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Effect of Apis Dorsata honey and honey sugars analogue on hematological and some biochemical parameters in albino rats model

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Abstract

Honey has been recognized for its wound-healing, anti-microbial, anti-oxidant, anti-diabetic and anticancer effects. This study was designed to examine the effect of daily oral administration of Malaysian Tualang honey (TH) and honey sugars analogue (HSA) namely fructose, glucose, maltose and sucrose in albino Sprague-Dawley (SD) rats model, and their comparative effectiveness. Thirty nulliparous female rats were divided into three groups: Group 0 (negative control); Groups 1 and 2 received 1.0 g/kg body weight/day of TH and HSA respectively. After 120 days treatment, the rats were subjected to necropsy and blood samples were collected for analyses. The body weight, fasting blood glucose, haematological parameters and serum level expression of proteins such as Apaf-1, IFN-γ, TNF-α and E2 were determined. Results show that an increased body weight was observed as the treatment progressed over days ($p>0.05$). TH and HSA showed an increasing effect on the level of haematological parameters such as red blood cells (RBC), haemoglobin (Hb), packed cell volume (PCV) ($p>0.05$), lymphocytes, TWBC, RDW, eosinophils, monocytes and platelets ($p<0.05$) compared to the non-treated negative control. HSA showed a slightly comparable effect to the negative control for fasting blood glucose level, while TH presented a slightly hypoglycemic effect ($p>0.05$). The treatments also showed an increasing effect on serum level of Apaf-1, IFN-γ, TNF-α and a reduced level for E2. In conclusion, daily supplementation of TH and HSA may modulate haematological and serological parameters. HSA may act akin to honey.

Keywords: Tualang honey; honey sugars analogue; haematological parameters

1. Introduction

Honey has been used for nutritional and medicinal purposes since ancient times. The first reference to honey is dated back to 2100–2000 BC, describing the use of honey as an ointment and a drug [1]. It has been recognized as a potential medicinal agent with potential antimicrobial, anti-inflammatory, anti-cancer, anti-angiogenic, ant-imetastatic, , immune-stimulant, antiulcer and wound healing effects [2]. It comprises primarily of sugars such as monosaccharaides, disaccharides and polysaccharides. It contains enzymes such as glucose oxidase, diastase, invertase, catalase and peroxidase [3]. Other contents which are reported to be present in honey are flavonoids and phenolic acids; vitamins such as niacin, pyridoxine, ascorbic acid and vitamin B12; minerals such as sulfur, chlorine, potassium, calcium, phosphorous, magnesium and sodium; little traces of proteins or free amino acids and a miscellaneous class of volatile and organic components [4].

Tualang honey (TH) is a multi-floral Malaysian jungle honey. It is produced by bees "*Apis dorsata*" which build their hives on Tualang trees (*Kompassia excelsa*) in Malaysian tropical rainforests [5 & 6]. Literature shows that it exhibits wound-healing [7], antimicrobial [7], anti-oxidant [6], anti-diabetic [5 & 8], anti-inflammatory [6], anti-aging [5] and anticancer [9] effects.

The estimated percentage composition of HSA or common sugars in honey has been described as follows; fructose (30-35 %), glucose (30-35 %), maltose (7-10%) and sucrose (1-3%) [5, 10 & 11]. The major components of honey, i.e., sugar, particularly glucose and fructose, have also been demonstrated to inhibit the yield of mutagenic activity in different models [12]. Fructose as one of the honey sugars analogue induces apoptosis in malignant hepatocytes with no cytotoxic effects [13]. Glucose is also by far one of the major sugars in honey. The possible health benefits of consuming glucose have been well documented [14]. High sugar content of honey provokes killing of bacteria and it also prevents the growth of bacteria by literally drying out or the osmotic effect on bacteria [15]. Earlier studies have shown that glucose and fructose possess either carcinogenic and anti-carcinogenic or mutagenic and anti-mutagenic properties. Though the phenolic, flavonoids and other constituents of honey have been well studied, so far none has taken the sugars part of honey into cognizance for its possible biological effects [16-19].

Apaf-1 plays a role in regulating centrosome maturation, other than apoptosis [20]. It also regulates the recruitment of HCA66 protein which is critical for ribosomes synthesis and centriole duplication [20]. Thus, Apaf1 expression might be considered a pro-survival molecule, and its absence impairs cells performance and may cause a higher responsiveness to stressful conditions [20]. Apaf-1 regulates caspases and may indirectly be involved in regulation of immunological responses [21]. IFN-γ is the major cytokine involved in the protective immune response [22]. TNF-α seems to have a primordial role which acts upon a wide variety of cells. The main producing cells are activated macrophages, T lymphocytes, and dendritic cells. This cytokine acts in synergy with IFN-γ to regulate immune system [22-24]. Data shows that estradiol regulates various facets of the immune system via complex molecular mechanisms. Estradiol and progesterone have also been reported to regulate TNFα release by macrophages [25 & 26].

This study was undertaken to investigate the effects of daily administration of honey with a view to ascertain that what is its effect on body weight, fasting blood glucose level, hematological and some biochemical parameters? We also investigated to determine whether honey sugars analogue (a mixture of sugars having composition similar to the sugars of honey) has the same effect as real honey or not.

2. Materials and Methods

2.1. Animals

The experimental protocol used in this study was approved by the animal ethics committee of Universiti Sains Malaysia, Malaysia [USM/Animal Ethics Approval/2011/ (68) (306). Sprague-Dawley (SD) female rats aged between 28-33 days old were obtained from Animal Research and Service Centre (ARASC), Universiti Sains Malaysia (USM).

2.2. Source of honey

Malaysian jungle Tualang honey was supplied by Federal Agricultural Marketing Authority (FAMA), Ministry of Agriculture and Agro-based Industry, Malaysia. The honey samples were filtrated, evaporated at 40 ^{0}C (to achieve 20% water content) and were subjected to gamma irradiation at 25 kGy for sterilization by STERILE GAMA™, Selangor, Malaysia.

2.3. Quality Assessment of the TH

The quality of TH was assessed through hydroxylmethylfurfural (HMF) level and diastase number (DN) using spectrophotometric method (Thermo Scientific™ Evolution 60S UV-Visible) and is briefly described below;

2.3.1. Determination of hydroxylmethylfurfural (HMF)

Approximately 5g of TH was dissolved in 25 mL of distilled H_2O and treated with a clarifying agent (0.5 mL of Carrez I and 0.5 mL of Carrez II solutions) and final volume was made up to 50 mL. The solution was filtered by discarding the first 10 mL. The absorbance of the filtered solution was measured at 284 and 336 nm against an aliquot of the filtered solution treated with NaHSO3. HMF value was determined using following formula: mg of HMF/100g of honey = (A284nm - A336nm) × 14.97 × 5/g of test sample [27].

2.3.2. Determination of Diastase number (DN)

Diastase number or diastase activity was determined by incubating TH and buffered solution of soluble starch in a thermostatic bath at approximate temperature of 40 ^{0}C. Later, 1 mL aliquot of TH and buffered starch solution mixture was removed at 5 min intervals and the absorption was measured at 660 nm. The diastase value was calculated using the time taken for the absorbance to get reach at 0.235, and the results were presented in Gothe degrees as the amount (mL) of 1% starch hydrolyzed by an enzyme in 1 g of each honey in 1 h.
The DN was calculated using following formula: DN (units/g of honey) = 28.2 × change in A660nm + 2.64 [27].

2.4. Preparation of Honey sugars analogue (HSA)

The estimated percentage composition of sugars in honey has been described as follows; fructose 30-35 %, glucose 30-35 %, maltose 7-10% and sucrose 1-3% (O. O. Erejuwa et al., 2011; Shin & Ustunol, 2005). Honey sugars analogue was prepared using mentioned percentage of sugars; fructose, glucose, maltose and sucrose (Merck, Germany) with water in ratio of 1:1:0.25:0.03 respectively.

2.5. Animals housing and treatment plan

A total of 30 virgin female SD rats were divided into 3 groups with 10 animals in each group. These rats were housed in a standard cage with commercial pine chip bedding in a well-ventilated animal room with a 12 h day/night cycle, maintained on standard and balanced rat feed diet and had free access to water ad libitum. The rats were acclimatized to the animal room conditions for at least one week prior to the experimentation. TH and HSA treatment by oral feeding was started to rats at age 40 days old. The treatment was planned to be continued till day 120th. The grouping of the rats was as follows;

a) Group 0: Negative control (normal rats).
b) Group 1: treated group; rats receiving TH 1.0 g/kg body weight/day treatment.
c) Group 2: treated group; rats receiving HSA 1.0 g/kg body weight/day treatment.

2.6. Determination of body weights

The total body weight of rats was measured using a digital analytical balance (Sartorius AG, Germany) weekly from start of treatment till day terminated. The percentage body weight changes or percentage weight gain were calculated at the end of study (week 16). The formula used to calculate percentage weight gain is described as follows;

Percentage body weight change or gain (BW change %)= [(FBW – IBW) × 100] /IBW

Legends: BW=weight, IBW=initial body weight, FBW=final body weight.

2.7. Samples collection

After 120th day of honey treatment, all the rats used in the present study were subjected to necropsy after intra-peritoneal (i.p) injection of pentobarbital 100mg/kg body weight. The blood samples were collected into EDTA and plain tubes by cardiac puncture using 10ml syringe and 23G needle. Blood samples in plain tubes were left to clot for 2 hours prior to centrifugation for 15 minutes at 4000 rpm (Eppendorf centrifuge, Germany). The serum was collected and stored at -80C^0 until assayed.

2.8. Determination of full blood count (FBC)

FBC was carried out using an automated cell count analyzer (Sysmex KX-21, Japan) via non-cyanide hemoglobin analysis. Auto-analyzer was proficient to run several parameters for each sample such as hemoglobin (Hb) concentration, packed cell volume (PCV), red blood cells (RBCs), mean cell volume (MCV), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular haemoglobin (MCH), platelet and white blood cells (WBCs) (counts. The equipment of sampling probe aspirated 20 µl with well mixed blood samples and the result of analysis was obtained accordingly. A total of 8-9 samples were run for FBC for each group.

2.9. Determination of Apaf-1, IFN-γ, TNF-a and E2 at serum level

Seven to eight serum samples per treatment and control group were analyzed to determine the level of Apaf-1, IFN-γ, TNF-α and E2 in 50µl serum using Apaf-1, IFN-γ, TNF-α and E2 ELISA kits (Catalog no. BG-RAT10190, Inc., Novate in Bio Sciences; CSB-E04579r; CSB-E11987r and CSB-E05110r Inc., COSMO BIO, USA respectively). Standards comprised serum of known concentrations of Apaf-1, IFN-γ, TNF-α and E2 and a serum blank. The ELISA procedure was performed according to the manufacturer's instructions. The results were obtained by calculating the mean absorbance at 450nm (Spectrophotometer, Thermo Fisher Scientific Inc, Waltham, MA, USA) for each of the duplicate standards, controls and samples as stated by the manufacturer. A standard curve was created by plotting with the absorbance value as the dependent variable (Y-axis) and concentration as the independent variable (X-axis), results in an equation formatted as follows: $y = ax^2 + bx + c$, with best-fit straight line, where solving for x determined the protein concentration of the sample.

2.10. Statistical analyses

Data were analysed using IBM SPSS, Statistics version 23. Comparisons between mean values of control and treatment groups were analysed using one-way ANNOVA with post-hoc test of Tukey Honest Significance Differences (Tukey's HSD). Mixed model two-way repeated measures ANOVA was conducted to evaluate the effect of treatments on the rats body weight gain. The time main effect and the experimental groups x time interaction effect were tested using the multivariate criterion of Wilk's lamda (Λ). Comparison of the median values between groups was done by Kruskal-Wallis H test followed by Benferroni's correction. P value <0.05 was considered statistically significant.

3. Results

3.1. Diastase number and hydroxylmethylfurfural level

The diastase number or activity of TH was 3.8 units/g and the level of hydroxylmethylfurfural was 0.53 mg/100 g (Table 1). This indicates that TH was of good quality as the values are far below the imposed limit of > 8 units/g of honey for DN and > 15 mg/100 g of honey for HMF.

Table 1 Diastase number and hydroxylmethylfurfural level for TH and MH.

Honey	Diastase number (units/g)	hydroxylmethylfurfural level (mg/100 g)
TH	3.8	0.53

3.2. Body weights

In general, body weights of the rats in all groups (non-treated negative control, TH and HSA treated groups) were found to be increased throughout the experimental period over time (Figure 1). Data for median body weights of rats in each group is presented in Table 2. No significant difference in the median body weights between all groups was observed at week 1 ($p > 0.05$). The rats in negative control showed a higher median body weight compared to TH and HSA treated groups. At week 16, no significant statistical difference was also observed in the median body weights of rats between control and TH and HSA treated groups ($p > 0.05$). But, the rats in negative control presented a lower median body weight compared to TH and HSA treated groups. The difference in percentage body weight change (BW change %) between all groups was found statistically not significant ($p > 0.05$). All the rats in TH and HSA treated groups showed a higher BW change % with no weight loss compared to the negative control (Table 1). The tested dose of HSA presented a higher body weight change % than a similar dose of TH (Table 2).

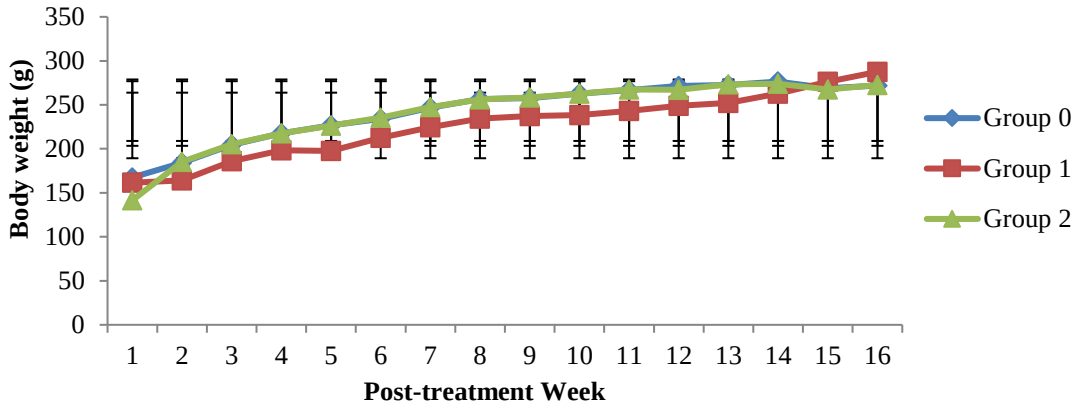

Figure 1 Body weight progression among all groups of rats during 16 weeks of experimental period. Data is presented as mean ± SEM and a mixed model two-way repeated measures ANOVA was conducted to analyze the results. A positive body weight progression was observed over time ($p>0.05$). Legends: TH= Tualang honey, HSA= Honey sugars analogue, Group 0= negative control (normal rats), Group 1= 1.0 g/kg TH and Group 2= 1.0 g/kg HSA.

Table 2 Body weight measurements of rats among all groups at week 1 and week 16.

	Groups			
Body weight	0 -ve control	1 (1.0 g/kg TH)	2 (1.0 g/kg HSA)	P value[a]
BW at week 1	167.5 (32.25)	161.5 (94.5)	141.5 (25.45)	0.300
BW at week 16	272 (32.25)	287.5 (31)	274.7 (20.87)	0.392
BW change (%)	62.38 (37.16)	78.01 (90.05)	94.13 (27.71)	0.182

[a]Kruskal-Wallis test. Data are expressed as median interquartile range (IqR). Values are statistically significant when $p \leq 0.05$. Legends: BW= Body weight, TH= Tualang honey, HSA= Honey sugars analogue, Group 0= negative control (normal rats), Group 1= 1.0 g/kg TH and Group 2= 1.0 g/kg HSA.

3.3. Haematological parameters

The results of haematological parameters of negative control were used to establish a normal or standard reference range. Treatment with TH and HSA showed a slightly potentiating effect on Hb, RBC, PCV, lymphocytes, RDW, eosinophils, monocytes and platelets compared to the non-treated negative control. While, it was observed that the level of MCV, MCH, MCHC and polymorphs was almost comparable for TH and HSA compared to the non-treated negative control. The difference for potentiating or lowering some parameters was minute between TH and HSA among themselves. The detailed results with statistical analyses are presented in Table 3. Treatment with TH presented a slightly reducing effect on fasting blood level, while, HSA showed almost comparable results when compared to the non-treated negative control (Figure 2). The statistical difference between treated and non-treated negative control groups was observed non-significant ($p>0.05$). The difference between all treatment groups among themselves was also not significant ($p>0.05$) (Figure 2).

3.4. Serum level concentration of Apaf-1, IFN-γ, TNF-a and E2

Serum levels of Apaf-1, IFN-γ, TNF-α and E2 in the negative control group (Group 0) were used to establish a normal reference range. The rats treated with TH and HSA (Groups 1, and 2) showed a higher median concentration of Apaf-1, IFN-γ and TNF-α, but a lower E2 concentration compared to those of non-treated negative control (Figure 2). A significant statistical difference was observed between all groups ($p<0.05$). The difference between all treatment groups among themselves was not significant ($p>0.05$). TH presented a slightly higher concentration when compared to a similar dose of HSA for Apaf-1 and TNF-α and almost comparable for IFN-γ. While, TH showed a slightly more decreasing effect for E2 compared to HSA (Figure 2).

Table 3 The haematological parameters of TH and HSA treated groups compared to the negative control.

	Groups			
	0 -ive control	1 (1.0 g/kg TH)	2 (1.0 g/kg HSA)	P value[a]
RBC (10^{12}/L)	7.25 (0.42)	7.98 (3.32)	8.33	0.003
Hb (g/dl)	14.97 (0.77)	15.95 (5.95)	15.93	0.003
PCV (%)	46 (3.25)	46.75 (17.75)	46.9	0.009
MCV (fl)	68.5 (3.25)	68.2 (11.75)	68.4	0.013
MCH (pg)	20.6 (1)	21 (3)	21.1	0.169
MCHC (g/L)	32 (1)	32 (3.5)	32.1	0.062
RDW (%)	11.4 (1.57)	12.25 (2.17)	11.96	0.01
TWBC (10^9/L)	4.75 (1.75)	5.15 (8.75)	5.53	0.02
Polymorphs (%)	32 (8.75)	31.87 (11.25)	32.1	0.01
Lymphocytes (%)	68 (8)	69 (9.75)	68.9	0.014
Monocytes (%)	1 (1.5)	1.5 (1)	1.5	0.231
Eosinophils (%)	0 (1)	0.5 (1)	1.1	0.102
Basophils (%)	0	0	0	1
Platelets (10^9/L)	839 (225.75)	866.5 (229.25)	843.3	0.01

[a]Kruskal-Wallis test. Data are expressed as median interquartile range (IqR). Values are statistically significant when $p \leq 0.05$. Legends: FBC=full blood count, RBC= Red blood cells, Hb= Haemoglobin, PCV= Packed cell volume, MCV= Mean corpuscular volume, MCH= Mean corpuscular haemoglobin, MCHC= Mean corpuscular haemoglobin concentration, RDW= Red cell distribution width, TH= Tualang honey, HSA= Honey sugars analogue, Group 0= negative control (normal rats), Group 1= 1.0 g/kg TH and Group 2= 1.0 g/kg HSA.

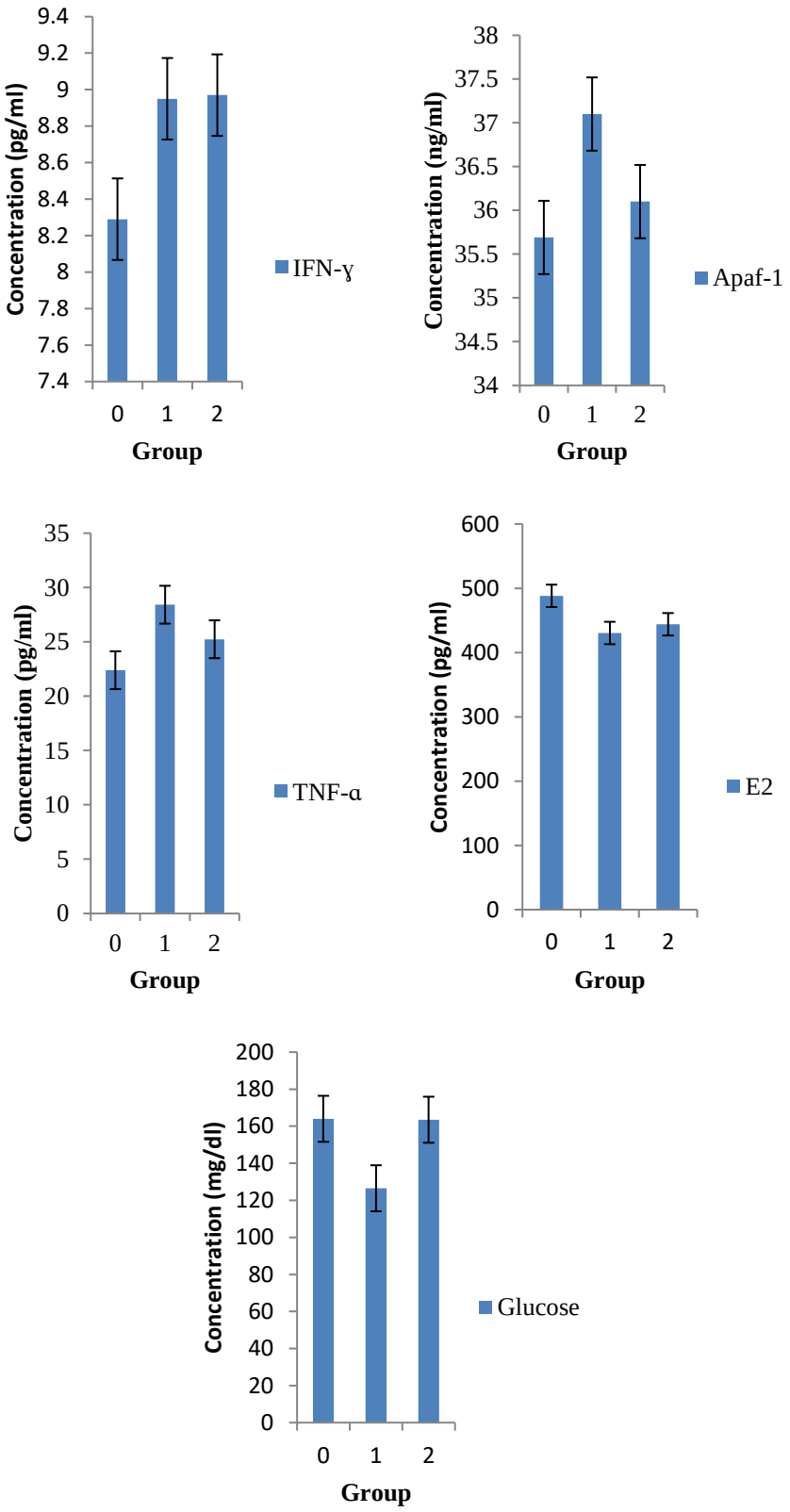

Figure 2 The serum level concentration of Apaf-1 (ng/ml), IFN-γ (pg/ml), TNF-α (pg/ml), E2 (pg/ml) and fasting blood glucose level (mg/dl) in the rats of TH and HSA groups compared to the rats of negative control. Group 0= negative control (normal rats), Group 1= 1.0 g/kg TH and Group 2= 1.0 g/kg HSA. Data are expressed as median interquartile range (IqR) using Kruskal-Wallis test. Values are statistically significant, $p<0.05$. Legends: Apaf-1=Apoptotic protease activating factor 1, IFN-γ=interferon gamma; TNF-α =tumour necrosis factor alpha; E2=estradiol; TH= Tualang honey, HSA= Honey sugars analogue.

4. Discussion

Honey has been used to cure several ailments. Currently, it has clinched the attention of researchers for its broad spectrum medicinal effects [28]. Is composition and physiochemical properties can vary from floral source to its origin [29]. Research has shown its several medicinal effects such as anti-microbial [30], anti-mutagenic [31], anti-inflammatory [32], antioxidant [33], antidiabetic [34] and anti-tumoural effects [35 & 36]. Our study highlights intriguing findings regarding the utilization of TH and HSA as potential agents on body weight, hematological and biochemical variables at various concentrations tested in normal albino female rats.

Quality assessment of honey has a vital importance to interpret its activity and efficacy [37]. The honey used in this study was of very fine quality to analyze the parameters. Our results report that the body weight of the rats in all groups increased steadily during the whole experimental period of 16 weeks (Figure 1). Treatment with TH and HSA presented a positive effect on percentage body weight gain. The rats showed no weight loss in either treatment group for each model. Thus, it can be presumed that the higher percentage of body weight gain in the treatment groups could be attributed to TH and HSA treatments. The negative control rats are not gaining as much weight as treated groups. This weight gain can be beneficial to make the use of honey in diseases such as in cancer where weight loss leads to worst prognosis, recurrence and death [38]. To rebut weight gain, one of the mechanisms explains that sugars in honey trigger a small spike in insulin levels, and insulin stimulates the release of tryptophan in the brain through insulin regulatory pathway. Tryptophan is converted to serotonin, which is then converted into melatonin at night. Melatonin in turn inhibits the release of insulin, thus further stabilizing blood sugar levels. This implication causes to down regulate the aerobic glycolytic pathway that is believed to play a vital role in lipogenesis, which may ultimately lead to an increase in body weight [39].

Full blood count is a prerequisite investigation in different diseases and poor blood parameters affect the outcome and prognosis of diseases [40]. Research has shown that the functioning of the immune system at haematological level has a direct influence on diseases [40]. We observed that treatment with similar strengths of TH and HSA had a slightly potentiating effect on the haematological parameters such as Hb, RBC, PCV, lymphocytes, RDW, eosinophils, monocytes and platelets compared to the non-treated negative control (Table 2). Researchers have reported an deranged level of RBC, Hb, PCV, MCV, RDW, TWBC, platelets and lymphocytes in diseases like cancer with acute anaemia [40 & 41]. Our results suggest that TH and HSA may modify these parameters to modulate different ailments. Exclusive honey feeding in the absence of any disease significantly modifies the haematological parameters [42].

Apoptotic protease-activating factor-1 (Apaf-1) is a human homolog of *C. elegans* CED-4 gene. It acts as a key regulator of the mitochondrial apoptosis pathway. It plays a role of central element in the apoptotic pathway via formation of apoptosome complex by procaspase-9, cytochrome c and Apaf-1 itself [43 & 44]. Loss of Apaf-1 expression can aid cells to evade immune attack-induced death and programmed cell death or apoptosis in diseases, especially in cancer [45 & 46]. Our data reported that TH and HSA cause to enhance the concentration of Apaf-1 at serum level (Figure 2.1). We can assume that TH and HSA may act as therapeutic agents to ameliorate the expression of Apaf-1, and thus can be used to modulate different diseases with Apaf-1 deficiency.

IFN-γ is a cytokine that has been described as a 17 kDa peptide. It is secreted by antigen activated lymphocytes or NK cells (natural killer cells). It is critical for innate and adaptive immunity against various types of diseases [47]. It has further been reported that higher IFN-γ concentrations at serum level predicted a favourable outcome or prognostic value in diseases [48]. Our study shows that the rats in TH and HSA treated groups have higher IFN-γ concentration than the non-treated negative control (Figure 2.2). Our findings are consistent with another study reporting that honey can cause high serum levels of IFN-γ [49]. Our results suggest that TH and HSA could be used as potential preventive immune-stimulating agents for diseases. Thus, honey acts by enhancing immunological activity of IFN-γ to make a profound effect [50 & 51]. IFN-γ is produced by lymphocytes [52], and our study also shows that TH and HSA cause to increase the level of lymphocytes. This validates that TH and HSA may hinder this signaling pathway by potentiating IFN-γ as well as lymphocytes.

The strengths tested of TH and HSA caused to increase the level of TNF-α concentration compared to the non-treated negative control (Figure 4.8). TNF-α has been shown to play both beneficial and deleterious role in the promotion or inhibition of diseases [53 & 54]. The primary role of TNF is to regulate immune cells. The increased concentration of TNF-α in TH and HSA treated groups compared to rats of non-treated negative control may be assumed to be due to effect of these treatments. Thus, TH and HSA may tend to increase TNF-α concentration at serum level, which may assist in ameliorating diseases. Several studies have reported the regulatory effect of honey on TNF-α [55 & 56]. Honey stimulates monocytes to release TNF-α [55 & 56]. TNF-α release is stimulated by monocytes, lymphocytes and eosinophils [57 & 58]. Our study also shows that TH causes to enhance the level of monocytes, lymphocytes and eosinophils in blood. This validates our findings that

honey modulates TNF-α as well as monocytes, lymphocytes and eosinophils, ultimately resulting in enhancing immunity.

Estradiol (17β-estradiol or E_2) is a female sex hormone. Primarily it acts as a key regulator of growth, differentiation and immune processes [59]. It has been shown that prolonged exposure of target cells or tissues to excessive estradiol may result in diseases such as cancer [60]. Our results demonstrate that the rats in TH and HSA treated groups showed inhibitory or decreasing effect on E2 concentrations at serum level. Research has shown that honey modulates estrogen through its antagonistic action and may be useful in estrogen-dependent cancers such as breast sand endometrial cancers [61 & 62]. This effect is attributed to its phenolic content [61]. It is also possible that honey, which is a natural phytoestrogen [2], plays its role in modulating the endogenous estrogen by stimulating immune system and other signaling pathways. TNF-α regulates the pathways of estrogen metabolism [63]. TH and HSA seem to modulate both E2 and TNF-α concentration at serum level, as observed in our study. Thus, our study suggests that TH and HSA may ameliorate E2 at serum level to inhibit its negative effects.

Considering honey as a sugar or sweetener, we would expect that the blood glucose level would rise after honey treatment, but our study shows otherwise. The rats of TH treated group showed a slightly lower fasting blood glucose level or hypoglycemic effect compared to those of non-treated control group (Figure 2.5). Elevated serum and fasting blood glucose in patients are associated with recurrence and worse outcomes [64-66]. Thus, honey does not raise blood glucose level which may be a favourable factor to use honey against diseases, with no hyperglycemic effects. Research has shown that honey exhibits hypoglycemic or anti-diabetic effects [33 & 67]. The proposed mechanism for hypoglycemic effect of honey may be through the role of honey in modulating the insulin signaling pathway [68]. Thus, our findings suggest that TH and HSA may modulate this insulin signaling pathway to bring a hypoglycemic effect.

Several factors may influence the effectiveness of honey. Some of these factors which enable honey to act as immune-stimulating may be its acidic PH, minerals, enzymes, osmotic properties and vitamins. These factors also promote tissue healing [69]. It can also be hypothesized that the phenolic acids and flavonoids in honey can also contribute to its protective effects against pathological conditions. Equally important to this finding was the fact that honey sugars analogue was found to have similar effects as honey for these parameters at concentrations tested. The superior effect of the tested dose of TH than a similar dose of HSA for some parameters, is perhaps due to the fact that honey is a mixture of several health boosting components compared to simple sugars or HAS.

5. Conclusions

Tualang honey and honey sugars analogue modulate body weight, fasting blood glucose level, hematological and biochemical variables such as Apaf-1, IFN-γ, TNF-α and E2. The most reliable study on the usefulness of TH and HSA as therapeutic agents warrants further research in clinical trials and other in vivo diseased models.

6. Acknowledgments

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