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Research Article
Enhancement of *Apis mellifera* L. Hypopharyngeal Gland using *Hyphaene thebaica* Ethanolic Extract as Supplement

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Abstract

Background and Objective: Hypopharyngeal gland (HPG) of *Apis mellifera* L. honey bee workers secrete the main proteinaceous substances, the royal jelly which acts a vital role in brood care by young workers and thus colony growth. Many factors may affect the development and function of the hypopharyngeal gland and consequently their role within the beehive. **Materials and Methods:** *Hyphaene thebaica* fruit powder (500 g) was soaked in one litter of 70% ethanol for 3 days at room temperature, then filtered and concentrated to dryness with a rotary evaporator. Bioactive compounds and biological activity of doum ethanolic extract were characterized to measure the extent of safety. Samples of workers bee feeding with the extract concentrations (0.5 and 1%) at 6, 10 and 14 days were examined for head weight, body weight, soluble protein content, DNA fragmentation and hypopharyngeal glands histology, the data were analyzed using analysis of variance (ANOVA), Tukey's least significant difference. **Results:** Ethanolic doum extract contains flavonoids (45.62 mg g^{-1}) and phenolic (27.24 mg g^{-1}) compounds, also possess antioxidant (147 µg mL^{-1}), anti-inflammatory (119.8 µg mL^{-1}) and antimicrobial activities. There was an elevation in soluble protein content and no DNA damage in the honey bee worker's genome after fourteen days of feeding. The histological studies exhibited no deformation in the structure of gland acini and an increase in gland size was detected. **Conclusion:** Ethanolic doum extract in the bee diet has a role in HPG enhancement. Studies on its safety profile and biological activities make it a good choice to attenuate honey bee diseases inside bee colonies.

Key words: *Apis mellifera*, *Hyphaene thebaica*, histology of hypopharyngeal glands, DNA fragmentation, total soluble protein, diet supplement, antimicrobial activity

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Royal jelly secreted by the Hypopharyngeal Gland (HPG) considers the most important food for brood and queen that is responsible for the differentiation among castes. Workers and drone larvae are fed on royal jelly for the first three days and only queen larvae continue to be fed with this special food during their development[1]. Consequently, the activity and development of the HPG affect directly the worker's behavior which in turn may affect the strength of the colony[2,3]. The hypopharyngeal gland is located below the pharynx in the head in front of the brain between the compound eyes of young worker honey bees, where the gland is well developed with large actively secreting acini. Active secretion reaches its peak in young nurse-workers at the age of 6-13 days, while in older honey bees that present foraging activity, the gland will tend to be atrophied[4].

In general, the status of HPG is used to describe the worker honey bees physiological conditions[5], measure bees fitness and reflect any inherent differences in young workers or brood[6]. Several beekeeping problems caused by chemicals used against honey bee pathogens are associated with harmful effects on bees and brood[7], besides health risk to beekeepers and bee product consumers[8].

Moreover, some protection crop products were detected for their harmful impact on workers hypopharyngeal glands which in turn affect the production of royal jelly which would seriously hamper the growth and brood care of the colony[9]. Therefore, the healthy and strong honey bee colonies could be maintained by using some harmless natural-based supplements that help to improve colony health and reduce bee mortality. There are fewer studies that tend to evaluate different plant extracts as supplementary feeding in bee colonies. The current study evaluates the ethanolic extract of Egyptian doum palm tree, *Hyphaene thebaica* belongs to the family Palmae (Arecaceae: Barassoideae) as a herbal supplement in honey bee feeding. Doum has a wood texture and edible oval fruits[10]. Doum fruit contains high levels of essential minerals such as potassium, sodium, calcium, magnesium, phosphorus. As well as, B-complex vitamins, carbohydrates, protein and dietary fiber which are essential for good nutrition[11].

Biological, Histological and biochemical studies were investigated to throw light on the role of *H. thebaica* as a supplement on the size and development of HPG. Besides, the tested doum ethanolic extract was estimated for its antioxidant, anti-inflammatory and antimicrobial activities.

MATERIALS AND METHODS

Study area: This research project was conducted from 3/2019 to 2/2020 at the Pest Physiology Research Department Lab. Plant Protection Research institute Sharkia Governorate, Agriculture Research Centre, Egypt. The sample of tested honeybees collected from the apiary of the same institute.

Preparation of *Hyphaene thebaica* ethanolic extract: *Hyphaene thebaica* fruit powder (500 g) was soaked in one litter of 70% ethanol in a glass jar for 3 days at room temperature. The extract was then filtered, concentrated to dryness in a rotary evaporator (RV 05 Janke and Kunkel, IKA-WERK, German) and kept at 4°C till use.

Evaluation of *Hyphaene thebaica* ethanolic extract: Biological activities (anti-inflammatory, antioxidant and antimicrobial) and bioactive compounds (totals of flavonoids, phenolic and alkaloids) were determined at the Regional Centre for Mycology and Biotechnology (RCMB) at Al-Azhar University. These analyses ensure the safety of tested doum ethanolic extract.

Bioactive compounds of doum ethanolic extract

Determination of total flavonoids content: The content of flavonoids in the ethanolic doum extract sample was determined using spectrophotometric method[12] at λmax = 415 nm as rutin equivalence (mg of RU g^{-1} of the sample).

Determination of total phenolics content: The content of phenolics in ethanolic doum extract was measured using the spectrophotometric method according to Singleton *et al.*[13] at λmax = 765 nm as gallic acid equivalence (mg of GA g^{-1} of the sample).

Determination of total alkaloid content: Alkaloids were precipitated in the sample using dragendorff reagent[14] and the absorbance was read at 435 nm. The amount of alkaloids content was expressed as mg atropine (AE) mL^{-1}.

Biological activities of doum ethanolic extract

Anti-inflammatory activity: The human RBCs membrane stabilization method was adopted by Parvin *et al.*[15] to determine the anti-inflammatory activity of *H. thebaica* ethanolic extract. The calculation of membrane stabilization percentage was determined using the following formula, Where, OD is optical density:

$$\text{Protection}(\%) = \text{OD of } \frac{\text{Test}}{\text{Control}} \times 100$$

Antioxidant activity: The antioxidant activity of the extract was estimated by Yen and Duh[16] using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay. The calculation of inhibition percentage (PI) of the DPPH free radical was estimated according to the formula:

$$PI = \frac{AC - AT}{AC} \times 100$$

Where:

AC = Absorbance of the control at t = 0 min

AT = Absorbance of sample+DPPH at t = 16 min

Antimicrobial activity: Antimicrobial activity was assayed by the well diffusion agar method[17]. The sample of doum ethanolic extract was tested at 10 mg mL^{-1} concentration against six human pathogenic microorganisms.

Bioassay: The experiment was conducted on *Apis mellifera* workers under laboratory conditions. Three colonies from the experimental apiary belonging to Plant Protection Research Institute, Agriculture Research Centre were used to collect frames of sealed brood close to bee emergence. The frames were incubated in darkness at 34°C and 65% RH to obtain newly emerged adult worker bees.

The emerged bees were manually collected from the combs and confined to rearing jars (1 kg). The top of the jar was perforated to allow ventilation and provided with a piece of wax comb for sugar solution. Caged workers were fed on pure sugar syrup as control (3 parts of sugar: 2 parts of water) and sugar syrup enriched with the two concentrations of *H. thebaica* extract (1 and 0.5%). Each concentration was replicated five times with 15 bees in each rearing jar. Caged bees were kept for 14 days at 30°C, 50% RH, in darkness to simulate conditions within the colony and the syrup was changed daily.

Bee sampling: Workers bee at different ages of 6, 10 and 14 days were sampled from each experimental group. Subsequently, the bees were examined for head weight, body weight, soluble protein content, DNA fragmentation and histological study on hypopharyngeal glands.

Head and body fresh weights: All bees used for the examination of HPG were weighed then decapitated and the fresh weights of the heads were measured. Weights were determined immediately after collecting the bees from each treatment.

Histological evaluation of hypopharyngeal gland: Five head capsules of honey bee workers were collected from each group (control group and the two groups fed previously on 0.5 and 1% of *H. thebaica* supplemented syrup) after 6, 10 and 14 caging periods. Samples were embedded in paraffin, sliced (5 µm thickness), placed onto glass slides and stained with hematoxylin-eosin for 15 min. Stained samples were observed and HPGs were photographed using an optical microscope (NLCD-120, PN 14003, LAMP S-LED W1, China).

Total soluble protein (TSP) of workers bee: Samples of bees were homogenized in distilled water using a Teflon homogenizer (MPW-309 Mechanic–Preczyina, Poland). Homogenates were centrifuged (Hettich, Germany) at 5000 rpm for 10 min at 5°C. The supernatants were used directly for the biochemical analysis of total soluble protein. Colorimetric determination of TSP in supernatants of workers homogenate was estimated as mentioned by Bradford[18].

DNA fragmentation analysis of *Apis mellifera* worker: DNA damage determined by DNA fragmentation assay[19]. Extracted DNA samples workers from control and the two concentrations (1 and 0.5%) of ethanolic doum extract after 14 days of feeding with the method of Phillips and Simon[20] were run on electrophoresis (NANOPAC-300, Cleaver Scientific Ltd, UK) using 1.2% agarose gel. The gel was run at a low voltage which improves the resolution of DNA fragments (50.0 volts), then the gel was stained using ethidium bromide. Samples were analyzed using Gel Analyzer 19.1, an image analysis software[21].

Statistical analysis: The data obtained were presented as Mean and Standard error of the mean (Mean ± SE). Differences among mean were analyzed using analysis of variance (ANOVA), Tukey's least significant difference by computer statistical software[22]. Probability value (p-value) <0.05 was considered significant.

RESULTS

Bioactive compounds and biological activity of doum ethanolic extract: The ethanolic *H. thebaica* extract contained a large number of flavonoids (45.62 mg RU g^{-1}) followed by phenolic (27 mg GA g^{-1}) and alkaloid compounds

Table 1: Total flavonoid, phenolic and alkaloid contents of ethanolic *H. thebaica* extract

Ethanolic extract/Bioactive contents	*Hyphaene thebaica* (Doum)
Total flavonoid	45.62 mg RU g^{-1}
Total phenolic	27.24 mg GA g^{-1}
Total alkaloid	12.10 mg AE g^{-1}

Table 2: Antioxidant and anti-inflammatory (membrane stabilizing protection %) activities of *H. thebaica* extract

Tested samples	IC_{50} (μg mL^{-1})
Antioxidant activity	147
Anti-inflammatory activity (Membrane stabilizing protection %)	119.8

Table 3: Head fresh mass and body weight of honeybee workers

	Head weight (Mean±SE)			Body weight (Mean±SE)		
Concentration	6 day	10 day	14 day	6 day	10 day	14 day
0.5%	0.0086±0.0001^{ab}	0.0114±0.0002^{a}	0.0115±0.0001^{b}	0.0729±0.0047^{b}	0.0777±0.0056^{b}	0.0860±0.0018^{b}
1%	0.0088±0.0006^{a}	0.0119±0.0002^{a}	0.0125±0.0002^{a}	0.0879±0.0025^{a}	0.0936±0.0050^{a}	0.1034±0.0031^{a}
Control	0.0072±0.0004^{b}	0.0101±0.0002^{b}	0.0111±0.0002^{b}	0.0697±0.0021^{b}	0.0750±0.0015^{b}	0.0752±0.0003^{c}
P	0.0772^{ns}	0.0008 ***	0.0007 ***	0.0053 **	0.0259 *	0.0000 ***

(12.10 mg AE g^{-1}) as shown in (Table 1). *Hyphaene thebaica* had antioxidant activity and scavenging ability for free radicals. The test showed that the scavenging ability of DPPH radical, increased by increasing doum extract concentrations. The 50% inhibitory concentration (IC_{50}), the concentration required to inhibit DPPH radical by 50% is 147 μg mL^{-1} (Table 2). Regarding the anti-inflammatory activity of doum extract, the IC_{50} value was defined as the concentration of membrane stabilization protector to protect 50% of HRBC membrane stabilization under the assay conditions, this means 119.8 μg mL^{-1} of doum extract protected 50% of the human RBCs membrane stabilization (Table 2).

The ethanolic extract of *Hyphaene thebacica* showed antimicrobial activity of *Staphylococcus aureus* (RCMB010010) as Gram-positive bacteria with 11 mm inhibition zone as data report from RCMB.

Head fresh mass and body weight: The obtained results from Table 3 indicated that 0.5 and 1% concentrations of *H. thebaica* extract caused an increase in the average head weight of the caged bees over time as compared to those fed on pure syrup. After 6 days increasing in head weight did not differ significantly from that of the control group but gradually such an increase recorded a significant difference after 10 and 14 caging periods (Table 3).

Fortunately, after 6, 10 and 14 days a significant increase in the average body weights were observed in the caged bee that were fed on syrup enriched with *H. thebaica* extract, where the maximum increase recorded with 1% concentration (0.0879±0.0025, 0.0936±0.0050 and 0.1034±0.0031 g) compared to control group (0.0697±0.0021, 0.0750±0.0015 and 0.0751±0.0003 g) after 6, 10 and 14 caging periods, respectively. The result also cleared a highly significant difference ($p<0.05$) between concentrations of *H. thebaica* extract and control after 14 days of feeding (Table 3).

Histological features of hypopharyngeal gland: Light microscopic observations showed that the shape of HPG looks like a long cluster of numerous pear shape lobules surrounding an elongated central axial duct. The gland was paired structures composed of numerous secretory units acinus (Fig. 1a). The size and structure of HPG in honey bee workers that fed on syrup supplemented with the two concentrations (0.5 and 1%) of doum extract after 6, 10 and 14 feeding days were compared with control. Doum extract did not negatively alter the structure of acini in the HPG moreover gland's size increased from the 6th day remained at a high level until the 14th day.

On the 6th day age, the control bees have similarly acini characterized by numerous secretory vesicles connected to the central axial duct by a thin, individual, secretory duct (Fig. 1b). Workers at 6 days age that supplemented with (0.5 and 1%) of doum extract had more developed acini with regular secretory vesicles beside many ducts and more obvious nucleus (Fig. 1c-d). On the 10th day, the glands achieved maximum development (Fig. 1e) while glands of members supplemented with (0.5%) doum extract showed wider intracellular duct (Fig. 1f) in acini however, larger secretory duct observed in glands of members supplemented with (1%) doum extract (Fig. 1g). Furthermore, both of the supplemented group had a fully developed nucleus than that originated from control members. At the 14th day age of

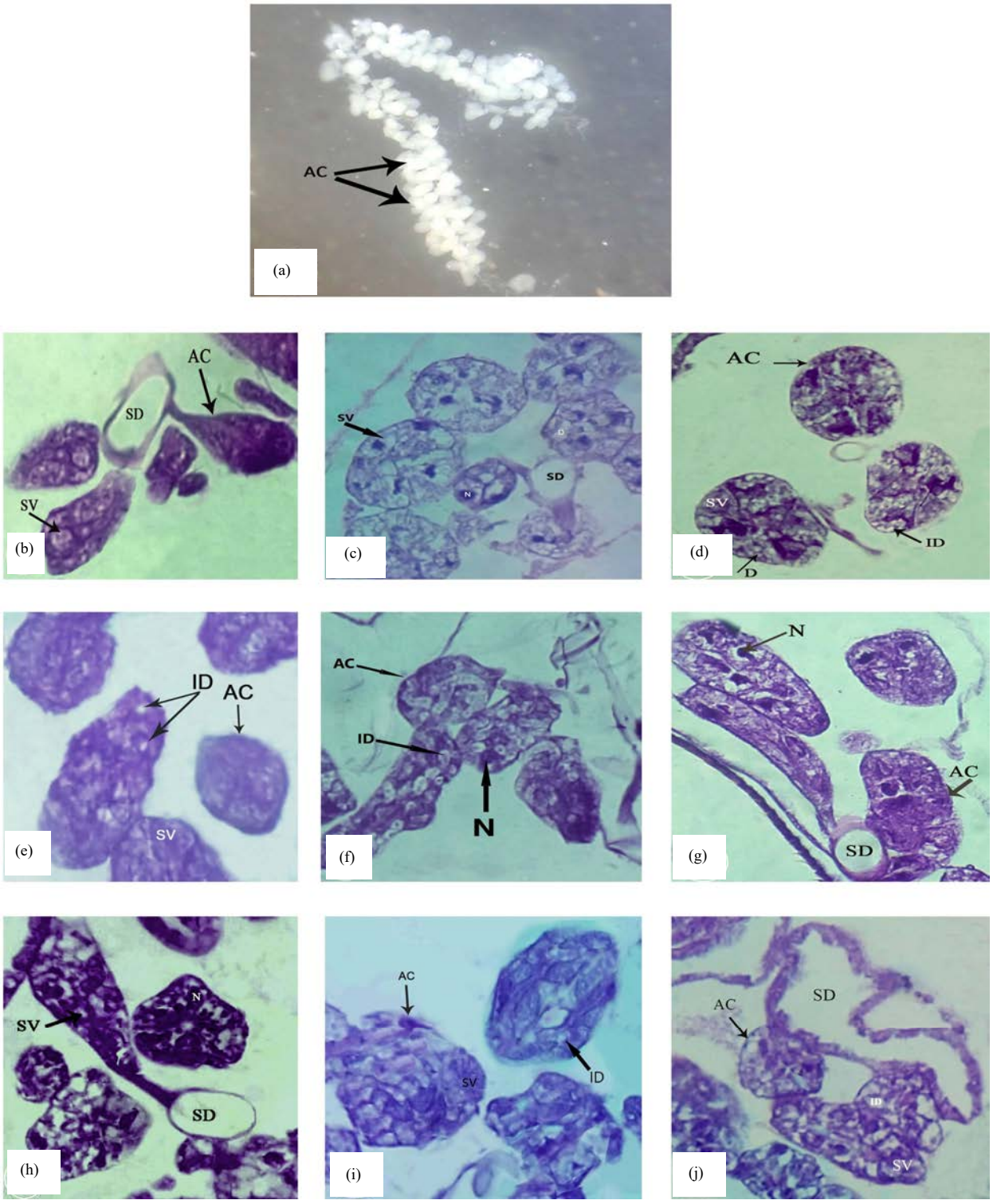

Fig. 1: Photomicrograph showed HPGs of *Apis mellifera* workers

(a): HPGs morghpology of honeybee worker, (b): control HPGs after 6 days, (c): HPGs of groups supplement with 0.5% concentrations of *H. thebaica* after 6 days, (d): HPGs of groups supplement with 1% concentrations of *H. thebaica* after 6 days, (e): control HPGs after 10 days, (f): HPGs of groups supplement with 0.5% concentrations of *H. thebaica* after 10 days, (g): HPGs of groups supplement with 1% concentrations of *H. thebaica* after 10 days, (h): control HPGs after 14 days, (i): HPGs of groups supplement with 0.5% concentrations of *H. thebaica* after 14 days, (j): HPGs of groups supplement with 1% concentrations of *H. thebaica* after 14 days, (AC) acini, (D) secretory ducts (ID) intracellular ducts, (N) nuclei of the gland cell, (SD) excretory duct and (SV) secretory vesicles

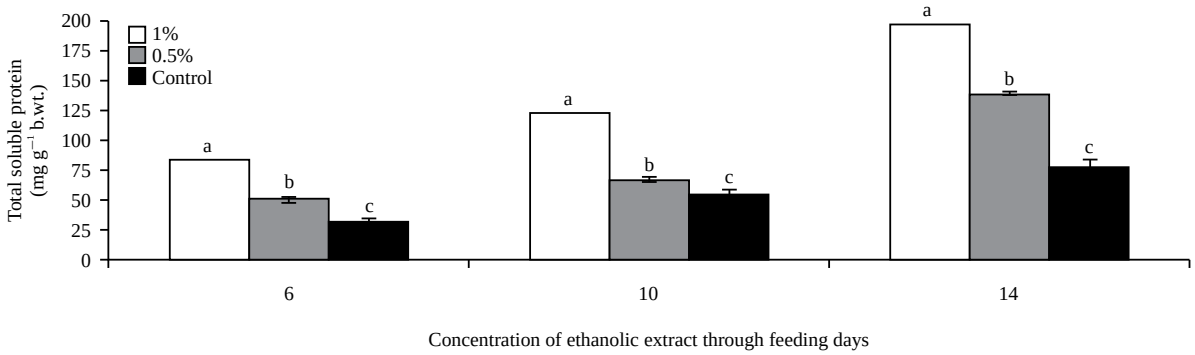

Fig. 2: Total soluble protein content of honeybee worker after intervals days of feeding with doum extract

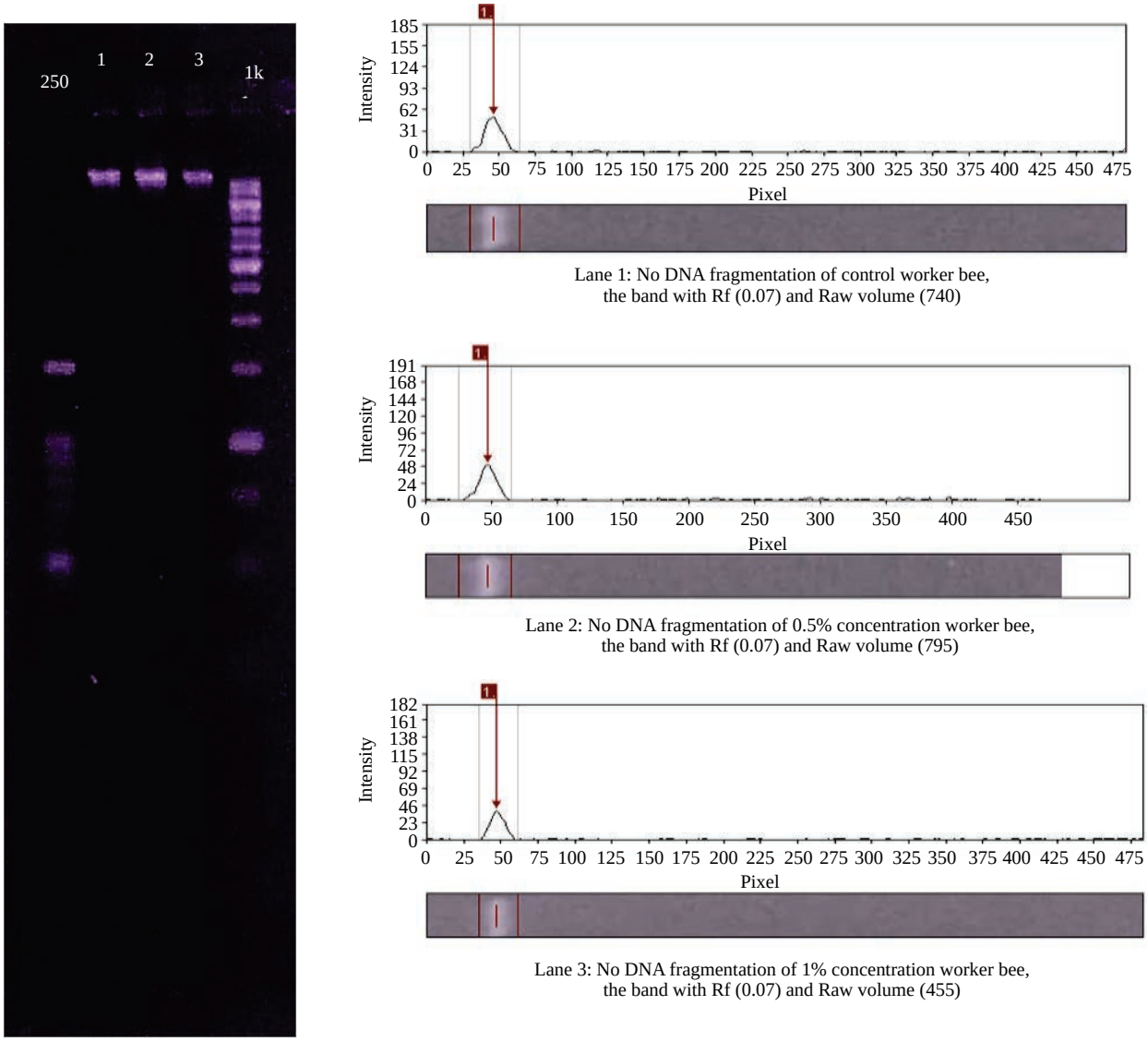

Fig. 3: Effect of two different concentrations of ethanolic doum extract (0.5 and 1%) on DNA fragmentation of total genomic worker bee body after 14 days of feeding

control, the HPG showed irregularity in secretory vesicles shape and condensation of the nucleus (Fig. 1h) in comparison with group supplemented with (0.5 and 1%) which still had well developed large acini with numerous secretory vesicles containing accumulated secretion (Fig. 1i-j).

Total soluble protein of honey bee workers: The change in the Total Soluble Proteins (TSP) was detected in the supernatants of homogenate worker bees fed on supplemented syrup with plant extract at 0.5 and 1% concentrations (Fig. 2). The two concentrations induced a highly significant increase in TSP as compared to control. The TSP gradually increased after 6, 10 and 14 days of caging periods and the maximum value recorded after 14 days at 1% concentration (196.37 mg g^{-1} b.wt.) as compared to control and 0.5% concentration (78.14 and 139.24 mg g^{-1} b.wt.) of 14 days. The data were expressed a highly significant difference at $p<0.05$.

Effect of doum ethanolic extract on total genomic of DNA: The electrophoretic pattern of worker bees is shown in Fig. 3. Total genomic DNA fragmentation of control (lane 1), worker feeding on 0.5% (lane 2) and 1% (lane 3) of ethanolic doum extract after 14 days of caging period. All the tested groups of workers did not reveal any fragmentation of DNA means no DNA damage occurred in the total genome of a tested worker bee.

DISCUSSION

The hypopharyngeal gland which is considered the main gland that secretes royal jelly is located below the pharynx in the head of young honey bee workers. At 3-4 days after emergence, the worker bees were engaged in nursing the young bees with the protein-rich substance released from their HPGs. The development and activity secretion of the HPG in bees is associated with worker's age, the kind of ingested feed and the function performed in the colony. Under normal conditions, HPGs start to function between the 3rd and 6th day of a worker bee's life whereas, become fully developed and show the highest secreting activity between the 6th and the 14th day. In our research, we explained the ability of glands to enlarge their size and in turn increase their activity using doum herb extract as a supplement. Sugar syrup enriched with the two concentrations of *H. thebaica* extract (1 and 0.5%) was tested for its effect on head fresh weight. The results showed a significant gradual increase in head weight after 10 and 14 caging periods as compared to those fed on the pure sugar syrup. Fresh mass of the head has been exhibited as a simple and rapid method to describe glandular development[5]. Additionally, Rahman *et al.*[23] found a significant positive correlation between fresh head weights and HPG acini diameters. So, the weights of fresh heads can serve as an indicator for the HPGs development.

The body weight of bees as determined after 6, 10 and14 days of feeding *H. thebaica* extract enriched syrups, was significantly higher than that measured in control bees. This may due to the doum fruits contains high percentages of total carbohydrates (84.87%), total sugars (29.39%) and crude fiber (22.36%) as described[24]. The higher maximum flight speeds of naturally reared honey bees occurred when fed on energy-rich 2 molar glucose solutions where the good nourishment of honey bee larvae in vitro can be developing into healthy adults without any morphologic deformations[25].

The total phenolic compounds of doum ethanolic extract are 27.24 mg GAE g^{-1} while it ranged from 45.08-64.90 mg GAE g^{-1} in different studies[26], on the other hand, it varied from 116.26-139.48 mg GAE g^{-1} in pitted doum fruit extracts[27]. The bioactive potential of doum is related to their high content of polyphenols[28]. Also, the ethanolic extract of doum under observation revealed that it holds 45.62 mg rutin/gm of total flavonoid content. In accordance, Mohamed *et al.*[26] exhibited that the doum methanolic extract contains 46.28 mg quercetin/g dry weight of flavonoids content. The total flavonoid content in different extracts of doum fruit extracts varied widely ranging from 24.04-47.17 mg rutin/g[27].

The histological study was performed to evaluate the effect of doum extract on the development of the HPG size and structure of the worker's honey bee concerning age. Our results revealed that doum extract affected the size of acini, where an increase in acini size was observed in workers fed on supplemented syrup with doum extract as compared to those fed on pure syrup after 6, 10 and 14 caging periods. Moreover, the extract has no adverse effects on the histological structure of the HPG acini of the worker honey bee. Previous findings showed that small-sized glands were less functional than the larger glands[6]. Similar results were described in *A. mellifera* and *A. cerana*[29,30]. When workers switched to performing outside tasks such as guarding the HPG began to shrink and accordingly, the production of protein also diminished. The HPGs are well developed depending on the age of workers, the colony conditions, ingested food and the time of the year. The current result suggested that the increase in acini size may be related to the protein and carbohydrate contents of

ethanolic doum extract where the doum fruit contains 2.86 and 5.01% of a good-quality amount of protein besides a high percentage of mannose which may be reached to 75.9% as important carbohydrates component[31].

Protein is the major biochemical component necessary for an organism's development, growth and performance of its vital activities. Also, plays the most important role in all biological processes including reproduction[32]. The results revealed a highly significant increase in total soluble protein in the supernatants of worker bee homogenate fed on syrup supplemented with plant extract at both tested concentrations after 6, 10 and 14 caging periods.

This finding suggested that during this feeding period protein synthesis was highly active this may be related to the increase in HFG size where workers with larger acini actively produce proteins more than workers, which have smaller gland size[33]. In accordance, the increase in soluble protein content may also explain the increase in body weight where, protein has been shown to affect important individual associated traits such as body size, growth rate and fecundity[34].

Biological activities of doum fruit extract revealed that the 50% inhibitory concentration (IC_{50}), the concentration required to inhibit DPPH radical by 50% is 147 μg mL^{-1}, this result are in harmony with Aboshora *et al.*[27], who exhibited the IC_{50} values of doum extracts in between 107.6 and 172.7 μg mL^{-1}. Gharb and Fadhel[35] indicated that doum fruits can be utilized as natural antioxidants and considered as important free radicals removal and disease prevention so it can be used as food additives. The antiradical activity of the *H. thebaica* extracts are probably related to its phenolic compounds[36], in the same context, there was a correlation between total phenolics content and total antioxidant activity[37]. Physiologically, antioxidants play a major role in preventing the formation of free radicals, which are responsible for many harmful oxidative processes were the reaction of free radicals with biomolecules inducing extensive damage to DNA, protein and lipid[38]. The DNA fragmentation assay of an adult honey bee worker proved that there was no damage in DNA after 14 days of two sugary ethanolic doum concentrations supplement. This observation may be related to the high antioxidant activity of ethanolic doum extract. This explanation was emphasized by Iweala *et al.*[39] confirmed that the antioxidant will blockade cytosolic free radicals and then inhibit the DNA mutations.

The analysis of *H. thebaica* ethanolic extract recorded IC_{50} (119.8 μg mL^{-1}), the concentration which protects 50% of membrane stabilization of the cell. This ability may be due to it contains flavonoids, coumarins and saponins which responsible for their anti-inflammatory and anti-oxidant properties. The fruit of doum enriched with flavonoid conjugates, oxygenated fatty acids and sphingolipids intercede in the anti-inflammatory activity of doum[40]. On the other side, the treatment with doum extracts suppresses the activity of cyclooxygenase (COX-1) enzyme as a contributor to the inflammation process[41]. Finally, bee diet supplementation with ethanolic doum extract may be used in attenuating honey bee diseases because of its anti-inflammatory properties.

The ethanolic extract of *H. thebacica* showed antimicrobial activity of *Staphylococcus aureus* (RCMB010010) as Gram-positive bacteria with an 11 mm inhibition zone. This result is in harmony with Dosumu *et al.*[42] emphasized that the doum fruit extracted with ethyl acetate was active against *Staphylococcus aureus* bacteria. Also, methanol extract of doum fruit was revealed higher antibacterial activity against Gram-positive bacteria, antifungal and anti-yeast activities than aqueous extracts[26].

Therefore, further studies are needed to evaluate the role of doum extract in protecting honey bee colonies from the two Gram-positive bacterium *Paenibacillus larvae* and *Melissococcus plutonius* which causing the most serious brood disease of honey bees, American foulbrood (AFB) and European foulbrood (EFB) where, this extract contains high amounts of flavonoids and phenols which frustrated hydrolytic enzymes like proteases, cell envelope transport proteins and non-specific interactions with carbohydrates. Notably, El-Sohaimy *et al.*[43] illustrated the different food sources of the honey bee as they visited several flowers in all seasons may be correlated with lacking a bacterial profile like *Lactobacillus plantarum* and others in its stomach which aiding as a defense mechanism in the treatment of some honeybee diseases (AFB and EFB). So that, the honey bee feeding with doum ethanolic extract is considered as promising management in bacterial honey bee diseases to avoid antibiotics treatments. These results are supported by Aboshora *et al.*[27] as they indicated the use of doum extract as its antioxidant and antibacterial properties to relieve adverse effects of oxidative stress and inhibit pathogenic bacterial diseases.

In vivo, the conduct experiments were preceded by feeding the honey bee worker with ethanolic doum extract, *H. thebacica* as a supplement at (0.5 and 1%) concentration. The doum supplement was demonstrated a high significance growth in body weight, head weight, total soluble protein content, increase in the hypopharyngeal gland size with no

deformation in its structure and no genomic defect on honey bee workers in a dose-dependent manner. The results encourage the beekeepers to use the doum extract in the hive instead of antibiotics in microbial disease treatment also, to elevate apiary immunity. There will be more further study in hives to study the effect of doum on the honey bee diseases to improve a control treatment for the *A. mellifera* worker's diseases.

CONCLUSION

The current research exhibited that the addition of *H. thebacica* ethanolic extract as a novel supplement to sugar syrup enhances the HPG development which tended to present larger sizes during the entire experimental period this, in turn, leads to an increase in their activity secretion of royal jelly the main substance for colony maintenance. Based on bioactive compounds and biological activity of doum palm extract it can use promising safety management inside beehives as an antioxidant and antimicrobial agent for healthy and strong colonies as a natural-based saved compound instead of antibiotics.

SIGNIFICANCE STATEMENT

This study discovers a new natural supplement assigned as doum extract that can be beneficial for the enhancement of the hypopharyngeal gland in honeybee workers which important in the production of royal jelly as food for all members of the beehive in the apiary and affect directly the behavior of honeybee worker. This study will help the researcher to develop eco-friendly apiculture feeding by using the doum extract instead of chemical compounds and antibiotics that many researchers were not able to explore. Thus, a new theory on the natural supplement combination in the apiary may be arrived at.

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