

Investigation of Anti-Inflammatory, Antipyretic and Analgesic Effect of Yemeni Sidr Honey

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Abstract—Traditionally, Yemeni Sidr honey has been reported to cure liver problems, stomach ulcers, and respiratory disorders. In this experiment, we evaluated Yemeni Sidr honey for its ability to protect inflammations caused by acetic acid and formalin -induced writhing, carrageenan and histamine-induced paw oedema in experimental rat model. Hyperpyrexia, membrane stabilizing activity, and phytochemical screening of the honey was also examined. Yemeni Sidr Honey at (100, 200 and 500 mg/kg) exhibited a concentration dependant inhibition of acetic acid induced and formalin induced writhing, paw oedema induced by carrageenan & histamine, and hyperpyrexia induced by brewer's yeast, it also inhibited membrane stabilizing activity. Phytochemical screenings of the honey reveal the presence of flavonoids, steroid, alkaloids, saponins and tannins. This study suggested that Yemeni Sidr honey possess very strong anti-inflammatory, analgesic and antipyretic effects and these effects would be a result of the phytochemicals present.

Keywords—Anti-inflammatory, Analgesic, Carrageenan, Acetic acid, Histamine, Yemeni Sidr Honey

I. INTRODUCTION

SINCE ancient times, honey was not only valued as a flavorful sweetener, it also has an extensive history of traditional human medicinal use in a large number of societies among them is Yemeni Sidr Honey.

At present, studies have shown that honey has a significant natural source of antioxidants and has potential therapeutic value in the treatment of cancer, heart disease, cataracts, and several inflammatory diseases [1]. The therapeutic potential of honey involves antioxidant capacity and antimicrobial properties, as well as wound-healing and anti-inflammatory activities [2]. It is reported that honey contains at least 181 substances. Essentially, it is composed of a complex mixture of sugars, enzymes, wax, lipid and other minor substances, such as minerals, amino acids, organic acids, proteins, vitamins, ash, pollen and propolis [3][4]. Honey is rich in phenolic acids, flavonoids and other phytochemicals which exhibit a wide range of biological effect. However, the actual health benefits derived from honey depend on the quality of the honey. Yemeni sidr honey is considered as the finest and of the best quality all around the world and that is because of the Yemen's climate and environment are good habitats for making honey. Besides, bee itself builds its hive without human interference, thus preserving its natural specifications

and its vital live enzymatic constituents. The best quality and the most highly appreciated Yemeni honey come from Hadhramaut. Yemen ancestors used Sidr honey to promote health, healing and to boost immune system in various ways. As it is a well-known anti-biotic and anti-bacterial agent. They believed that this honey cures sore throat, cough and cold, stomach ulcers and other stomach ailments, digestive problems and all types of inflammations. This study was carried out to investigate the traditional claims of the therapeutic effect of Yemeni sidr honey and to find alternatives substances for controlling inflammation with minimal side effect.

II. MATERIAL

A. Apparatus, Chemicals and solvents

Fresh honey was obtained from Duan Mountain Hadramaut Yemen. The honey was stored at laboratory room temperature. Acetic Acid and carrageenan (Sigma chemicals Co, USA) Aspirin and Indomethacin (Sigma chemicals Co, USA) Formaline (Sigma - Aldrich -USA), Histaminedi hydrochloride (Sigma -aldrich, China), Acetylcholine (Sigma -aldrich, China), Brewer yeast (AB. Mauri Malaysia), Trisodium citrate and sodium chloride (Fisher Scientific, UK). Thermalent TH-5 (physitemp, USA), Centrifuge (Hellich Zentrifugen), Water bath (Memmert), Spectrophotometer (Anthelie light, secoman), Incubator (Memmert), Vernier caliper and stop watch.

B. Animal Model

Sprague Dawley rats of either sex weighing 250-280g obtained from Institute of Medical Research (IMR) were used throughout the investigations. They were housed in groups of five in standard cages in animal holdings units, UCSI University, Kuala Lumpur, Malaysia and maintained under standard environmental condition (temperature: 22 ± 1°C; humidity 14 ± 1 and light / dark schedule 12/12 hour) and were fed with standard pellet diet and water ad libitum. They were left to acclimatise for 2 weeks before starting of experiment. All experiments were carried out in accordance UCSI University ethical guidelines on the usage of Laboratory Animals.

III. METHODS

A. Toxicity Test

The toxicity test of honey was studied according to [15]. High concentration of honey (1000mg/kg/rat) prepared in saline was administered orally to group of five rats each for 7 consecutive days. The rats were observed for any abnormal behavior such as diarrhea, salivation, respiratory distress,

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motor impairment, hyperexcitability and incidence of mortality. Furthermore, at the end of the seventh day, the animals were sacrificed and the internal organs (stomach, small intestine, kidneys and liver) were observed, weighted and compared with normal rat's organs.

B. Acetic Acid-Induced Writhing

Analgesia of honey was evaluated by writhing test in rat using the method of [5] and [6]. Acetic acid (0.6%.v/v) was injected into the peritoneal cavities of rats (n=5 per group) in a volume of 0.1 ml/10g. Vehicle (saline), Indomethacin (5mg/kg) and honey (100,200,500mg/kg) were orally administered 30min prior to acetic acid injection. The intensity of analgesic behavior was quantified by counting the total number of writhing response which consists of a contraction of the abdominal muscle together with a stretching of the hind limbs which was compared to the response in the control group as described by [7]. Immediately after stimulus injection of the allergic compound, each animal was isolated in an individual box (24cm x 11cm x 10cm) to be observed over a period of 30 min. A significant reduction in the number of writhes by drug treatments as compared to vehicle control animals was considered as a positive analgesic response. The percentage inhibition of writhing was then calculated as follow:

$$\text{Inhibition (\%)} = \frac{(\text{Control mean} - \text{treated mean})}{\text{Control mean}} \times 100$$

C. Formalin Test

Tonic and localized inflammatory pain were induced by subcutaneous injection at diluted (2.5%) formalin (0.05ml) into the plantar surface of ipsilateral right hind paw (i.pl.) of rats. The animals were individually place into transparent observation chambers as previously described [8] and [9]. Oral treatment (p.o) with vehicle, indomethacin (5m/kg) and honey (100, 200, and 500 mg/kg) were giving 30 mins prior to formalin injection (n=5 per group). The duration of hind paw licking, flexing and the number of paw jerking were counted as the number of nocifensive events (NNE) over 30 mins immediately after formalin injection. These behaviors were sometimes exhibited in long bouts; i.e more than 3 second of the event was counted as one event, plus one event when the paw returned to the resting position. The data collected between 0-5 min posts -formalin injection were considered as the first phase and the data collected between 15-30 min post-formalin injections were considered as the second phase. These phases represented neurogenic and inflammatory pain response, respectively. Inhibition was calculated with the following equation

$$\text{Inhibition (\%)} = \frac{(\text{Control mean} - \text{treated mean})}{\text{Control mean}} \times 100$$

D. Carrageenan Induced Paw Oedema

Anti-inflammatory activity was assessed in rats on the basis of the inhibition of the Carrageenan induced hind paw oedema [10], following an overnight fast with free access to water. Rats (n = 5 per group) were divided into five

experimental group and the basal volume of the right hind paw was determined before the administration of any drug. Honey (100, 200, 500, mg/kg), (Indomethacin 10mg/kg, p.o) and vehicle control were orally administered 1hr prior the carrageenan administration. Acute inflammation was produced by the subplanter administration of 0. 1ml of 1% carrageenan suspension in 0. 9% NaCl in the right hind paw of the rats. The volume at the oedema was monitored by measuring the thickness at hind paw swelling at 1, 2, 3, 4, and 5hr after carrageenan injection by using vernier caliper. The results are presented as the paw volume (ml) variation in relation to the basal Values and the inhibitory activity was calculated According to the following formula:

$$\text{Inhibition (\%)} = \frac{[(C_t - C_0) \text{ control} - (C_t - C_0) \text{ treated}]}{[(C_t - C_0) \text{ control}]} \times 100$$

Where C_t = mean paw volume for each group at time and C_0 = mean paw volume for each group before carrageenan injection.

E. Histamine Induced Paw Edema

Three different doses of honey (100,200 and 500mg /kg), indomethacin (5 mg/kg) and saline vehicle were administrated to rats (n=5 per group) orally through the feeding tube 1h prior histamine injection. Oedema was induced by subcutaneously injection of 0.05 ml of 1% histamine into the plantar side of the right hind paw. The paw volume was measured using a vernier caliper; before injection and 5 times at 1 h intervals after the injection [11]. Values were calculated as percent difference between saline vehicle and histamine injected paw. The obtained results were compared with indomethacin and control groups.

F. Antipyretic Activity

Hyperpyrexia was induced by subcutaneous administration of 10 ml/kg-body weight 15% brewer's yeast in normal saline [12]. The selected animals were grouped into five (n = 5) and were fasted overnight with water ad libitum before the experiments. Initial rectal temperature of the rats was recorded [13]. After 24h, animals that showed an increase of 0.3–0.5 °C in rectal temperature were selected for the antipyretic activity. Subsequently, vehicle, Aspirin (100 mg/kg) and honey (100,200,500 mg/kg) were orally given to the animals and their rectal temperature was measured using a digital thermometer at 1 h intervals for 4 h. The results are expressed as percentage [14].

G. Effect on Heat -Induced Erythrocytes (HH Assay).

Rat erythrocyte was prepared as previously described [16] and [17]. Whole blood was collected from rats under ether anaesthesia into an anticoagulant containing trisodium citrate (3.8%, w/v) and mixed thoroughly. The blood samples were then centrifuged at 3000 rpm for 5 min at room temperature. The supernatant was decanted and the blood was washed three times with 0.9% saline until the supernatant was clear followed by centrifugation. Then, 2% (v/v) erythrocyte

suspension was prepared in normal saline solution.

The inhibitory activity towards rat erythrocyte hemolysis was analyzed [16] and [18]. The reaction mixture contained of 2ml of 0.25% (w/v) sodium chloride, 1.0 ml of 0.15M sodium phosphate buffer (pH 7.4), 0.5 ml of 2% (v/v) erythrocyte suspension, 0–1.0 ml of drugs or honey and the final mixture was made up to 4.5 ml with saline. The blank control lacked the erythrocyte suspension. Subsequently the reaction mixtures were incubated at 56 °C for 30 min, cooled under running water and centrifuged at 5000 rpm. The supernatant was collected and the color of the released haemoglobin was determined by reading the absorbance at 560 nm on the Spectrophotometer.

The percentage membrane stability was estimated from the expression:

$$\text{Inhibition (\%)} = \frac{(\text{Absorbance drug test} - \text{absorbance drug control})}{\text{Absorbance drug Control}} \times 100$$

Where the control represents 100% lysis or zero membrane stability

H. Preliminary Phytochemical Screening:

Yemeni Sidr honey was further subjected to Preliminary phytochemical screening for the presence or absence of different chemical constituents which include flavonoids, alkaloids, tannins, steroids and saponins using the method of [19].

I. Statistical Analysis

The data were expressed as mean values $\pm$ standard error mean (S.E.M.). Statistically significant differences between groups were measured using one-way analysis of variance (ANOVA) followed by Dunnett's test. Values of * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ was considered statistically significant.

IV. RESULTS

A. Toxicity Test

The toxicity study showed no occurrence of death over the period of seven days treatment with the highest concentration of (1000mg/kg). There was no observed abnormality in any of the organs indicating that Yemeni Sidr honey is not toxic at high concentration.

B. Acetic Acid-Induced Writhing

The oral administration of increasing concentrations (100, 200, 500 mg/kg) of Yemeni Sidr honey and Indomethacin (5mg/kg) caused a significant reduction ($p < 0.001$) in the number of writhing episodes induced by acetic acid compared to the -vecontrol. The results of acetic acid induced writhing are shown in Fig. 1.

C. Formalin Test

The subcutaneous injection of diluted (2.5%) formalin (0.05ml) into the plantar surface induced a nociceptive response, characterized by an increase in licking time. The oral

administration of increasing concentrations (100, 200, 500 mg/kg) of Yemeni Sidr honey did not show any significant reduction in the licking time during the first phase (neurogenic phase). In addition Yemeni Sidr honey concentrations (100, 200, 500 mg/kg) elicited an inhibition of the formalin response during the second phase (inflammatory phase). Results of formalin test are shown in Fig. 2.

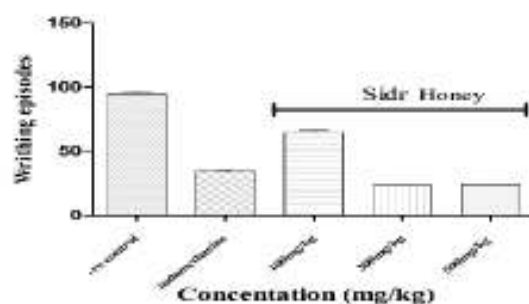


Fig 1 Effects of Yemeni Sidr honey administered orally against acetic acid induced writhing movements in rats. Animals were pretreated orally with saline solution (-vecontrol), honey doses (100, 200, and 500 mg/kg), and indomethacin (5 mg/kg) prior to the acetic acid (0.6 %, i.p.). Each column represents mean $\pm$ SEM, N=5. Statistical significance One Way ANOVA followed by Dunnett test ($p < 0.001$), In comparison to negative control.

D. Carrageenan Induced Paw Oedema

Fig. 3 shows that Yemeni Sidr honey at (100, 200, and 500mg/kg) and indomethacin (10 mg/kg) significantly inhibited Carrageenan-induced rat paw oedema ($P < 0.05$ and $P < 0.01$). The inhibitory values of oedema were based on 5hr post carrageenan treatment.

E. Histamine Induced Paw Oedema

Histamine induced paw Oedema was significantly ($p < 0.05$) reduced in a concentration- dependent manner by treatment with Yemeni Sidr honey at (100, 200, and 500 mg/ kg) and indomethacin (5mg/kg) at 1, 2, 3, 4, 5 hr after injection time of histamine. The result of histamine - induced paw oedema is shown in (Fig.4).

F. Antipyretic Activity

Subcutaneous injection of yeast suspension markedly elevated the rectal temperature after 24hr of administration. Treatment with the increasing concentrations of Yemeni Sidr honey (200, and 500 mg/kg) and aspirin (100mg/kg) significantly ($P < 0.001$ and $P < 0.01$) decreased the rectal temperature of the rats in a concentration – dependant manner, there was no significant difference between the negative control and honey at 100mg/kg. The antipyretic effect started as from the first hour and the effect was maintained for 4hr, after administration of Yemeni Sidr honey. The result obtained from the both the reference drug and Yemeni Sidr honey treated rats were compared with that of control and significant reduction in the yeast induced elevated rectal temperature was observed. The result of Antipyretic activity is shown in (Fig. 5).

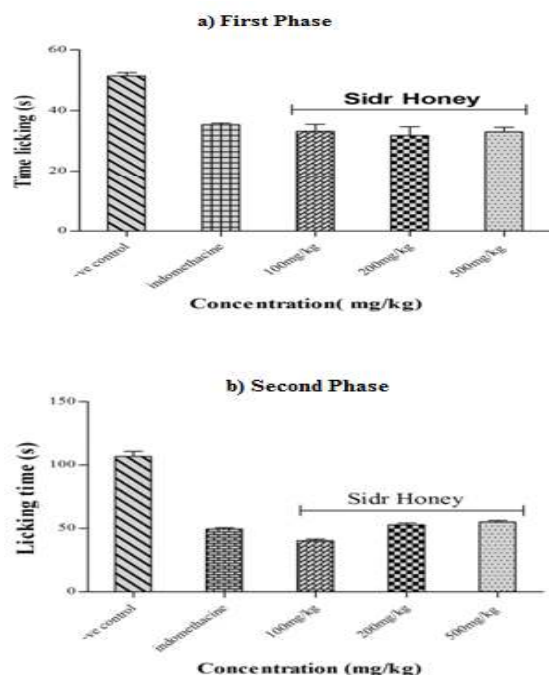


Fig. 2 Subcutaneous injection of 2.5% formalin (0.05ml) into the right hind paw of rats caused swelling. Phase 1 of nociceptive response began immediately after the formalin stimulation, while phase 2 started about 15 min after the chemical stimuli. Orally administrated Yemeni Sidr honey (100,200, and 500 mg/kg) has reduced formalin induced flinching and licking during phase 1 and 2. Indomethacine significantly suppressed only in the second phase of formalin test. Each column represents the mean with SEM for N=5. ($p < 0.001$).

G. Effect on Heat -Induced Erythrocytes (HH Assay).

Yemeni sidr Honey ((1, 2, and 3 mg/mL) and indomethacine (2mg/mL) was able to significantly ($P < 0.001$) protect erythrocytes against heat-induced lysis in a dose-dependent manner. The result of Heat -Induced Erythrocytes activity is shown in (Fig.6)

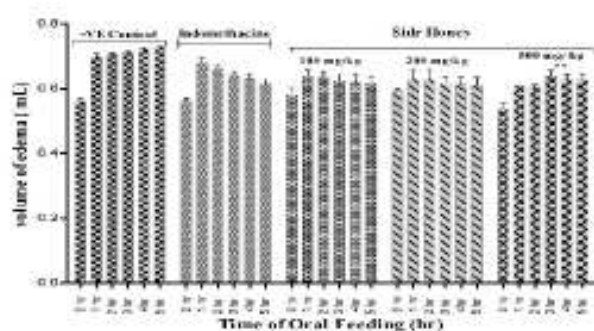


Fig.3 Effects of administration of Yemeni sidr honey (100,200,500 mg/kg) or indomethacine (10mg/kg) on rat paw oedema induced by intraplantar injection of 0.1 ml of 1% carrageenan suspension. Each column represents the mean $\pm$ SEM for n=5. The significance level when compared with the -ve control (Saline solution), $p < 0.05$ for honey concentration (100, and 200 mg/kg) and $p < 0.01$ for honey concentration (500 mg/Kg).

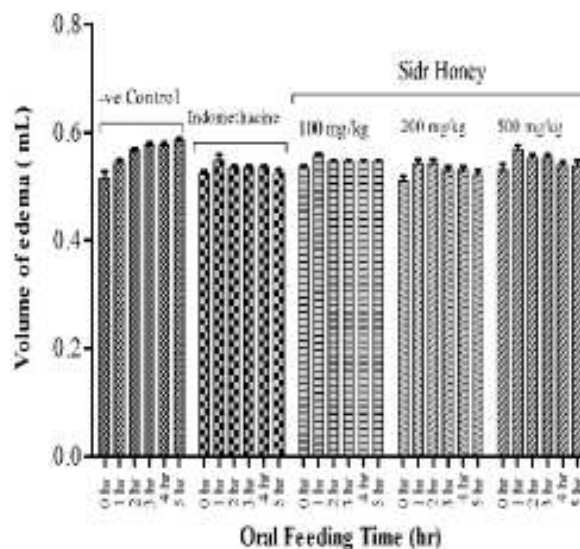


Fig.4. Effect of Yemeni Sidr honey (100,200, and 500 mg/kg) or indomethacine on rat paw oedema induced by histamine. Each column represents the mean $\pm$ SEM for N=5. ($p < 0.05$ for honey 100,200 mg/kg and indomethacine compare to the negative control and $P < 0.001$ for 500mg/kg compare to negative control).

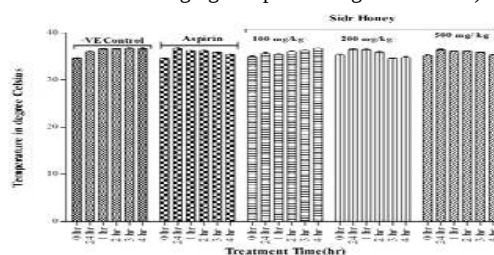


Fig. 5 Antipyretic effect of Yemeni Sidr Honey (100,200, and 500 mg/kg) and NSAID, Aspirin in yeast induced hyperpyrexia at 4th hr of rectal temperature of the rats. Each column represents the mean $\pm$ SEM for N=5. (-ve vs. Aspirin & 500mg/kg $P < 0.01$, -ve vs 200mg /kg Yemeni sidr honey, $P < 0.001$, there was no significant difference (NS) between -ve vs 100mg/kg and aspirin (100mg/kg) vs 200 & 500mg/kg of Yemeni sidr honey)

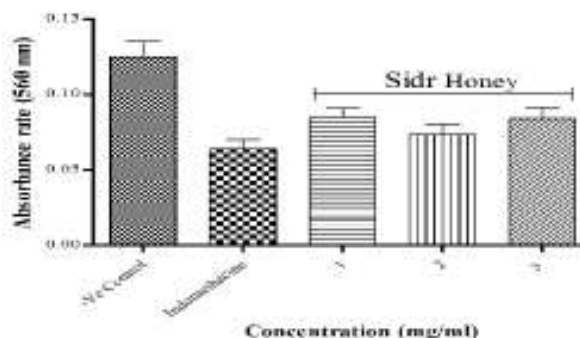


Fig. 6 the absorbance reading at 560 nm, used to determine the inhibition of heat induced hemolysis of rat erythrocytes. Values are expressed as mean $\pm$ SEM, n=4/ concentration. Yemeni sidr Honey doses were (1- 3 mg/mL). Each column represents the mean $\pm$ SEM for N=5. ($p < 0.001$, compare to the negative control)

H. Preliminary Phytochemical Screening

The Preliminary phytochemical screening revealed the presence of flavonoids, alkaloids, tannins, steroids and saponins.

V. DISCUSSION

Various traditional claims have been made about the usefulness of Yemeni Sidr honey in the treatment of inflammatory related disorders, therefore this study was undertaken to give scientific authentication to these traditional claims. The toxicity study of Yemeni Sidr honey shows that it is safe to consume and show no threat of toxicity at high concentration. The acetic acid induced abdominal constriction assay is regarded as a very sensitive method that uses minimal noxious stimulus, and even weak analgesics can be detected with this test [20]. In this model, pain is generated indirectly via endogenous mediators like bradykinin, serotonin, histamine, substance P and prostaglandin, which stimulated peripheral nociceptive neurons that are sensitive to narcotic analgesics and non-steroid anti-inflammatory drugs (NSAIDs) [21] and [22]. These mediators are able to increase vascular permeability, reduce the threshold of nociception and stimulate the nervous terminal of nociceptive fibers [23]. Injected acetic acid can also directly activate non-selective cation channels located at primary afferent pathways [24]. Pre-treatment of the rats with Yemeni Sidr honey reduced the acetic acid-induced writhing response, and this analgesic role was similar to those of the reference drugs suggesting the potential to reduce the liberation of inflammatory mediators or block receptors, resulting in a peripheral antinociceptive effect. These explanations are corroborated by the results obtained in the second phase of formalin testing. The formalin test is a model consisting of two distinct phases. The first transient phase is caused by direct effect of formalin on sensory C-fiber, and the second prolonged phase is associated with the development of an inflammatory response and the release of nociceptive mediators [25]. Phase 2 corresponds to inflammatory pain and is inhibited by nonsteroidal anti-inflammatory drugs [25] and [26]. In this test the peripherally acting drug indomethacin and Yemeni Sidr honey inhibited both the first and second phase but the inhibition of second phase was more significant than the first phase. The carrageenan-induced paw oedema formation is a classical model of acute inflammation and it is believed to involve a biphasic event. It is well known that the early phase (1-2 hr) is mediated by the release of histamine and serotonin [27], while the second phase (3-5hr) is the result of the release of kinins and mainly prostaglandins [27, 28]. In general, development of oedema induced by carrageenan is correlated with the early exudative stage of inflammation, one of the important processes of inflammation [29]. In this study we observed that Yemeni Sidr honey inhibited both phases of carrageenan-induced paw oedema, based on the present results it can be suggested that the inhibitory effect of Yemeni Sidr honey on carrageenan-induced paw oedema in rat may be due to the suppression of the release of mediators responsible for

inflammation including histamine, serotonin, bradykinin and prostaglandin. Among the several models of acute inflammation, histamine-induced inflammation has been well established as a valid model to study acute inflammation. It's a well known that the acute inflammatory process, in which vascular permeability increased and leukocyte migration occurs, involves several mediators including neutrophil-derived active oxygen species and free radicals, such as hydrogen peroxide, superoxide and the hydroxyl radicals [30] and [31]. Paw oedema formation is a result of a synergism between various inflammatory mediators that increase blood flow [32]. Several experimental models of paw oedema have been described. Rat paw oedema is characterized by an early phase caused by the release of histamine, serotonin and bradykinin followed by late phase mainly sustained by prostaglandin release [33]. In various studies, free radicals [34] and [35] and, in recent years, the l-arginine NO pathway has been proposed to play an important role in the inflammatory response [36] and [37]. Oral administration of Yemeni Sidr honey inhibited the histamine-induced paw oedema in the rat. Subcutaneous injection of yeast induces pyrexia by increasing synthesis of prostaglandin which ultimately increases body temperature [38]. The present results showed that the oral administration of sidr honey was able to significantly ($P < 0.01$) reduce yeast-induced pyrexia in experimental rats when compared to aspirin (100mg/kg) and Sidr honey (500mg/kg), and $P < 0.001$ when compared to negative control. There was no significant difference (NS) between negative control and 100mg/kg, aspirin (100mg/kg) and Yemeni sidr honey 200 & 500mg/kg. Honey (200mg/kg) showed the most significant antipyretic activity compared to the negative control. Honey seems to show more efficacy than aspirin, but aspirin is more potent. Yemeni Sidr honey at concentration 1-3mg/mL protects the rat red blood cell (RBC) membrane against lysis induced by heat. Since there is a close similarity of the RBC membrane system to the lysosomal membrane system, protection against hypotonicity or heat-induced lysis of RBC is often extrapolated to stabilization of lysosomal membrane, and used as a biochemical index of anti-inflammatory activity [39]. The stabilization of lysosomal membrane ability of Yemeni Sidr honey may account for the inhibition of the inflammation induced by carrageenan [40]. Drugs or compounds with membrane stabilizing properties are well known to interfere with the early phase of inflammatory mediator release, specifically by mediating the release of phospholipase A_2 that triggers the formation of such inflammatory mediators [41]. In addition, Preliminary phytochemical screening of Yemeni Sidr honey revealed the presences of chemical constituents such as flavonoids, alkaloids, tannins, steroids and saponins which has been widely reported to possess the observed pharmacological effects.

VI. CONCLUSION

From this study we may conclude that Yemeni Sidr honey possess a potent anti-inflammatory, antipyretic and analgesic

properties. These properties may be as a result of the phytochemicals present.

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