Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Medicine

journal homepage:www.elsevier.com/locate/apjtm

Document heading doi:

Status of carbohydrate, protein and lipid profile in the mosquito larvae treated with certain phytoextracts

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ARTICLE INFO

Article history:
Received 23 November 2010
Received in revised form 27 December 2010
Accepted 15 January 2011
Available online 20 April 2011

Keywords:
Anopheles
Culex
Artemisia
Azadirachta
Biochemical profile
Phytoextract

ABSTRACT

Objective: To investigate the impact of the most potent petroleum ether extract of *Artemisia annua (A. annua)* and *Azadirachta indica (Az. indica)* on total carbohydrate, lipid and protein level of *Anopheles stephens*i and *Culex quinquefasciatus* larvae. **Methods:** Mosquito larvae were exposed to the extracts selected as per standard WHO procedure. Carbohydrate (glucose), total lipid and protein were estimated by the methods as Nelson, Bragdon and Lowry described, respectively. **Results:** The glucose levels were increased to 27.87% and 46.8%, respectively in anopheline larval tissues after treatment with petroleum ether extract of *A. annua* and methanolic extract of *Az. indica*. In culicine larvae, glucose levels were reduced to 58.96% and 24.65%, respectively. After treatment with *A. annua* extract, lipid contents in anopheline and culicine larvae decreased by 28.57% and 25.0%, respectively and increased by 14.29% and 50.00% in the *Anopheles* and *Culex* larvae, respectively after treatment with methanolic extract of *Az. indica*. Total protein levels were reduced to 63.13% and 92.62% in anopheline and to 32.39% and 48.12% in culicine larvae after treatment with *A. annua* and *Az. Indica* extracts, respectively. **Conclusions:** Two extracts produce significant alterations in the biochemical profiles of anopheline and culicine larvae. Further, the impacting factors of extracts on carbohydrate, lipid and protein contents of larvae are species and specific extraction. It indicates the disturbed metabolic activity of the larvae.

1. Introduction

Phytochemicals are nowadays considered as ideal alternatives to hazardous and non-biodegradable synthetic pesticides. Moreover, it is reported that plant derivatives are target specific[1] and not toxic to non-target organisms of the environment in which they are applied[2]. A lot of plant products have been tested as larvicides, adulticides and antifeedants against agricultural pests and vectors, chiefly mosquitoes. However, the mechanism of these products is not fully known. Therefore, investigation on the changes of basic biochemicals in the target specimens treated by phytochemicals is very essential. The biochemical changes induced by these chemicals have been reported[3,4]. The biochemical parameters are valuable in assessing and predicting the toxical effect on the insects. Further, the potential effects of botanical insecticides on biochemical milieu of insects is of great interest in biological control applications[5]. Many researchers observed larvicidal activities of various extracts of *Artemisia annua (A. annua)* and *Azadirachta indica (Az. Indica)*, and found petroleum ether extract of *A. annua* and methanol extract of *Az. indica* were the most effective against *Anopheles stephensi (An. Stephensi)* and *Culex quinquefasciatus (Cx. quinquefasciatus)*[6-9]. It is also reported that *A. annua*[10] and *Az. indica*[11] can affect biochemistry and physiology of insects. Depending upon these reportes, this study focuses on the impact of petroleum ether extract of *A. annua* (Linn.) and methanol extract of *Az. indica (A. juss)* on carbohydrate, lipid and protein contents of *An. stephensi* (Liston) and *Cx. quinquefasciatus* (Say) larvae. The alterations induced by these plant extracts may be helpful in establishing the larvicidal mode of the extracts against the target vectors.

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2. Materials and methods

2.1. Mosquito rearing

An. stephensi and *Cx. quinquefasciatus* were reared in our laboratory under controlled conditions of (27±1) ℃, relative humidity (85±5)% and normal photoperiod (natural light: dark=14:10) from eggs collected from cyclic colony of National Institute of Malaria Research (NIMR), New Delhi[12].

2.2. Preparation of botanicals

2.2.1. Collection of plant material

Leaves of *A. annua* and seeds of *Az. indica* were collected during their availability period, from adjoining areas of Dayalbagh Educational Institute, Dayalbagh, Agra. After proper washing and drying leaves were powdered manually and stored in air tight glass jars. *Az. indica* fruits were soaked in water for four hours and washed thoroughly to remove outer skin and flesh[13]. Seeds were air dried and their seed coats were removed to obtained kernels which were crushed by pestle and mortar and stored.

2.2.2. Preparation of extracts

2.5 kg dried leaves of *A. annua* and dried seed kernels of *Az. indica* were extracted in Soxhlet apparatus (Borosil, Mumbai, India) with petroleum ether, carbon tetrachloride and methanol successively for 72 hrs each[14]. Extracts were concentrated by vacuum rotary evaporator (Biocraft Scientific Industries, India) until solvents were completely evaporated and crude extracts were solidified. In case of *A. annua*, 97.75 g residue was obtained from petroleum ether extract, while *Az. indica* methanol fraction yielded 38.75 g residue.

2.3. Preparation of stock and test concentrations

2.5 g petroleum ether crude extract of *A. annua* was dissolved in 100 mL of 99.9% pure analytical grade acetone (25 000 ppm stock). While 2.5 g methanol residue of *Az. indica* was dissolved in 100 mL ethyl alcohol (25 000 ppm stock). These stocks were further diluted by adding different solvents to the working concentration. Desired amount of extraction was added to 249 mL water to concentrations equivalent to the LC_{50} of the extracts

2.4. Treatment of larvae

100 anopheline and culicine larvae each were treated with petroleum ether extract of *A. annua* and methanol extract *Az. indica* at LC_{50} values of 16.85 and 18.20 ppm against anopheline larvae and 78.20 and 21.95 ppm against culicine larvae for 24 hours. A pinch of larval food (yeast powder) was supplied during experimentation. Similar conditions were maintained for control with 1 mL solvent. After the exposure of 24 hours, the larvae were removed, washed with chilled normal saline solution, dried and were weighed. Larval tissue homogenate (10%) was prepared in 0.25 M chilled sucrose solution by homogenizer. The homogenate was centrifuged at 700 × *g* for 10 minutes to remove cell debris. Clear supernatant was used for determination of total carbohydrates, lipids and proteins. All the experiments were conducted in triplicate.

2.5. Estimation of total carbohydrates

Carbohydrate (glucose) was estimated by the method of Nelson[15]. Proteins were removed from the tissue homogenate and the filtrate containing glucose only as reducing substrate was heated with alkaline copper reagent and subsequently treated with Arsenomolybdate reagent. The blue colour thus developed was read at 540 nm. Glucose was calculated as:

$$\text{Glucose concentration in larvae (mg/g)} = \frac{\text{Concentration of test solution}}{\text{Volumn of test solution taken}} \times 10$$

2.6. Estimation of total lipids

Total lipids was estimated by Bragdon's method[16]. Lipids separated from non-lipid components by chloroform-methanol solution was estimated in the aqueous phase by the reducing action of fatty acids on a sulphuric acid – dichromate mixture and the resulting green colour was then read at 600 nm. Total lipid was calculated as:

$$\text{Total lipids concentration in larrae (mg/g)} = \frac{\text{Concentration of test solution}}{\text{Volumn of test solution taken}} \times 10$$

2.7. Estimation of total proteins

Protein was estimated by the method of Lowry[17]. Proteins reacting with Folin – Coicalteu reagent become purple blue proportional to the amount of proteins which can be read at 620 nm. Total protein was calculated as:

$$\text{Protein concentration in larrae (mg/g)} = \frac{\text{Concentration of test solution}}{\text{Volumn of test solution taken}} \times 10$$

2.8. Statistical analysis

The estimation of each biochemical parameter was done five times and the results were analysed by *t*- test.

3. Results

The result revealed that carbohydrate level increased from 12.81 mg/g in control anopheline larval tissue to 16.38 and 18.32 mg/g in anopheline larval tissue after treatment with *A. annua* and *Az. indica* extract, respectively. However, carbohydrate level of *Cx. quinquefasciatus* larvae was reduced from 15.01 mg/g in control to 11.31 and 6.16 mg/ g in larvae after treatment with *A. annua* and *Az. indica* extract, respectively.

A decrease in lipid content from 0.000 07 mg/g of control larval tissue was observed in anopheline larvae when treated with *A. annua* extract and it was declined to 0.000 05 mg/g in anopheline larval tissue. However, after treatment with

Az. indica extract, lipid profile increased to 0.000 08 mg/g in anopheline larval tissue. *Culex* larvae treated with *A. annua* extract also showed a reduction in lipid quantity and declined from 0.000 04 mg/g in control to 0.000 03 mg/g. Contrarily, when culicine larvae were treated with *Az. indica* extract, lipid content increased to 0.000 06 mg/g.

Protein level of *Anopheles* larvae treated with *A. annua* extract was lowered from 63.31 mg/g in control to 23.34 mg/g. Similarly, when treated with *Az. indica* extract protein level of anopheline larval tissue decreased to 4.67 mg/g. *Culex* larvae was reduced from 49.31 mg/g to 33.34 mg/g when treated with *A. annua* extract and to 25.58 mg/gm under *Az. indica* treatment, respectively.

4. Discussion

All types of insecticides have some negative impact on the growth and development of the insect, and also affect the metabolic and biochemical processes. This investigation shows that after treatment of *A. annua* extract, the glucose level of *An. stephensi* larvae increased by 27.87% over control. After treatment of *Az. indica* glucose level of anopheline larvae showed more increase by 46.8%. This increase in glucose level may be attributed to the insecticidal stress induced by these extracts in both the larvae. The stress might induce glycogenolysis resulting in hyperglycaemia. Nath *et al* also reported increase in glucose level due to insecticidal stress[18] when *Bombyx mori* was subjected to fenthion and fenitrothion at sub *lethal doses*. The results are also supported by Razak *et al*[19]. They found that on exposure to *Manduca oil*, adult female *Chrysoperea carnea* experienced hyperglycaemia and there was 8.64% increase comparing with the control. While carbohydrate quantity increased by 44.70% and 1.42% when final instar of *Chrysoperea carnea* larvae were exposed to *Pungam* and *Manduca* oil, respectively. Likewise the glucose level was elevated in third instar larvae of *Xanthogaleruca luteola* after treatment with methanolic extract of *A. annua* at 2.55 ppm for 24 and 48 hours[20]. However, ethanolic and water extract of *A. annua* reduced level of carbohydrate in third instar larvae of *An. stephensi*. It suggested that it may be due to lowered feeding and improper utilization of digested food[10].

The effects of the extracts on *Culex* sp. were contradictory to that on *Anopheles* sp. and the treated larvae displayed reduction in glucose content. Glucose level of the larvae treated with *A. annua* extract was decreased by 24.65%. The reduction in glucose content was more significant in larvae exposed to *Az. indica* extract by 58.96% decline over control. This depletion in glucose content may be due to utilization of the reserved glucose sources of larval tissues as a result of insecticidal stress. Likewise, the glycogen levels of juveniles and adults of *Pimpala turionellae* tended to decrease significantly as exposure to cypermethrin[5]. It is suggested that the effect of the extract on the carbohydrate content of treated larvae is specie-specific, depending on the variation in the physiology of these two larval species. Further, it was noted in our previous studies that during the extract treatments, alimentary canal was highly damaged in *Anopheles* species[6,7]. The larvae, thus, were unable to assimilate the food resulting in increase of carbohydrate content. In *Culex* species, however, alimentary canal was ruptured comparatively less seriously and it was found blocked with the extract[8,9]. The blockage probably inhibited the larvae from feeding, thus, lowering the glucose level. Inhibition of feeding process induced by neem extract from Spodoptera litura has been also reported by Koul *et al*[21].

After treatment with *Artemisia* extract, lipid level was declined by 28.57% as compared to control in *Anopheles* larvae. Similar trend was noted in *A. annua* treated *Culex* larvae and lipid content showed 25.0% reduction. This reduction in lipid profile indicates a negative effect of the extract on lipid metabolism and peroxidation. The decline in lipid quantity may be due to shift in energy metabolism towards lipid catabolism as the result of insecticidal stress induced by the extract. This observation is identical to the findings of Lohar *et al*, who found that *Tenebrio molitor* suffered lipid depletion in haemolymph, fat bodies and oocytes when exposed to malathion[22]. Sak *et al* reported the decline in lipid content due to shift in energy metabolism to lipid catabolism due to insecticidal stress induced by *Pimpala turionellae*[5]. Moreover, it has reported by Senthilkumar *et al* that total lipid were reduced in *An. stephensi* larvae treated with some plant extracts and it is suggested that it might be due to physiological stress conditions induced by the extracts[10].

In contrast to *Artemisia* extract, larvae treated with methanolic extract of *Az. indica* displayed an increase in lipid content. This increase, however, was significant in case of treated *Anopheles* larvae and the level was elevated by 14.29% above the control value. *Culex* larvae showed almost 50.00% elevations over control in lipid level after treatment with the extract. This increase in lipid content may be due to alteration in lipid peroxidation rate during the detoxification of the *An. stephensi*, induced by the insecticidal stress[10].

Total protein level of larvae under all the treatments was significantly decreased. After treatment with *A. annua* extract, *Anopheles* larvae showed 63.13% reduction in protein quantity. The reduction in protein level was comparatively lesser in *Culex* larvae and the proteins were lowered by 32.39%. This decline in protein content is probably due to insecticidal interference of the extract with the hormones regulating protein synthesis. These results are supported by our earlier studies regarding the developmental deformities induced by *A. annua* extract in *An. stephensi*[6] and the growth inhibitory effect of the extract on the developmental profile of *Cx. quinquefasciatus*[8], where body wall and larval tissues were found ruptured and degenerated in both the larval species. Senthilkumar *et al* noticed that protein levels in *An. stephensi* larvae treated with some phytoextracts were reduced and resumed that it was the result of interference of the extract with normal protein synthesis mechanism[10]. After treatment with methanol extract of *Az. indica*, protein level was was lowered by 92.62%. Similarly, in *Culex* larvae, the extract induced comparatively less decline in protein amount and it was dropped 48.12% below control level. Neem extract contains azadirachtin that has been known to affect protein amount and expression. For instance, azadirachtin have been known to interfere with protein synthesis in *Schistocerca gregaria*[23] and *Spodoptera litura (S. litura)*[24]. Further, it is reported that protein expression in *S. litura* was significantly lowered under azadirachtin treatment[25]. The diminution of protein

profile is probably due to structural deformities produced in *Anopheles* larvae when they are exposed to methanol extract of *Az. indica*[7]. Likewise, Sharma *et al* reported that *Culex* larvae also damaged body wall and larval tissues[9]. Body wall made of chitin, a protein, and other protenious tissues were destroyed in both the species resulting in over all decrease in protein levels of the larvae. Qadri *et al* reported the decreased protein content of the pesticide treated *Periplaneta americana*[26]. Rao *et al* found disturbance in the hormone that regulate protein synthesis due to azadirachtin in *Schistocerca gregaria*[27].

It is concluded that the impacting factors of extracts on carbohydrate, lipid and protein contents in treated larvae are species and specific extraction. The lowering of these biochemical components indicates that these extracts can lowered the feeding and proper digestion of food. They further interrupt with protein synthesis hormones resulting in its decline. In contrast, the increase in certain profiles demonstrates the physiological stress induced by the extract and the disturbed metabolic activity of the larvae. The effect of extracts on the metabolism of treated larvae depends on their nature and probably also on the action of different phytochemicals in these extracts.

Conflict of interest statement

We declare that we have no conflict of interest.

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