg) respectively intraperitoneally. After 2 minutes of administration of the drugs the hind limb of each rat was widely parted and the time taken for the limbs to return to its normal position was noted. Diazepam-Induced Sleeping Time Determination

The sleep latency (time between diazepam administration and loss of righting reflex) and duration of sleep (from loss of righting reflex to recovery of the reflex) were recorded for each animal. The control animals were treated with diazepam alone. Group 1 diazepam (4 mg/Kg) served as the control.

Statistical analysis

Data used, were expressed as Mean ±Standard Error of the Mean (SEM). Data were also analyzed using one-way Analysis of Variance (ANOVA) and by Dennett's post hoc test for multiple comparisons between the control and treated groups. Values of $P \leq 0.05$ were deemed significant.

III. Result

Effect of Thymus vulgaris on diazepam induced sedation in rat

No significant ($P<0.05$) change was observed in the time taken for rat in all groups to return their widely parted hind limb to normal position when compare to the control (Table1).

Table 1: Effect of caffeine on diazepam induced sedation in rat

S/N	Group	Dose	Sedative effects (sec
1	Group 1	Diazepam 4 mg/kg	15.0±0.5
2	Group 2	Diazepam 4 mg/kg + T. Vulgaris 100 mg/kg	13.0±0.7
3	Group 3	Diazepam 4 mg/kg + T. Vulgaris 200 mg/kg	14.0±0.4
4	Group 4	Diazepam 4 mg/kg + T. Vulgaris 400 mg/kg	14.0±0.5

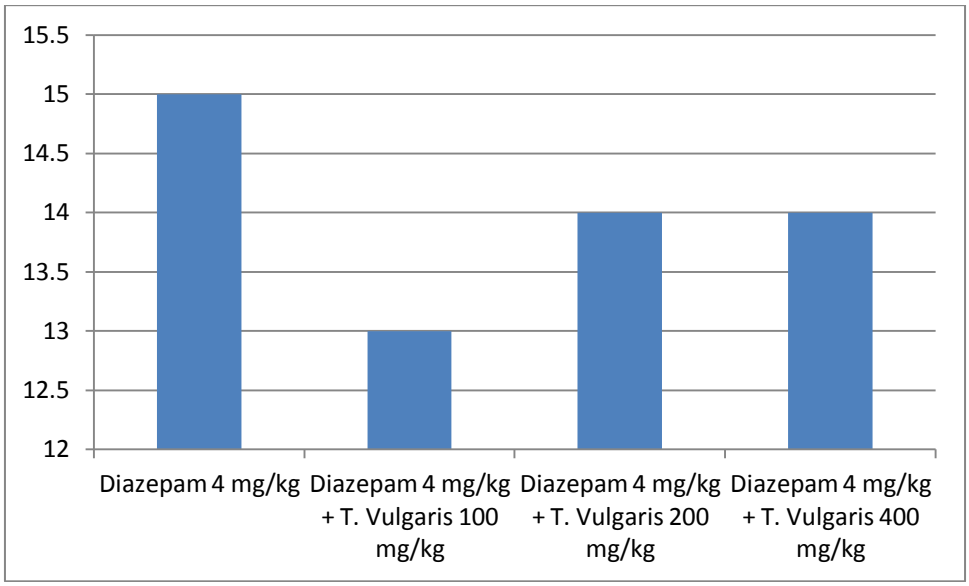

Fig. 1: Effect of Thymus vulgaris on diazepam induced sedation in rat

Effect of Thymus vulgaris on diazepam induced rat on sleep latency

There was significant (P<0.05) dose dependent increase in sleep latency in all group administered Thymus vulgaris when compare to control group that received diazepam alone.

Table 2: Effect of caffeine on diazepam induced rat on sleep latency

S/N	Group	Dose	Sleep latency (seconds)
1	Group 1	Diazepam 4 mg/kg	67.0±0.5
2	Group 2	Diazepam 4 mg/kg + T. Vulgaris 100 mg/kg	71.0±0.4
3	Group 3	Diazepam 4 mg/kg + T. Vulgaris 200 mg/kg	112.0±0.6*
4	Group 4	Diazepam 4 mg/kg + T. Vulgaris 400 mg/kg	119.0±0.2*

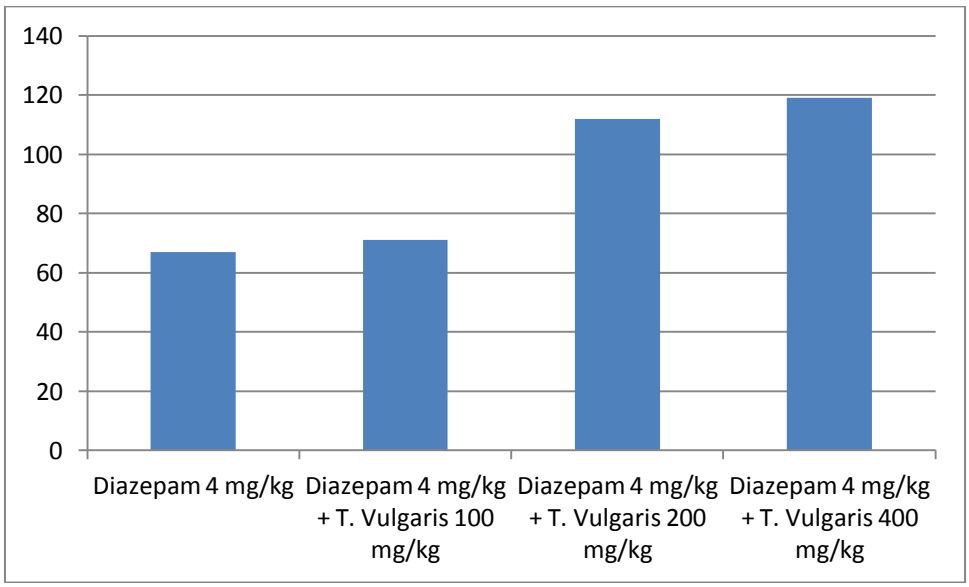

Fig. 2: Effect of Thymus vulgaris on diazepam induced rat on sleep latency

Effect of Thymus vulgaris on diazepam induced rat on duration of sleep

There was significantly (P<0.05) dose dependent increase in duration of sleep in all group administered extracts when compare to control group that received diazepam alone .

Table 3: Effect of caffeine on diazepam induced rat on duration of sleep

S/N	Group	Dose	Duration of sleep (seconds)
1	Group 1	Diazepam 4 mg/kg	1200±12.5
2	Group 2	Diazepam 4 mg/kg + T. Vulgaris 100 mg/kg	1230.0±22.4
3	Group 3	Diazepam 4 mg/kg + T. Vulgaris 200 mg/kg	1460.0±18.9*
4	Group 4	Diazepam 4 mg/kg + T. Vulgaris 400 mg/kg	1600.0±27.7*

*significantly different from the diazepam alone adminstered control at p <0.05.

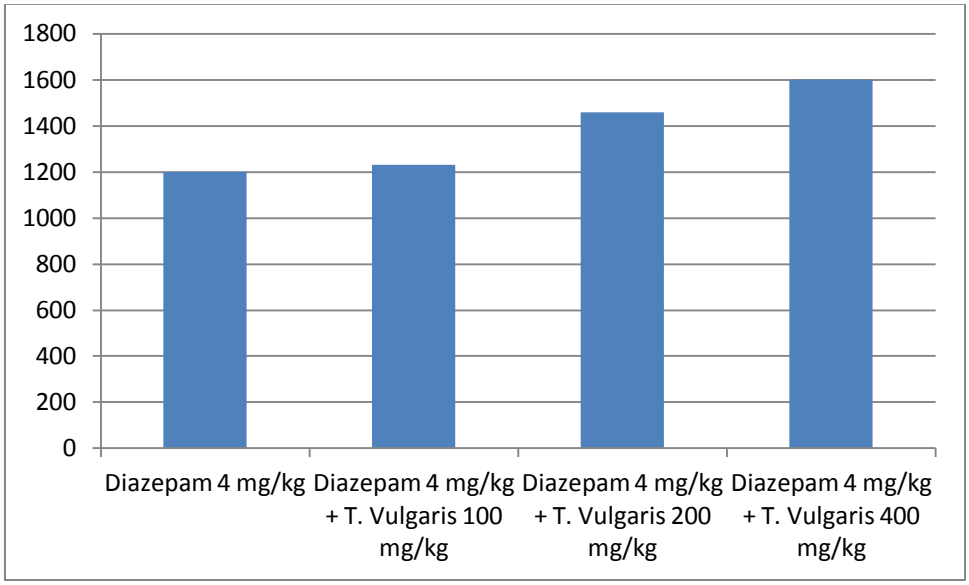

Fig 3: Effect of Thymus vulgaris on diazepam induced rat on duration of sleep

IV. Discussion

World Health Organization Drugs Strategy, claimed that 70% of the world's population has been using local plants as an alternative approach to convention drugs. Their relevance has improved tremendously in many parts of the world for more than the past 20 years [17]. . Herbal medicines, is a combination of active plant component that are assumed to work synergistically, resulting in effect greater than the sum of the effects of the single chemical entity[18]. There is the believed that herbal medicines, are safe because they are of natural origin. Many different unpleasant effects to herbs have been reported, and are been studied [15,17], including adverse events caused by drug-to-herb interactions[18,19]. Since all herbal medicines, are mixtures of different active ingredient, there is the potential of increase in the likelihood of interactions. Therefore, the possibility of drug-to-herb interactions is higher than drug-to-drug interactions, mostly because synthetic drugs usually contain just a single active ingredient.

In this study, diazepam causes sedation and hypnosis in administered rats. Diazepam is a benzodiazepine tranquilliser, with anticonvulsant, muscle relaxant, sedative, and amnesic effect [20,21]. Diazepam, similar to most benzodiazepine, bind to receptors in different area of the spinal cord and brain. This binding promote inhibitory effects of gamma-aminobutyric acid (GABA), to increase [20]. GABAs functions include CNS involvement in sleep induction [22] , control of hypnosis, anxiety, epilepsy , memory and neuronal excitability. Benzodiazepines, are known as positive allosteric modulators of the GABA type A receptors (GABAA) [23,34]. The GABAA receptors are ligand-gated chloride-selective ion channels that are activated by GABA, the main inhibitory neurotransmitter in the brain [25]. Binding of diazepam to this receptor complex, encourages the binding of GABA, which result to increased total conduction of chloride ions across neuronal cell membrane. This increased chloride ion influx, result in hyper polarization of the neuron's membrane potential [26]. This result, to increase in the difference between resting potential and threshold potential and firing is less likely. The arousal of the cortical and limbic systems in the central nervous system, is therefore reduced [27,28,29].

Several study, have shown that Thymus vulgaris has potential effect and influence on CNS activities. previous study has revealed that T. vulgaris extract has the potential effect in repairing and recovery effects on memory and behavioral disorders caused by scopolamine and may be useful in the clinical treatment of Alzheimer's disease. The study, also revealed that T. vulgaris reduced brain MDA levels in experimental groups [30,31]. Phytochemical analysis showed the presence of flavonoids, alkaloids phlobatannins, terpenoids, reducing sugars, thymol and carvacrol [32]. In another work, T. vulgaris, was found to manifest an anxiolytic profile in behavior of rat, which is not influenced by the locomotor activity [33,34]. This work, showed that

Thymus vulgaris had no significant effect in interfering with sedative time of the diazepam induce rat, while there was significant dose dependent increase in sleep latency in all groups and there was dose dependent increase in duration of sleep in all group administered with extracts. This means that T. vulgaris extract, may not interfere with sedative effect of CNS acting drugs, while still having the potential to cause difficulty in falling asleep. The study, also revealed that Thymus vulgaris, at high dose, may promote hypnotic activity of diazepam and possibly other CNS depressants. As previously mentioned, thyme among other active ingredient contains among other chemical molecules, thymol and carvacrol compounds [17,35]. It has been reported that thymol possess significant anticonvulsant and antiepileptogenic properties [36]. The similarity of action of thymol and GABA, could infer that this terpenoid acts centrally by mimicking or facilitating GABA action. Furthermore, it has been reported that thymol acts, as a positive modulator of the GABA (A) receptor [37]. Also, it has been established that anxiolytic effect of carvacrol in mice is involvement with GABAergic transmission [37].

V. Conclusion

Result indicates, that Thymus vulgaris, a regular condiment in Africa, Asian and Mediterranean food may have the ability to interfere with the activity of CNS depressants. The identification of several compounds such as polyphenols, flavonoids, and essential oil in the T. vulgaris further explains the possible CNS effects observed. It is possible, that the CNS activity observed in this work, was not only dependent on the flavonoid or essential oil content, but may be related to other molecules with antioxidant activity. Further study should be extended towards the activity of the plant on CNS stimulating agents. There is therefore the need for special attention on patients who have been expose to this plant while expecting a prescription on CNS acting drugs

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