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Evaluation of Acute Toxicity and Anti-Ulcer Activity of Malaysian *Apis Mellifera* Bee Venom in Experimental Animals

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Abstract**: The use bee products is know from thousands of years and its usage in treating various pathological condition has been well documented in various religious texts. Apis Mellifera Bee Venom (BV) have 18 pharmacological active compound and is known to treat many diseases such as arthritis and pain. No proven studies of Apis Mellifera Bee Venom on toxicity and peptic ulcer have been carried out. Hence, this study was undertaken to screen BV for acute toxicity and anti-ulcer activity. The Bee Venom was extracted from the bee in a bee farm in Melaka from the bee species of Apis Mellifera. The acute toxicity studies were carried out on mice. Peptic ulcer was induced by 80% ethanol orally and by pyloric ligation. The Sprague Daley rats were pre-treated with bee venom for 7 days via intra-peritoneal (IP). Two different doses of Apis Mellifera Bee Venom, 0.28mg/kg and 0.56mg/kg were used to screen the anti-ulcer activity. The results were evaluated for statistical significant difference by one-way ANOVA with a post hoc Dunnett test. No mortality was witnessed in the animals throughout the treatment for acute toxicity test. Both the dose which is 0.28mg/kg and 0.56mg/kg showed anti-ulcer properties which was evaluated through ulcer index, determination of total acidity and histopathology studies. This result is probably due to the melitin in the Apis Mellifera Bee Venom which reduces the reactive oxidative stress and protects the gastric mucous membrane

Keywords: Peptic Ulcer Disease, Apis Mellifera Bee Venom, Anti- ulcer, Acute toxicity

1. INTRODUCTION:

In the present scenario many of the analgesic and anti-inflammatory drugs are being developed and are used for many of the pathological conditions. One of the most common problem with these drugs is GIT bleeding and ulceration. Stress is also one of the major contributors for the gastric ulcer. The usage of natural products that originate from either plant, animal or marine sources have been shown great interest. The concept of food as medicine has been in practice centuries ago[1]. Peptic ulcers diseases is one the most common diseases affecting many people through the world. It is caused because of the imbalance in the defensive system like mucosal membrane, prostaglandins ect and aggressive factors like gastric acid, pepsin ect. In addition to this *Helicobacter pylori* and many drugs like analgesic and NSUIDs also responsible for causing peptic ulcer[2]. These elements can cause submucosal disintegration and repress cyclooxygenase, in this manner irritating the insurance

of the gastric mucosal layer. Anatomically, peptic ulcers happen for the most part in the stomach and proximal duodenum. Peptic ulcers are caused by an irregularity between the gastric mucous membrane discharge, mucosal obstruction, blood stream, cell recovery and endogenous defensive operators and dangerous which are corrosive and pepsin emission elements of the gastric framework. Liquor actuated gastric sores impede gastric barrier factors, for example, bodily fluid discharge and mucosal dissemination. Ethanol causes necrotic injuries in the gastric mucosa through numerous pathways, straightforwardly delivering necrotic sores, which thusly lessens protective elements, bicarbonate emission and bodily fluid generation. The gastric divider bodily fluid is thought to assume an essential part as a cautious obstruction against gastrointestinal harm. Bodily fluid emission is thought to be a significant cautious factor that shields the gastric mucosa from injuries which incorporate an epithelial obstruction, bodily fluid discharge, bicarbonate, prostaglandins, nitric oxide, development factors, warm stun proteins and consistent blood stream. Mucosal damage may happen when toxic elements overpower an in place mucosal safeguard or when the mucosal guard is by one means or another disabled . The level of gastric divider bodily fluid has been assessed beforehand and is utilized as a pointer of gastric bodily fluid emission.[3]

Peptic ulcer ailment is regularly a non-deadly illness that principally gives side effects of epigastric agony normally calmed by nourishment or salt. Side effects regularly show periodicity implying that they happen, melt away, and repeat over long stretches.[4] When gastric mucosa is presented to harming specialists, it incorporates the disturbance of the unstirred bodily fluid, bicarbonate, phospholipid layer, peeling of the surface epithelium with loss of its boundary and the more profound gastric mucosal layers, including microvascular endothelial cells, forebear, parietal and boss cells. At the point when the slender endothelium was harmed, it prompted microvascular stasis with discontinuance of oxygen and supplement conveyance and hypoxia.

There are few drug classes available for the treatment of gastric ulcers, that includes proton pump inhibitors, M_1-receptor blockers, and H_2-receptor blockers[5]. However these drugs possess side effects including arrhythmia, gynaecomastia, and hematopoietic changes. Additionally, there is a high backslide rate of 80% at first year and 100% in the second year of treatment. Different issues incorporate the long haul span of the treatment time frame in where treatment with H2-receptor enemies for 1 year and the deficient destruction of ulcers. Consequently, new medications have been tried to improve the viability of current medications or to find potential new specialists that are more powerful and more affordable and have less wellbeing related reactions than those at present utilized.[6]

The use of honey and other bee products have been use for thousands of years and the healing properties of these products have been included in many religious texts including the Veda, Quaran and Bible. Since ancient time in traditional medicine bee venom therapy have been utilize in the application to treat various diseases. *BV* have been reported to possesses a many of different peptides that includs melittin, apamin, adolapin and mast cell degranulating peptide[7] (Son et al., 2007). Bee venom has been used as an alternative medicine in treating many diseases like cancer related problems and also in rheumatoid arthritis problems. It has also been reported to use as a cosmetic ingredient for anti-ageing agent.[8] For the purpose of accessing bee venom for further pharmacological testing this study aimed to test for toxicity and anti-ulcer activity in experimental animals.

2. MATERIALS AND METHODS

Collection of Venom

The venom of the bee species Apis Mellifera was collected from a bee farm in Melaka, Malaysia with the assistance of the beekeepers. The venom was collected via the Electrically Stimulated (ESV) method which uses very mild electric shock. The tray was then put on the

hive top and the steal wires were facing downwards as it was being assessed. Maximum of 3 volts was used to charge the wire alternately. The venom collected was scrapped and stored at -20°C for further use[9].

Experimental Animals

Experiments were performed on twenty-four healthy, young 9 weeks old male Spargue-Dawley (SD) rats for anti-ulcer activity and Swiss albino mice for acute toxicity testing. All animals were examined individually on procurement and used after 7 days of acclimatization.

Housing Conditions

All the animals under the study were maintained in temperature 22 ± 5°C, humidity 50 ± 10%, 12 : 12 h light : dark cycle. The animals were housed 3 per cages wire-mesh cage (255 W× 465 L × 200 H mm). All the Cages were Maximum efforts were made so that the animals have minimum suffering and distress [10].

Acute Toxicity Study

The Swiss albino mice either sex were used for acute toxicity with reference to the OECD guidelines. The test animals (n=3) were divided into two groups. Group 1and 2 were administered with *Apis Mellifera* bee venom of 0.28mg/kg and 0.56mg/kg ip respectively for 3 days. The sane dose was used for screening the anti-ulcer activity. Behavioral changes, body weight, mortality and toxicity signs for 14 days after dosing were recorded.[11]

Anti – Ulcer Activity by Pyrolic Ligation

Anti-ulcer activity was carried out on SD rats. The animals were divided into four groups (n=6). Group 1is negative control and administered with vehicle. Group 2 is standard and administered with Ranitidine 100mg/kg. Group 3 and 4 is test group which is administered with *Apis Mellifera* bee venom of 0.28mg/kg and 0.56mg/kg respectively. The administration was done by ip route for 7 days. The abdomen was opened under light after induction of anesthesia by ketamine HCl (75 mg/kg, ip). The pyrolic portion of the stomach was exposed for further surgical procedure. Cotton thread was placed around the exposed pyrolic portion and tied around. The rats were then placed separately to avoid any disturbance to the suture upon consciousness of the rats. After 4 hours, the rats were sacrificed by cervical dislocation. The rat abdomen was opened and the content was drained out into a glass tube which was centrifuged at 2000rpm for 10 minutes. Tthe supernatant was taken to determine the total acidity. Each animal stomach was examined for ulcer lesion and indexed according to severity.[12]

Microscopic Evaluation of Stomach

Along a great curvature, the stomachs were opened and was rinsed with saline to remove the gastric content and blood clots and by using a 10X dissecting microscope the stomachs were examined. The numbers of ulcers were counted. The scoring as done for normal coloured stomach- 0, Red colouration- 0.5, Spot ulcer- 1, Haemorrhagic streak- 1.5, Deep ulcers- 2, Perforation- 3. From the mean ulcer score, the score was expressed as ulcer index. The percentage of ulcer protection was determined as the formula UI= UN+US+UP X 10-1 where UI= Ulcer index, UN= Average number of ulcer per animal, US= Average number of severity score, UP= Percentage of animals with ulcers[12].

Determination of Total Acidity

1ml of gastric acid was diluted with 1ml of distilled water in a 50ml conical flask. The phenolphthalein was used as an indicator in where two drops were added in the dilution and it was titrated with 0.01NaOH until a light pink was observed. The volume of NaOH used in

the titration for the light pink to be produced was noted. The total acidity was calculated by the following formula and was expressed in meq/L.[12]

Histopathological Evaluation

The stomachs were removed and then cut into small pieces. Stomach sections were fixed in 10% formaldehyde, dehydrated in gradual ethanol (50% to 100%), cleared in xylene and embedded in paraffin. Sections (4 to 5 µm thick) were prepared and stained with hematoxylin and eosin (HE) dye for histopathological examination and observed under a microscope at a magnification of 100.

3. RESULTS

Acute Toxicity

All the treatment animals did not show much significant in the behavioural changes, body weight, mortality and toxicity signs. The monitoring parameters such as fur and skin, eyes, salivation, urination, faeces consistency remained unchanged when compared with those of control group. No significant toxicity sign and body weight changes were recorded during the 14 days.

Ulcer Index

Both the ulcer index and total acidity were significantly reduced when the animals were treated with *Apis Mellifera* bee venom of 0.28mg/kg and 0.56mg/kg for 7 days as compared to the control group. The results are shown in the fig 1 and 2.

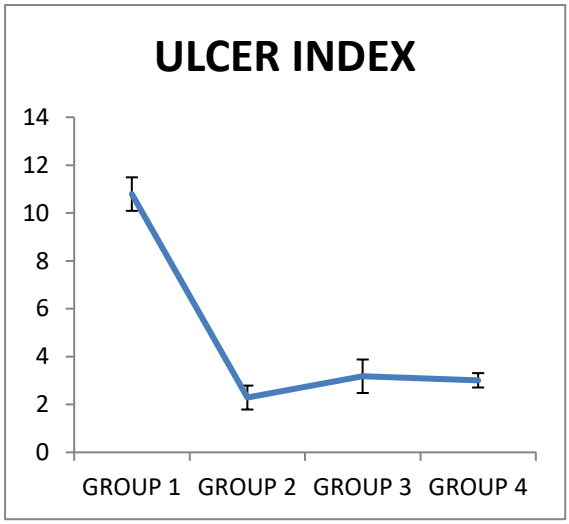


Figure 1: Average ulcer index expressed as SEM. Group 1: Negative control administered with vehicle. Group 2: Standard control administered with Ranitidine 100mg/kg. Group 3 and 4 : Test group administered with *Apis Mellifera* bee venom of 0.28mg/kg and 0.56mg/kg respectively .

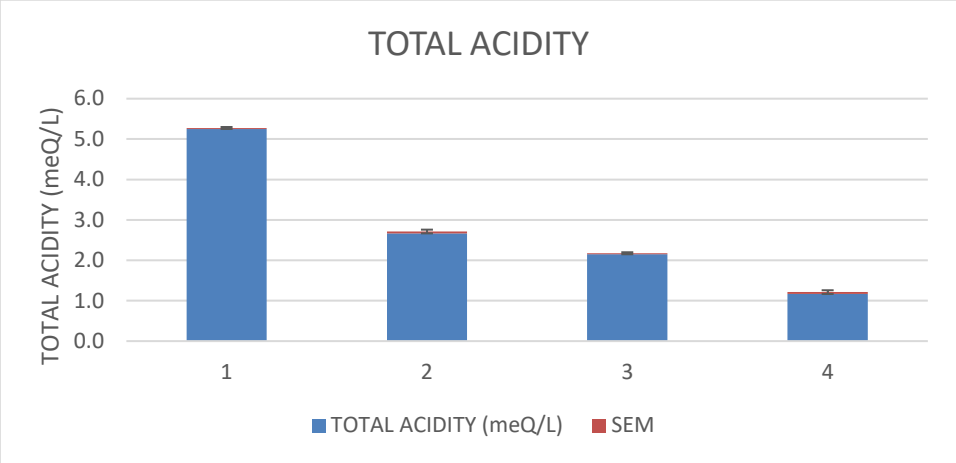


Figure 2 : Average total acidity expressed as SEM Group 1: Negative control administered with vehicle. Group 2: Standard control administered with Ranitidine 100mg/kg. Group 3 and 4 : Test group administered with *Apis Mellifera* bee venom of 0.28mg/kg and 0.56mg/kg respectively.

Histopathological Evaluation

The microscopic evaluation of the tissue sections BV treated rats did not show any histopathological changes of ulcer formation with haemorrhagic streak as compared to their control groups. The results are shown in the figure 3

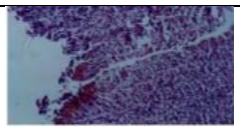
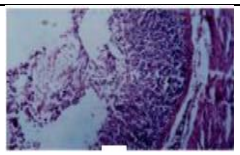
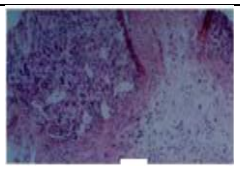
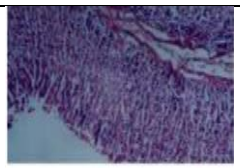
GR	SAMPLE	RESULTS
1		Ulcer formation with haemorrhagic streak.
2		No ulcer formation with red colourations
3		No ulcer formation with mild red colouration
4		No ulcer formation with very mild red colouration

Figure 3: Histopathological evaluation of rats. Group 1: Negative control administered with vehicle. Group 2: Standard control administered with Ranitidine 100mg/kg. Group 3 and 4 : Test group administered with *Apis Mellifera* bee venom of 0.28mg/kg and 0.56mg/kg respectively.

4. DISCUSSION

There are many methods are available for the induction of ulcer in rats model. One of the methid is ethanol-induced gastric ulceration model, HCl is responsible for causing severe

injury to gastric mucous membrane. Alcohol induced ulcer by imposing haemorrhagic gastric lesions characterised by tissue layer friability and cellular exfoliation.[13]

Pyloric ligation induce ulceration by accumulation as the pyloric region will be tied. This in turn results into the activation of pepsin and results into ulceration. Moreover, pyloric ligation has been found to cause decrease in defence system of the stomach which could be due to starvation or may be due to the increase in pneumogastric discharge which leads to the degranulation of mast cells and in turn results in depletion of amine in gastric tissue. peptic ulceration elicited by pyloric ligation is believed to ensue to worry elicited inflated in gastric acid secretion and/or stasis of acid. The amount of secretion is additionally a crucial consider the formation of ulceration because of exposure of the unprotected lumen of the abdomen to the accumulating acid, pyloric ligation – elicited gastric ulcers happens due to a rise in acid-pepsin accumulation because of opening obstruction and later tissue layer digestion and breakdown of the gastric tissue layer barrier. Oxidative stress plays a crucial role within the pathologic process of varied diseases together with peptic ulceration, with antioxidants being reported to play a big role within the protection of gastric mucous membrane against numerous death agents. Reactive chemical element species area unit concerned within the pathologic process of pyloric ligation – induced gastric tissue layer injury in vivo. As compared to traditional rats, pyrolic ligation was found to extend lipoid peroxidation and reduce SOD, CAT and GSH as compared to traditional management teams, therefore resulting in oxidative stress.[14]

Bee venom has been reported to contain many active compounds such as phospholipase A2, hyaluronidase, acid phosphomonoesterase, α-D-glucosidase, and lysophospholipase. Among them, melittin, a soluble cationic amphipathic twenty six amino acid α-helical amide, could be a very nonspecific lysis amide that attacks all lipoid membranes resulting in vital toxicity. Phospholipase A2 includes 10-12% of peptides and it's the foremost damaging part of apitoxin. it's an accelerator that degrades the phospholipids.[15] Melittin is known to decreases Cox_2 and phospholipase A_2 and which in turn reduces levels of tumor necrosis factor α, interleukin-1, interleukin-6, Nitric oxide and ROS system. Melittin suppresses the expression of pro-inflammatory cytokines through the NF-κβ signalling pathway. [16]

In contrast, as noted in the results the ulcer index and total acidity of the rats treated with the both doses of bee venom which is 0.28mg/kg and 0.56mg/kg prior to pyrolic ligation tends to tends to be lower than the pyrolic ligated control group. Thus, these results indicated that there might be an antioxidant activity in the bee venom as in pyrolic ligated animals the ulcer is caused mainly by oxidative stress. In bee venom, there is an compound called melitin in where it tends to reduce the reactive oxygen species which in return reduces the oxidative stress. Moreover, the bee venom tends to also reduce the acid secretion in the pre-treated animals with bee venom which is another factor for low ulcer index and total acidity.

5. CONCLUSION

The results in this study have confirmed the absence of acute toxicity in mice and presence of anti-ulcer activity in rats. In the study the group treated with dose of 0.56mg/kg showed a more potent anti-gastric ulcer activity when compared to the control. Further studies can be carried out which can focus on isolation of specific toxin and elucidating mechanisms of action of *Apis Mellifera* Bee Venom.

6. REFERENCES

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