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# SYNERGISTIC POTENTIAL OF HONEY BEE PRODUCTS WITH RIVASTIGMINE AS NOVEL NEUROPHARMACOLOGICAL APPROACH IN RODENT MODEL OF SCOPOLAMINE-INDUCED DEMENTIA

Zoology

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## ABSTRACT

The appearance of cognitive decline as a major hallmark of neurological and neurodegenerative disorders make it an intriguing pathophysiological state for which there is an urgent need for the development of effective pharmacotherapy. Decades of research have uncovered a multitude of promising factors that can serve as the starting point for research and development in this domain of research. Depleted cholinergic neurotransmission and oxidative damage are two such causative factors for cognitive impairment associated with neurodegeneration. Approved treatments for neurodegenerative disorders like Alzheimer's Disease (AD) provide only symptomatic relief and are known to cause unwanted side effects. Honeybee products are made up of bioactive substances, which have long been known for their medicinal and health-promoting effects. The purpose of the present study was to explore the neuroprotective potential of bee venom and bee propolis separately and in combination with the standard drug rivastigmine as a novel treatment regimen against scopolamine-induced cognitive deficits. Intraperitoneal administration of bee venom (0.5 mg/Kg), propolis (250 mg/Kg, oral) and rivastigmine (0.5 mg/Kg) or their combinations showed improved cognitive functions in scopolamine exposed mice as assessed by passive avoidance test. Administration of honeybee products (venom and propolis) alone and in combination significantly decreased the activity of acetylcholinesterase in the brain of scopolamine treated animals. Bee products either alone or in combination further decreased the lipid peroxidation with a concomitant increase in the activity of antioxidant enzymes (SOD, catalase, GPx, GR, and GST) in the brains of the animals treated with scopolamine. The data obtained suggests that the treatment with combination of bee venom and propolis could be developed into a novel and effective therapy against cognitive dementia associated with neurological and neurodegenerative disorders including Alzheimer's disease.

## KEYWORDS

Alzheimer's disease, Bee venom, Oxidative stress, Propolis, Rivastigmine, Scopolamine

## INTRODUCTION:

Cognition is the process of acquiring and comprehending information via the use of one's intellect, experiences, and senses [1]. Cognitive impairment observed in various neurodegenerative disorders like Alzheimer's Disease (AD), Parkinsons's Disease (PD), Amyotrophic lateral Sclerosis (ALS) etc. Alzheimer's disease (AD) is one of the most common health problems seen in geriatric population. AD is a progressive neurological disease that is linked to the irreparable decline of cognitive function with age. This is the most prevalent type of dementia, accounting for over 60% of all instances of memory impairment [2]. Till date, over 30 million individuals are affected by dementia worldwide, and it is anticipated thatmore than 115 million people would be affected by 2050 [3]. There are several theories associated with the cognitive decline associated with neurodegenerative disorders.

The current treatment options available for AD, envisages the importance of cholinergic neurotransmission in cognitive deterioration. Acetylcholine (ACh) is a neurotransmitter that is known to be a major player in maintaining cognitive balance. Acetylcholinesterase (AChE) is an enzyme that breaks down ACh to acetate and choline, thereby causing decreased cholinergic neurotransmission [4]. Therefore, AChE inhibition is one of the viable approaches for the amelioration of cognitive impairment. Till date, there is no effective cure for AD, however, some AChE inhibitors such as rivastigmine, physostigmine, tacrine and donepezil have shown to provide only symptomatic relief to the patients of AD [5]. None of these medications can slow the progression of the disease or stop and reverse the damage done by this disease. Therefore, it is imminent to find an effective alternative approach for the treatment cognitive decline associated with the neurodegeneration as observed in AD, which pose a significant clinical challenge to the researchers and medicinal chemists working in this field [6, 7].

Another prominent theory associated with the pathophysiology of cognitive decline is the oxidative stress. Reactive oxygen species (ROS) produces deleterious effects on the lipid and protein content of the neuronal network, causing alterations in the properties of neuronal structure and function, ultimately resulting in neurodegeneration. Therefore, managing oxidative stress is also a promising avenue of research for the management of cognitive decline [8].

Medicinal Chemists have explored a variety of synthetic compounds that have exhibited therapeutic potential, but, apart from synthetic compounds, natural products also play an important role in drug discovery and provide promising safe alternatives for the treatment of various diseases. Researchers have discovered antibacterial, hemolytic, cytolytic, anti-paralytic, insecticidal, and pain-relieving pharmacological activities in diverse natural chemicals (i.e., peptides) found in insects [9, 10]. Bee products found in *Apis mellifera* L. are potential source of natural antioxidants capable of counteracting the effects of oxidative stress, which is thought to have a role in the development of a variety of medical conditions [11]. Propolis is an antioxidant-rich honeybee product with a complicated chemical composition. It includes a high percentage of polyphenols, flavonoids, tannins, terpenoids, and phenolic chemicals, which also have antioxidant properties [12, 13]. Propolis has a wide range of biological and pharmacological activities[13], Antibacterial, antifungal, anti-inflammatory, antioxidant, immunomodulatory, antiviral and anticarcinogenic characteristics are only a few of them. Because of its broad-spectrum biological capabilities, propolis is becoming more popular as a reliable alternative therapy [14, 15].

Bee venom (BV) has a peculiarchemical structure, made up of organic and inorganic components that give it distinct bioactive properties [16]. Intrinsic bee-related parameters like age, strain, and caste, as well as external factors such as seasons and BV collecting techniques, have a major impact on BV content[17]. Proteins, peptides, enzymes, phospholipids, amines, carbohy drates, mineral salts, etheric oils, and other volatile compounds are all found in BV [18, 19]. Melittin is the most significant component of the bee venom. It can cure malignancies and infectious disorders by increasing capillary permeability,

increasing blood circulation, and lowering blood pressure[20]. Phospholipase-A2 and hyaluronidase are two more well-represented fractions. Hyaluronidase (an anti-inflammatoryenzyme) is made up of 349 amino acids and is a single polypeptide [21-23]. Apamin is BV's tiniest neuropeptide, with only 18 amino acids [24]. Hyaluronidase breaks down hyaluronic acid in various tissues and is used as an anti-inflammatory agent. In addition, tertiapin is also a small peptide with methionine residue that makes it extremely vulnerable to oxidation [19]. Bee venom has been shown to have protective effect against oxidative-based DNA damage from radiation in rat lymphocytes [25].

Thus, in view of the above facts the present study was designed to investigate the pharmacological effect of propolis and bee venomas a neuro-protectant in neurobehavioral deficits, oxidative damage, and antioxidant enzymesactivity in scopolamine-induced cognitive deficits and dementia. Scopolamine is a muscarinic cholinergic blocker that has been widely used to develop pre-clinical model of dementia and is a frequent paradigm for determining the efficacy of putative cognition-enhancing medicines. Several recent studies have found that scopolamine-induced memory loss in animal models is intimately linked to the brain [26, 27].

## Experimental

### Chemicals

The chemicals of analytical and molecular biology grade were used in this study. Bee venom was procured from Bee Hi Tech, New Delhi. Scopolamine and the standard drug Rivastigmine (>98% HPLC purified) was purchased from Merck company (Mumbai, India).

### Animals And Treatment Schedule

Six to eight weeks old Balb/c female mice (N=48, 25-30 g) were procured from Central Animal House, Panjab University, Chandigarh. The mice were housed in plastic acrylic cage at room temperature in well ventilated rooms for one week to acclimatize the experimental conditions. There was a free access to food (Ashirwad Industries, Kharar, Punjab, India) and water to all the animals of each cage. The experimental procedures of the current study were fist approved from Institutional Animal Ethics Committee (IAEC) of Panjab University and the study was commenced after obtaining the ethical clearance certificate (PU/45/99/CPCSEA/IAEC/2019/246) from the committee. The mice were randomly segregated (n=6) into eight groups. Blinding of experiment at each stage was followed to avoid any biasness in the data.

**Group 1 (Control):** The animals of this group were administered with normal saline (NS).

**Group 2 (Scopolamine):** Animals of this group received SCO (intraperitoneally, 2 mg/Kg body weight in normal saline) on 8$^{th}$ day.

**Group 3 (SCO+RIV):** The animals of this group were administered RIV (intraperitoneally; 0.5 mg/Kg body weight in normal saline) daily for 7 days and SCO on the 8$^{th}$ day in the dose given above.

**Group 4 (SCO+BV):** This group of animals were given BV (intraperitoneally; 0.5 mg/Kg body weight in NS) daily for 7 days and then received SCO injection on the 8$^{th}$day in dose mentioned above.

**Group 5 (SCO+PROP):** The animals of this group received bee propolis (PROP) orally (250 mg/Kg body weight) daily for 7 days and SCO on 8$^{th}$ day as per given dose.

**Group 6 (SCO+BV+PROP):** The animals of this group were administered with a combination of BV and PROP for a period of 7 days and then SCO was injected on the 8$^{th}$day according to dose given above.

**Group 7 (SCO+RIV+BV):** The animals of this group were administered with a combination of RIV and BV for a period of 7 days and SCO was injected on the 8$^{th}$ day according to the dose given above.

**Group 8 (SCO+RIV+PROP):** The animals of this group were administered with a combination of RIV and PROP for a period of 7 days and then SCO on the 8$^{th}$ day according to the dose mentioned above.

Doses of SCO, RIV, BV and PROP used in current study are already standardized in the previous reports from our laboratory [24, 28, 29]. The animals were sacrificed after the behavioral test on 8$^{th}$day of study. The treatment regimen of the present study is represented in Table 1.

### Extraction Of Propolis

Bee propolis was extracted from beehives of *Apis mellifera* L maintained by Department of Zoology, Panjab University Chandigarh. The method of Mani *et al*. [30] was used to obtain clean propolis from beehives.

### Collection Of Serum And Tissue Samples

Fresh blood was collected from retro-orbital sinus of each mice using capillary. To obtain serum, the whole blood was left undisturbed at room temperature for 30 mins. Subsequently, the clotted blood was centrifuged at 3000 g for 10 min. The clear yellowish serum was collected as supernatant. Additionally, tissue samples including brain were also collected and reperfused with ice cold isotonic saline. All the serum and tissue samples were stored at -80ºC for further experiments.

### Behavioral Assessment

#### Passive Avoidance Test:

To conduct the passive avoidance test, themethod described by Vohra and Hui [31] was followed. A wooden chamberwas prepared with a rubber platform (8 x 8 x 0.5 cm) in one corner. The floor of the chamber was covered with an electric grid. Briefly, a typical passive avoidance test consists of three phases:

**1**. **Adaptation**: The animals were acclimatized to the compartment for 90 sec.

**2. Training:** The animals were trained to stay on the rubber platform for a minimum time of 60sec. to avoid electric shock. Thereafter, the animals were subjected to a second long continuous electric shock (0.8 mA) from the grid floor whenever they went out from the safety zone. Basal step-down latency and escape down latency were recorded during this training session.

**3. Retention test:** On 8$^{th}$day, after scopolamine treatment, the animals were evaluated for the passive avoidance task following a 24 hr training period. Each mouse was placed individually on the rubber platform and the step-down latency was calculated for how long they don't get down from the platform. The time taken up by the animals to return to the rubber platform after being placed on the grid floor and subjected to electric current was measured as the step-down latency and escape latency. Numbers of mistakes were also recorded for all the animals during this test.

### Preparation Of Tissue Homogenate

To assess biochemical and oxidative stress parameters, tissue homogenate (10% w/v) of brain was prepared in ice cold 50 mM buffered PBS (pH 7.4). The tissues were placed in glass tube and homogenized with glass rod. The homogenate was placed on ice for 15 min and then centrifuged at 1000 g for 10 min at 4°C. The clear supernatant was collected and used for various biochemical assays.

### Biochemical Analysis For Oxidative Stress And Antioxidant Parameters:

#### *Lipid Peroxidation*

The lipid peroxidation was assessed in tissue homogenate by following the method of Ohkawa *et al*. [32]. The quantification of malondialdehyde (MDA) produced is an indicator of lipid peroxidation which reacts with thiobarbituric acid to form red adduct. The absorbance of the red adducts was measured at 532 nm and the results were expressed as nmoles MDA/mg protein.

#### *Reduced Glutathione (GSH)*

GSH levels were assessed in the homogenate by the method of Roberts and Francetic [33]. The assay mixture of 1 ml homogenate with 1ml sulphosalicylic acid 4 % (w/v) was centrifuged at 1200 rpm for 5 min. The supernatant thus obtained was then mixed with Ellman's reagent (0.1 mM DTNB in 0.1 M phosphate buffer, pH 8.0). The optical density was measured at 412 nm and the results were expressed as µmoles of GSH/mg protein.

#### *Superoxide Dismutase (SOD)*

Enzyme activity of SOD was estimated by the method of Kono [34]. The inhibition of reduction of nitroblue tetrazolium (NBT) mediated by hydroxylamine hydrochloride is governed by SOD. This reaction is accompanied with the development of blue color. The optical density was measured at 560 nm for 3 min. The results were expressed as units/mg protein.

#### *Catalase (CAT)*

The activity of CAT was assessed by the method of Aebi [35]. Briefly, the change in the absorbance of assay mixture consisted of $H_2O_2$-

phosphate buffer (12.5 mM $H_2O_2$ in 0.067 M Phosphate buffer, pH 7.0) and homogenate (0.05 ml) was recorded at 240 nm for 3 min. The results of CAT activity were expressed as µmoles of $H_2O_2$ decomposed/min/mg protein.

***Glutathione Peroxidase (GPx)***

The activity of G Px enzyme was measured by the method of Flohe and Gunzler with slight modifications [36]. The reaction mixture (2.6 ml phosphate buffer (50 mM, pH 7.0), 0.1 ml NADPH (6 mM), 5.0 µl GR (1 U), 0.1 ml of sodium azide (30 mM), 0.1 ml GSH (30 mM) and homogenate (0.1 ml) was prepared and equilibrated at 20°C. Subsequently, 0.1 ml of $H_2O_2$ (2.2 mM) was added to the reaction and change in absorbance was recorded at 340 nm for 3 min. The results were expressed as µmoles NADPH oxidized/min/mg protein.

***Glutathione Reductase (GR)***

The activity of GR was analyzed by the method of Carlberg and Mannervik [37]. For this, the assay mixture was prepared by mixing 2.7 ml phosphate buffer (0.067 M, pH 6.6), 0.1 ml EDTA (15 mM), 0.05 ml oxidized glutathione (7.5 mM) and 0.1 ml NADPH (6 mM in 1% $NaHCO_3$). After this, homogenate (0.1 ml) was added to this reaction mixture to initiate the reaction and change in absorbance was recorded at 340 nm for 3 min. The results were expressed as nmoles NADPH oxidized/min/mg protein.

***Glutathione-S-transferase (GST)***

The activity of GST was quantified by the method of Gronwald and Plaisance [38]. 1-chloro- 2, 4-dinitrobenzene (CDNB) was used as substrate of GST in this assay. The reaction mixture was prepared by adding 2.7 ml phosphate buffer (0.3 M, pH 6.5), 0.1 ml of CDNB (30 mM) and 0.1 ml post-mitochondrial supernatant was used for the test. Subsequently, 0.1 ml GSH (30 mM) was added to this reaction mixture and change in absorbance was recorded at 340 nm for 3 min. The results were expressed as nmoles of GSH-CDNB conjugate formed/min/mg protein.

**Biochemical Analysis For Acetylcholinesterase Inhibition**

***Acetylcholinesterase (AChE)***

To assess the AChE activity in the tissue homogenate, the method of Ellman was used[39]. This method involves the colorimetric estimation of a yellow chromophore, 5-mercapto-2- nitrobenzoate detected at 412 nm. The assay mixture was prepared by adding 2.6 ml of phosphate buffer (0.1 M, pH 8.0 containing 0.013% Triton-X 100), and 0.1 ml of 10 mM DTNB and 0.1 ml of acetylthiocholine iodide (14.9 mM). The reaction was initiated by adding 0.4 ml of homogenate to reaction mixture. The optical density of reaction was recorded at 412 nm and the results were expressed as nmoles substrate hydrolyzed/ min/ mg protein.

**Estimation Of Protein**

The protein was isolated from tissue homogenate and quantified by the method of Lowry *et al.* [40]. For this purpose, Biuret reagent was prepared by mixing sodium potassium tartrate (4.5 g in 40 mL of 0.2 N NaOH), cupric sulphate pentahydrate and potassium iodide (0.5 g) with volume made up to 100 mL with 0.2 N sodium hydroxide solution. To estimate the protein in the tissue homogenate, 50 µl of homogenate, 2.9 mL of normal saline and 3 mL of Biuret reagent were mixed and the reaction was incubated at room temperature for 10 minutes. The absorbance was measured at 540 nm using UV/VIS spectrophotometer (PerkinElmer, USA). Bovine serum albumin (BSA) was used as standard protein for plot generation and calculating the protein content in tissue homogenates. The results were expressed as mg/ml.

**Statistical Analysis**

The results of this study were expressed as mean ± SEM of each observation per groups and. One way analysis of variance (ANOVA) followed by Newman-Keuls test was applied for multiple pair wise comparisons between the various treated groups. Values with $P<0.05$ were considered as statistically significant.

## RESULTS:

**Effects Of Bee Venom And Propolis, Separately And In Combination Onscopolamine-induced Dementia And Neuro behavioral Deficit In Mice**

Basal latency and escape latency were measured on the 7$^{th}$day after 30 min of training. The animals received pretreatment with bee venom, propolis and rivastigmine and showed good learning and memory in passive avoidance test as depicted in **Fig 1 and Table 2**. On the 8$^{th}$ day, animals were evaluated for cognition test after receiving scopolamine injection. The test measured step down latency (SDL; **Figure 1A**), escape latency (EL; **Figure 1B**) and number of errors **(Figure 1C)**. On day 8, SDL and EL were noted to check the retention power of animals after 24 h of training. Numbers of errors werealso recorded on day 8 when mice were left in the chamber for 180 sec to analyze the acquisition. Prolonged SDL, decreased EL, and reduced errors were regarded as good memory functions. These parameters were significantly better with the rivastigmine treatment as compared to the control. However, other treatments of honeybee products, alone or in combination also showed statistically significant results. The combination of bee venom and propolis had a more profound effect in preventing the adverse effect of scopolamine and the results were closer to the rivastigmine treated group.

**Effects Of Bee Venom And Propolis, Separately Andin Combination, On Acetylcholinesterase Enzyme Activity In The Brain Of Scopolamine Treated Mice**

AChE activity was significantly increased in the brain of the scopolamine administrated mice (32.08 ± 2.83) as compared to the control animals (20.12 ± 1.94) **(Figure 2, Table 3)**. Rivastigmine (FDA approved acetylcholinesterase inhibitor drug) profoundly down regulated enzyme activity in scopolamine treated mice. In addition, Bee venom, propolis and their combination treated groups were found markedly effective in reducing AChE activity. However, inhibition of the enzyme activity was more pronounced in the group of animals treated with the combination of bee venom with Rivastigmine.

**Effects of bee venom and propolis, alone and in combination, on the oxidative stress and antioxidant defense system in brain of scopolamine treated mice**

***Lipid peroxidation (LPO)***

LPO, a marker of oxidative stress, was found significantly increased in the brain of the scopolamine treated mice in comparison to the control animals as shown in **Figure 3 and Table 3**. Rivastigmine did not show a promising effect in the reduction of oxidative stress. However, natural product treated groupsshowed promising effective on oxidative management. Even though, the combination of Rivastigmine with bee venom and propolis demonstrated a significant reduction in the LPO levels, bee venom and propolis given in combination were more effective in ameliorating the oxidative stress in scopolamine treated mice.

***Superoxide Dismutase (SOD)***

Scopolmine treated mice exhibited a significant reduction in the SOD activity when compared to the control group **(Figure 4A, Table 3)**. Rivastigmine alone did not show promising effect on the SOD activity, while, Rivastigmine in combination with the honeybee products showed marked effectiveness on the enzyme activity. Moreover, bee venom and propolis, both alone and in combinations, had a significant positive effect on enzyme activity.

***Catalase***

Catalase activity was observed to be significantly reduced in the brain of scopolamine treated mice as compared to the control animals **(Figure 4B, Table 3)**. Treatment with rivastigmine alone and in combination with honey bee products was profoundly effective in improving the catalase activity.

***Glutathione And Related Enzymes***

Glutathione (GSH) levels and activities of associated enzymes (GPx, GR and GST) were found to be significantly decreased in the brain of scopolamine treated mice as compared to their control counterparts **(Figure 5, Table 4)**. Rivastigmine didn't have any effect on GSH related antioxidant activity alone but showed significant preventive effect in combination with honey bee products. Bee venom and propolis showed synergistic effect in enhancing the glutathione content and related enzymes activity in scopolamine treated mice.

## DISCUSSION:

Apitherapy is a complementary healthcare system that uses honeybee products, particularly bee venom, and propolis to treat a variety of human diseases. Bee venom and propolis include a variety of active compounds, including peptides and enzymes, which offer therapeutic potential for a variety of illnesses and ailments[16, 19].It is worth noting that a number of these compounds have the potential to become vital alternative sources for the synthesis of more effective drugs with

fewer side effects [41]. Treatment of neurodegenerative disorders and the associated cognitive dementia is one such area, where there are only a few treatment options available and current pharmacotherapy for these disorders entails numerous unwanted-side effects. Bioprospecting of these natural substances available from honeybee is significant and a viable approach because the medications currently administered to treat severe neurological illnesses provide only symptomatic alleviation and the prevalence of serious side effects remains high [42, 43]. This study presents the research on the promising effects of the bee venom and propolis in the alleviation of cognitive decline, which was comparable to the effects of Rivastigmine in scopolamine-induced pre-clinical model of dementia. Scopolamine, a well-knownsyntheticdrug which is non-selective muscarinic antagonist and blocks cholinergic signaling and produceslearning and memory impairment is considered as a good model of dementia AD [44, 45]. In this study, the effect of scopolamine on neurobehavioral dysfunction is examined by employing a passive avoidance test to measure the duration of step-down latency, escape latency, and reference memory errors. The present data demonstrated that bee venom and propolis could mitigate the cognitive impairment in scopolamine-induced cognitive deficits in mice as comparable to the rivastigmine treatment. Ye M *et al.* [46] have demonstrated neuroprotective effect of bee venom PLA2 in transgenic mouse model of AD due to its antioxidant and anti-inflammatory properties. Moreover, extensive studies have revealed that bee venom has ability to restore neurochemistry by down regulating the neuro-inflammation and apoptotic markers in Parkinson disease [41, 47]. A study on wild type *Drosophila melanogaster* reported propolis as a memory enhancer and it gave remarkable results with combination of donepezil [48]. Another study has reported the anti-inflammatory effects of propolis in acute and chronic conditions due to the presence of polyphenolic compound [19].

Attention and memory problems in AD patients are linked to reduced levels of acetylcholine, which are controlled by acetylcholinesterase enzyme in the brain. Blockage of acetylcholinesterase activity improves cholinergic depletion hence boosts cognitive performance [49]. In the present study acetylcholinesterase activity was significantly high (32.08 ± 2.83) in scopolamine induced mice which was significantly controlled with pre-treatments of bee venom (24.31 ± 1.89) and propolis (26.51 ± 1.54), effectiveness was near to rivastigmine (22.25 ± 2.80) treatment. Hyper-activation of the cholinergic anti-inflammatory pathway is mediated by cytokines, which further increase the AChE activity and might be associated with increased risk of AD [50]. Bee venom down regulates the production of NO, COX-2, PGE2 and pro-inflammatory cytokines (IL-1β, IL-6 and TNF-α) in lipopolysaccharide (LPS) exposed murine BV-2 microglia [51].One of the previous study has reported that AChE activity is profoundly inhibited by flavonoids, potential natural compounds for treating AD, since the therapy involves rise in acetylcholine levels [52]. Propolis is rich in polyphenols (flavonoids, phenolic acids, and esters), phenolic aldehydes, and ketones, as mentioned in literature [19, 53] and due to its components may reduce the acetylcholinesterase activity.

Haider *et al.* [54] have demonstrated that scopolamine administration induced the alteration in neurochemical functions and oxidative stress in the rodent brain. In addition, AChE inhibition also plays a key role to accelerate the oxidative stress [55]. In our results, a significant increase in LPO levels and profound degradation in antioxidants (SOD, CAT, GSH associated enzymes) were found in the brain of scopolamine treated mice, which were restored to normal by the honeybee products. Goschorska *et al.* [56] in their research have detailed that AChE inhibitors like donepezil and rivastigmine were not able to normalize the fluoride-induced oxidative stress, as evident lack of antioxidant property. El Adham *et al.* [19] in their study detailed the efficiency of bee venom and propolis as an antioxidant on gamma rays induced oxidative stress in rats. On one hand, bee venom can cause irritation but on the other hand it has high antioxidant profile due the presence of melittin and apamin (active constituents of bee venom) as reported in the study of Somwongin *et al.* [57].Various studies have reported that propolis has free radical scavenging properties [58].

In conclusion, the findings of the present study provide evidence that bee venom and propolis exert neuroprotective effect against the scopolamine induced dementia through free radical scavenging activity and maintenance of antioxidant enzymes which further aids to reduce the tissues toxicity. The findings from the study may at least in part explain the acetylcholinesterase inhibition activity of the honeybee products in experimental studies. It is noteworthy that approved drugs for the AD treatment may givemore effective results with the combination of honeybee products. Thus, bee venom and propolis synergistically may have the neuroprotective potential for the treatment of neurological diseases associated dementia including AD. This could serve to bridge the gap in the current paradigm of Alzheimer's research and open research avenues for other natural products for the alleviation of cognitive deficits.

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**Conflicts Of Interest**
The authors have no conflicts of interest to disclose.

**Table 1. The Experimental Protocol And The Treatment Groups Employed In Passive Avoidance Test.**

| Groups | Injection 1 | Injection 2 | Injection 3 |
|---|---|---|---|
| CONTROL | Saline | Saline | |
| SCOPOLAMINE | Saline | Scopolamine (2 mg/Kg) | |
| SCO+RIV | Rivastigmine (0.5 mg/Kg) | Scopolamine (2 mg/Kg) | |
| SCO+BV | Bee venom (0.5 mg/Kg) | Scopolamine (2 mg/Kg) | |
| SCO+PROP | Propolis(250 mg/Kg) orally | Scopolamine (2 mg/Kg) | |
| SCO+BV+PROP | Bee venom (0.5 mg/Kg) | Propolis(250 mg/Kg) orally | Scopolamine (2 mg/Kg) |
| SCO+RIV+BV | Rivastigmine (0.5 mg/Kg) | Bee venom (0.5 mg/Kg) | Scopolamine (2 mg/Kg) |
| SCO+RIV+PROP | Rivastigmine (0.5 mg/Kg) | Propolis (250 mg/Kg) orally | Scopolamine (2 mg/Kg) |

**Table 2. The Memory Parameters (Latency, Escape Latency And Number Of Mistakes) Of bee Venom And Propolis, Separately Or In Combination With Rivastigmine On Scopolamine-induced Cognitive Deficits And Dementia Assessed By Passive Avoidance Test.**

| Groups | Basal (seconds) | SDL (seconds) | Basal EL (seconds) | EL (seconds) | No. of Mistakes |
|---|---|---|---|---|---|
| CONTROL | 9.5 ± 1.04 | 153.83 ± 1.47 | 40.33 ± 1.63 | 20.33 ± 1.63 | 4.5 ± 1.04 |
| SCOPOLAMINE | 10.17 ± 1.47 | 12.67 ± 1.63* | 37.17 ± 2.63 | 31.33 ± 2.80* | 16.17 ± 1.47* |
| SCO+RIV | 11.33 ± 2.16 | 92.67 ± 2.58# | 33.83 ± 2.04 | 15.67 ± 2.3# | 6.33 ± 1.75# |
| SCO+BV | 9.83 ± 1.47 | 58.5 ± 4.13# | 36.5 ± 2.88 | 24.17 ± 2.31# | 9.67 ± 1.63# |
| SCO+PROP | 7.83 ± 1.16 | 46.83 ± 3.43# | 35.5 ± 4.08 | 25.67 ± 2.33# | 13.17 ± 1.94# |
| SCO+BV+PROP | 10.17 ± 1.47 | 62.17 ± 4.70# | 33.17 ± 3.18 | 23.5 ± 1.87# | 8.83 ± 1.47# |
| SCO+RIV+BV | 9.83 ± 1.47 | 93.33 ± 6.28# | 32.5 ± 3.27 | 19.83 ± 2.31# | 7.5 ± 1.04# |
| SCO+RIV+PROP | 7.83 ± 1.16 | 85.67 ± 4.96# | 33.67 ± 2.42 | 22.67 ± 1.63# | 7.83 ± 1.47# |

**Table 3. Effect Of Bee Venom And Propolis, Separately Or In Combination With Rivastigmine On Acetylcholinesterase (AChE), LPO, SOD And Catalase Activity In The Brain Of Scopolamine Induced Cognitive Deficit And Oxidative Stress.**

| Groups | AChE activity (nmoles/min./mg protein) | LPO (nmoles MDA/mg protein) | SOD (units/mg protein) | Catalase (µmoles of $H_2O_2$decomposed/min/mg protein) |
|---|---|---|---|---|
| CONTROL | 20.12 ± 1.94 | 0.85 ± 0.04 | 0.58 ± 0.04 | 21.71 ± 0.92 |
| SCOPOLAMINE | 32.08 ± 2.83* | 1.65 ± 0.05* | 0.15 ± 0.03* | 9.47 ± 1.06* |

| | | | | |
|---|---|---|---|---|
| SCO+RIV | 22.25 ± 2.80# | 1.44 ± 0.03# | 0.22 ± 0.03 | 13.60 ± 1.08# |
| SCO+BV | 24.31 ± 1.89# | 1.25 ± 0.04# | 0.41 ± 0.03# | 14.38 ± 1.68# |
| SCO+PROP | 26.51 ± 1.54# | 1.36 ± 0.04# | 0.38 ± 0.04# | 12.87 ± 1.66# |
| SCO+BV+PROP | 23.91 ± 2.24# | 1.04 ± 0.03# | 0.46 ± 0.04# | 18.31 ± 1.47# |
| SCO+RIV+BV | 21.01 ± 1.67# | 1.24 ± 0.04# | 0.38 ± 0.05# | 15.93 ± 1.69# |
| SCO+RIV+PROP | 21.92 ± 2.02# | 1.11 ± 0.04# | 0.35 ± 0.05# | 14.07 ± 1.93# |

**Table 4. Effect Of Bee Venom And Propolis, Separately Or In Combination With Rivastigmine On Glutathione Levels, Glutathione-s-transferase, Glutathione Reductaseand Glutathione Peroxidase Activity In The Brain Of Scopolamine Induced Cognitive Deficit Animals.**

| Groups | GSH (µmoles/mg protein) | GST (nmoles/min./mg protein) | GR (nmoles NADPH/min./mg protein) | GPx(µmoles NADPH oxidized/min./mg protein) |
|---|---|---|---|---|
| CONTROL | 45.68 ± 3.77 | 83.11 ± 2.75 | 1.51 ± 0.06 | 0.79 ± 0.04 |
| SCOPOLAMINE | 23.55 ± 3.74* | 33.60 ± 3.26* | 0.85 ± 0.05* | 0.40 ± 0.06* |
| SCO+RIV | 27.83 ± 2.29 | 36.82 ± 4.15 | 0.87 ± 0.04 | 0.42 ± 0.04 |
| SCO+BV | 32.21 ± 3.64# | 58.77 ± 3.43# | 1.21 ± 0.06# | 0.58 ± 0.04# |
| SCO+PROP | 33.12 ± 2.42# | 50.54 ± 3.25# | 1.13 ± 0.06# | 0.55 ± 0.04# |
| SCO+BV+PROP | 37.76 ± 4.03# | 66.42 ± 3.85# | 1.42 ± 0.04# | 0.66 ± 0.03# |
| SCO+RIV+BV | 31.33 ± 4.24# | 51.76 ± 4.10# | 1.28 ± 0.03# | 0.57 ± 0.05# |
| SCO+RIV+PROP | 29.15 ± 3.90# | 52.50 ± 3.81# | 1.26 ± 0.06# | 0.55 ± 0.05# |

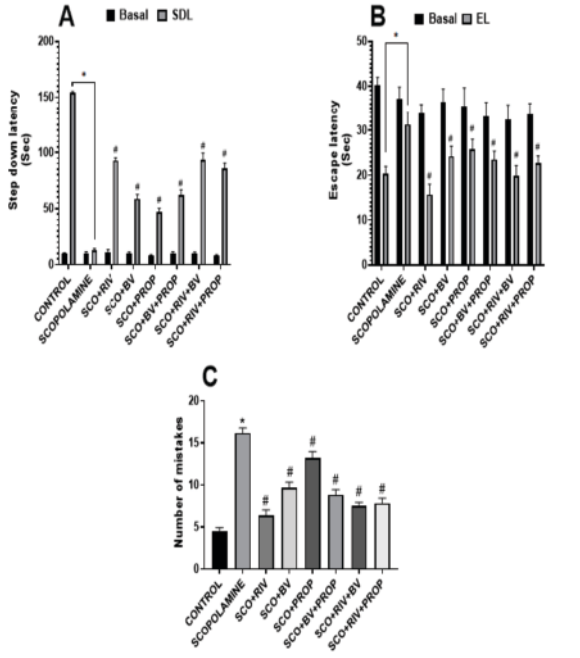


**Figure 1:** Effect of bee venom and propolis, separately or in combination with rivastigmineonscopolamine-induced cognitive deficits and dementia assessed by passive avoidance test. Bar graph showing step down latency (A);Escape latency (B); and number of mistakes (C) in passive avoidance task. Values are expressed as mean±SEM; n=6. *Significantly different from control group (p<0.05), #Significantly different from scopolamine treated group (p<0.05).

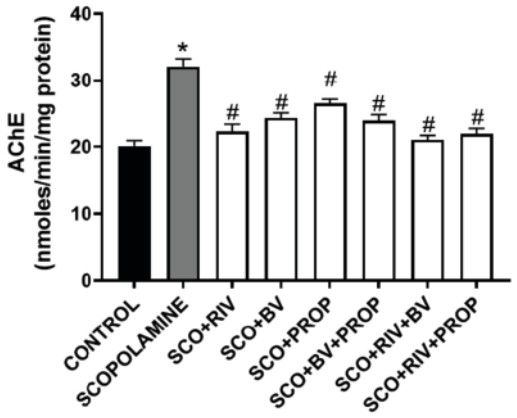


**Figure 2:** Effect of bee venom and propolis, separately or in combination with rivastigmine on acetylcholinesterase (AChE) enzyme activity in the brain of scopolamine treated animals. Values are expressed as mean±SEM; n=6. *Significantly different from control group (p<0.05), #Significantly different from scopolamine treated group (p<0.05).

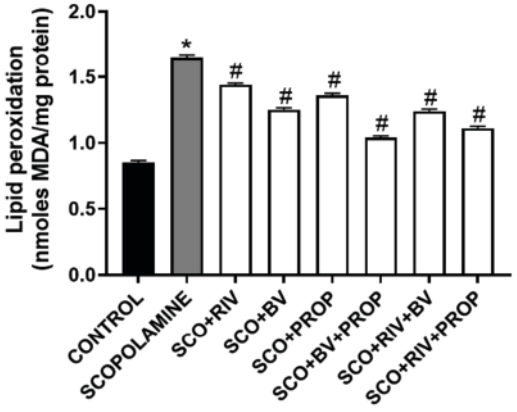


**Figure 3:** Effect of bee venom and propolis, separately or in combination with rivastigmine on lipid peroxidation (LPO) in the brain of scopolamine treated animals. Values are expressed as mean±SEM; n=6. *Significantly different from control group (p<0.05), #Significantly different from scopolamine treated group (p<0.05).

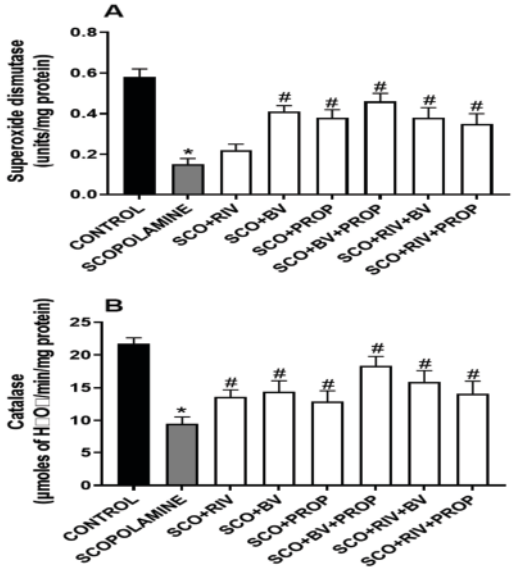


**Figure 4:** Effect of bee venom and propolis, separately or in

combination with rivastigmine on enzyme activity of superoxide dismutase (SOD) (A) and catalase (B) in the brain of scopolamine treated animals. Values are expressed as mean±SEM; n=6. *Significantly different from control group (p<0.05), #Significantly different from scopolamine treated group (p<0.05).

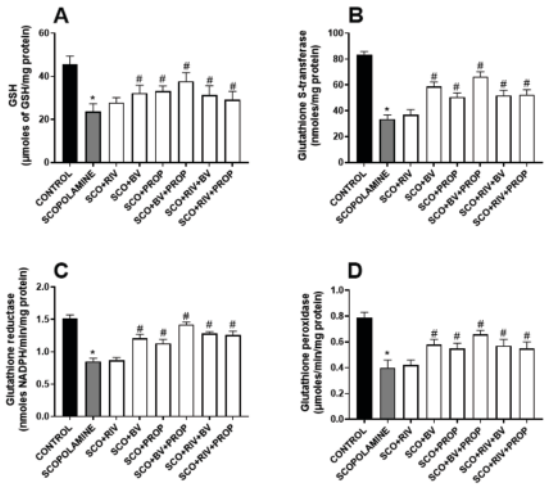


**Figure 5:** Effect of bee venom and propolis, separately or in combination with rivastigmine on glutathione levels (A); Enzyme activity of Glutathione-S-transferase (B); Glutathione reductase (C); and Glutathione peroxidase (D)in the brain of scopolamine treated animals. Values are expressed as mean±SEM; n=6. *Significantly different from control group (p<0.05), #Significantly different from scopolamine treated group (p<0.05).

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