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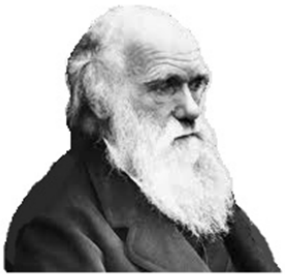

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Changes in the metabolism of freshwater snail *Lymnaea acuminata*, due to aqueous extract of stem bark of molluscicidal plant *Euphorbia tirucalli*

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ABSTRACT

The aim of the present study is to evaluate the potent molluscicidal activity of aqueous stem bark extracts of plant *Euphorbia tirucalli* (Family: Euphorbiaceae). *Lymnaea acuminata* were exposed for 24 hours to sublethal doses of crude aqueous stem bark extracts of *Euphorbia tirucalli*, i.e. 95.67 µM and 191.35 µM (40% and 80% of 24h LC_{50} of *L. acuminata*) respectively, and then were switched to extract-free water to determine the effects of withdrawal from treatment. For the following seven days, this water was replaced every 24 hours. Following that, biochemical parameters were assessed in various snail tissues which showed significant ($P < 0.05$) alteration in the carbohydrates and nitrogenous metabolisms in time and dose dependent manner. The snail tissues recovered in part after 7days completely after withdrawl of toxic aqueous extracts. The aqueous extracts of stem bark of *Euphorbia tirucalli* may be used as a potent source of molluscicides; being less expensive, easily available, easily soluble in water.

***Keywords*:** Snail, fascioliasis, Metabolism, Enzyme Activity

1. INTRODUCTION

Snails belong to the phylum Molluscs that comprise the second largest group of invertebrates. Many aquatic snails are well known carriers or vectors of larvae of trematodes and are thus known causing a number of diseases, among which fascioliasis ans schistosomiasis

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are responsible for causing immense harm to man and his domestic animals. For *Fasciola hepatica* and *Fasciola gigantica, Lymnaea acuminata* and *Indoplanorbis exustus* serve as vectors [1]. This snail is the intermediate host of *Fasciola hepatica* which causes endemic fascioliasis in cattle and sheep in Northern section of India. Snail-borne parasitic diseases are serious parasitic infections that continue to be a major public health concern around the world, particularly in impoverished areas. Snails are the transmission vectors and intermediate hosts for a number of parasitic diseases that have affected millions of humans in about 90 different countries. Thus, an alternative approach to prevent the transmission of snail-borne parasitic diseases can be to focus on the elimination or control of snails. Synthetic chemicals biodegrade slowly, and preliminary evidence suggests that some populations of snail hosts may have developed resistance to them. The hazardous nature of synthetic pesticides has prompted the scientists to find less disruptive, newer techniques in controlling pests. Heavy use of synthetic pesticides have caused high rate of toxicity levels in water bodies, owing to their bioaccumulation and long time persistent nature. Plant molluscicides are currently receiving more attention from national and international institutions in the hopes that they will prove to be less expensive and more readily available than synthetic chemicals. As a result, plant molluscicide research has become multidisciplinary.

Plant molluscicides have been studied since the 1930s, when Archibald and Wagner proposed for the planting of the desert palms *Balanites aegyptiaca* and *B. maughamii* along the Sudan's and Southern Africa's waterways, respectively [2]. Through cytotoxicity and molluscicidal activity assays, the leaves and stem-bark of *Cassia renigera* were assessed against *Lymnaea acuminata* Lamarck adults. All other leaf extracts were shown to be effective besides the Petroleum ether extract [3]. In another research, the molluscicidal activity of the essential oils of *Cymbopogon nervatus* and *Boswellia papyrifera* was evaluated and confirmed against the snail *Lymnaea acuminata*, as plant origin molluscicides [4].

Frequently grown as a hedge or decorative plant, *E. tirucalli* is most likely the most well-known and widely distributed. The medicinal folklore literature of several regions of the world (particularly tropical and subtropical countries where it is endemic) is polluted with *E. tirucalli's* healing properties, possibly because of the wide variety of chemical compounds contained in its tissues. The aim of the present study was to report the effect of sub lethal exposure of aqueous extracts of crude extracts of stem bark of the plant *Euphorbia tirucalli* on the carbohydrate and nitrogenous metabolism as well as *on the metabolic enzyme systems* of different tissues of the target snail species *Lymnaea acuminata.*

2. MATERIAL AND METHODS

2. 1. Test Plant

The plants under investigation, *Euphorbia tirucalli* were collected easily, from the Botanical Garden of Deen Dayal Upadhyay Gorakhpur University, Gorakhpur, India. *Euphorbia tirucalli* (commonly called pencil cactus), belongs to the family Euphorbiacae.

2. 2. Preparation of Aqueous Extracts of Stem leaves

Fresh stem barks of *Euphorbia tirucalli* were minced with distilled water, homogenized for 5 minutes, and then centrifuged at 1000g for around 10 minutes. The molluscicidal activity of the obtained supernatant was tested.

2. 3. Test Animals

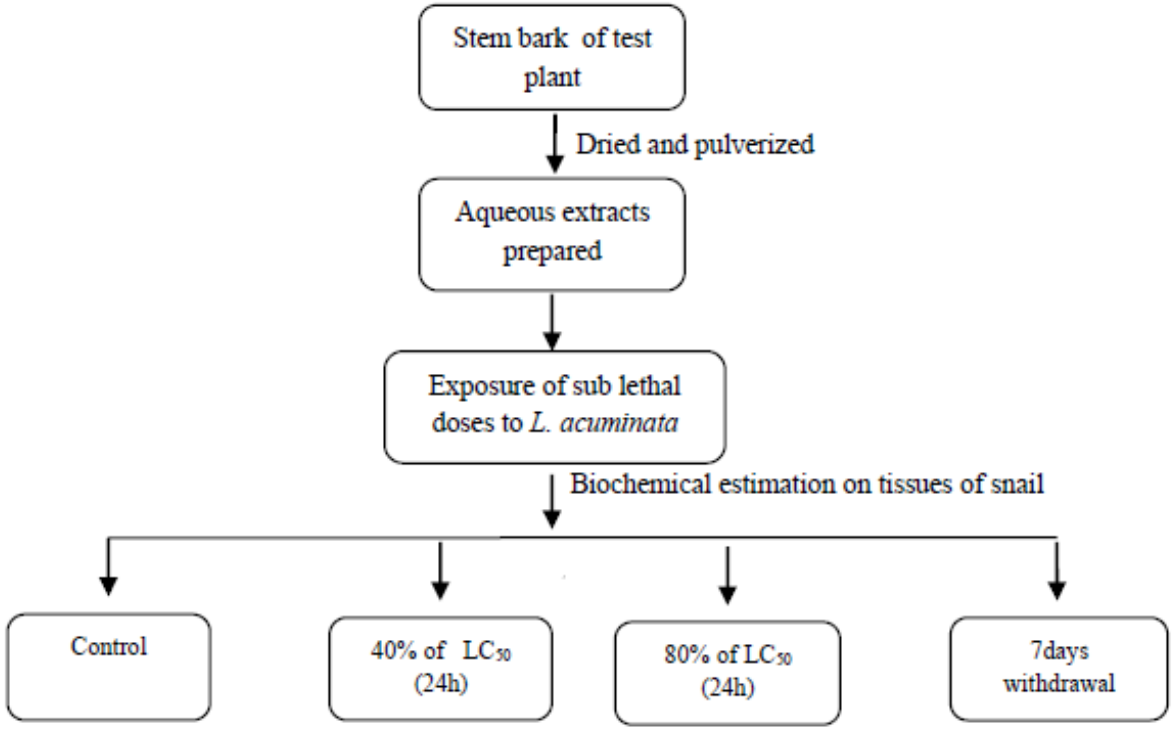
The target organisms for this research study, adult freshwater snails, *Lymnaea acuminata* (2.5 ± 0.9cm in shell height), were collected from pool alongside the campus of Veer Abdul Hameed P.G. College, Medical Road, Gorakhpur district. To acclimatise to laboratory settings, the collected creatures were maintained in glass aquariums with dechlorinated tap water. The water in the aquariums was changed for every 24 hours, Any dead animals were routinely removed to keep the water from being contaminated.

Experimental conditions

Experimental conditions of water were calculated using APHA/WPCF method. Accordingly, the parameters and their values determined were as follows:

Atmospheric temperature	27.0 - 29.0 °C
Water temperature	20.0 - 23.0 °C
pH of water	7.2 - 7.4
Dissolved Oxygen	6.9 - 7.4
Free carbon dioxide	4.6 - 6.7
Bicarbonate alkalinity	110.0 - 111.0

Treatment protocol for dose- response relationship



Lymnaea acuminata, the freshwater snail under investigation, was kept in glass aquaria containing 3L dechlorinated tap water. Each aquarium contained 30 experimental animals.

Lymnaea acuminata was exposed for 24 h to sublethal doses, 95.67 µM and 191.35 µM (40% and 80% of 24h LC_{50} of *L. acuminata*), of the crude aqueous stem bark extracts of *Euphorbia tirucalli*. Similar conditions, but without any treatment, were given to the control animals.

The test animals were taken out of the aquariums once the therapy was finished and given a freshwater wash. The tissues of *L. acuminata*'s ovotestis (OT), hepatopancreas (HP), and nervous systems (NT) were rapidly removed and placed in an ice tray for biochemical testing.

Lymnaea acuminata were exposed for 24 hours to sublethal doses of crude aqueous stem - bark extracts of *E. tirucalli*, i.e. 95.67 µM and 191.35µM (80% of 24 h LC_{50} of *L. acuminata*) respectively, and then were switched to extract-free water to determine the effects of withdrawal from treatment.

For the following seven days, this water was replaced every 24 hours. Following that, biochemical parameters were assessed in various snail tissues. Each experiment was replicated at least six times and the values have been expressed as means ± SE of six replicates. Student's t' test and analysis of variance were applied to locate significant changes.

3. BIOCHEMICAL ESTIMATION

Following biochemical estimations were done:

Protein: computed using the Lowry et al. (1951) technique and a standard of bovine serum albumin. In 10% TCA, homogenates (5 mg mL^{-1}, w/v) were created [5].

Total free amino acid: calculated using the Spies (1957) technique. In order to estimate amino acid content, homogenates (10 mg mL^{-1}, w/v) were produced in 95% ethanol, centrifuged at 6000 g, and then utilized [6].

Nucleic acids (DNA and RNA): calculated using the Schneider (1957) method with the reagents diphenylamine and orcinol, respectively. For estimation, homogenates (1 mg mL^{-1}, w/v) were produced. The DNA and RNA concentrations have both been given as g mg^{-1} tissue [7].

Glycogen: measured using the anthrone method developed by Van Der Vies in 1954 [8] and updated by Mahendru and Agarwal in 1982 [9] for the snail *L. acuminata*. In the current experiment, 5 mL of cold, 5% TCA were used to homogenise 50 mg of tissue. 1.0 mL of the filtrate from the homogenate's filtering was utilised for the test.

Pyruvate: based on estimates from Friedemann and Haugen (1943) [10]. In 10% TCA, homogenate (50 mg mL^{-1}, w/v) was made. Sodium pyruvate was used as the benchmark.

Lactate: calculated in accordance with Barker and Summerson (1941) [11], which Huckabee (1961) [12] amended. In 10% cold TCA, homogenate (50 mg mL^{-1}, w/v) was produced. use sodium lactate as a reference .

The activity of protease: based on an estimate from Moore and Stein (1954) [13] . Cold distilled water was used to make the homogenate (50 mg mL^{-1}, w/v), and the optical density was assessed at 570 nm. The enzyme's activity was measured in mol of tyrosine equivalents per milligramme of protein per hour .

The activities of acid and alkaline phosphatase: approximated by the Bergmeyer (1967) technique, which Singh and Agarwal (1983) [14, 15], modified. In ice-cold 0.9% saline, tissue homogenates (2% w/v) were made, and they were centrifuged at 5000 g and 0 °C for 15 min.

At 420 nm, optical density was evaluated in comparison to a simultaneously manufactured blank. The enzyme's activity was measured in terms of the amount of p-nitrophenol produced per mg of protein per minute.

The activity of lactic dehydrogenase (LDH): calculated using the Sigma Diagnostics (1984) approach. In 1 mL of 0.1 M phosphate buffer, pH 7.5, homogenates (50 mg mL^{-1}, w/v) were produced and incubated for 5 minutes in an ice bath. The enzyme's activity is given as mol of pyruvate reduced per minute per milligramme of $protein^{-1}$.

The activity of succinic dehydrogenase (SDH): using the Arrigoni and Singer (1962) [16] approach, measure. In 1 mL of 0.5 M potassium phosphate buffer, pH 7.6, homogenate (50 mg mL^{-1}, w/v) was produced for 5 min in an ice bath. At 600 nm, the optical density was determined. The enzyme's activity is given as mol of dye decreased per minute per milligramme of $protein^{-1}$.

The cytochrome oxidase activity: recorded using the Cooperstein and Lazarow (1951) [17] technique. In order to determine the enzyme activity, homogenates (50 mg mL^{-1}, w/v) were produced in 1 mL of 0.33 M phosphate buffer, pH 7.4, for 5 min in an ice bath. The enzyme activity was then expressed in arbitrary units min^{-1} mg $protein^{-1}$.

Acetylcholinesterase (AchE): calculated using the Ellman et al. (1961) technique [18]. After homogenising (50 mg mL^{-1}) in 0.1 M phosphate buffer, pH 8.0, for 5 min in an ice bath, the sample was centrifuged at 1000 g for 30 min at - 4 °C.

4. RESULT

Effect on Freshwater target snail

Data of sublethal doses of 40% and 80% of LC_{50} (95.67 µM and 191.35 µM) of aqueous stem bark extract exposure, and their recovery after 7^{th} day withdrawal experiment of treatment, to the freshwater snail *L. acuminata* are given in Tables 1 and 2. Exposure of snails to sublethal doses of aqueous stem bark extracts for 24h caused significant alterations in the nitrogenous and carbohydrate metabolism in different body tissues of the freshwater snail *L. acuminata*.

Effect of aqueous stem bark extract of *Euphorbia tirucalli* exposure and withdrawal effect on tissues of *L. acuminata.*

Total protein and nucleic acids (DNA and RNA) levels were significantly reduced, while the free amino acid level was significantly enhanced in all body tissues after exposure to sublethal doses. Acid and alkaline phosphatase activities were significantly reduced, while the protease activity was increased after exposure. Total protein levels were reduced to 36%, 37%, and 45% of controls after exposure to sublethal doses of aqueous extracts of stem of *Euphorbia tirucalli* in the nervous, hepatopancreas, and ovotestis tissue of *L. acuminata*, respectively. The DNA level was reduced to 53%, 53%, and 51% of controls after treatment in the nervous, hepatopancreas, and ovotestis tissue of *L. acuminata*, respectively. The RNA level was reduced to 51 %, 50 %, and 41% of controls after treatment with sublethal dose of 191.35 µM, respectively, in nervous, hepatopancreas, and ovotestis tissue of *L. acuminata*. Total free amino acid levels were induced to 109%, 106%, and 109% of controls after treatment with sublethal dose 191.35 µM of aqueous stem – extract of *Euphorbia tirucalli*, respectively, in the nervous, hepatopancreas, and ovotestis tissue of *L. acuminata*.

Table 1. Changes in total protein, total free amino acid, and nucleic acid (DNA and RNA) levels (μmg^{-1}), level activities of protease (μmol of tyrosine equivalents mg protein^{-1} h^{-1}) and acid and alkaline phosphatase (amount of *p*-nitrophenol formed from (30) min^{-1} mg protein-^{1}) in muscle, liver and gonad tissues of *Channa punctatus* tissues after exposure to sublethal doses of (40)% and(80)% of LC_{50} of aqueous stem bark extract of *Euphorbia tirucalli* for 24h and recovery after 7th day of withdrawal experiment of treatment.

Parameter	Tissue	Control	40% of LC_{50} (24h)	80% of LC_{50} (24h)	7th day of withdrawal
Protein	Muscle	98.53±0.012 (100)	68.97±0.011 (70)	56.84±0.520 (58)	91.63±0.018 (93)
	Liver	79.12± 0.001 (100)	53.80±0.068 (68)	47.47±0.610 (60)	74.37±0.045 (94)
	Gonad	84.28± 0.020 (100)	59.83±0.072 (71)	53.93±0.480 (64)	80.91±0.031 (96)
Amino acid	Muscle	36.28± 0.200 (100)	38.81±0.800 (107)	42.44±0.800 (117)	36.64±0.003^{+} (101)
	Liver	32.45± 0.010 (100)	36.02±0.710 (111)	38.61±0.520 (119)	33.74±0.008^{+} (104)
	Gonad	38.80± 0.180 (100)	40.74±0.540 (105)	44.23±0.610 (114)	39.58±0.180 (102)
DNA	Muscle	34.10±0.020 (100)	24.55±0.080 (72)	23.12±0.080 (68)	31.03±0.160 (91)
	Liver	30.62± 0.001 (100)	22.35±0.020 (73)	19.90±0.070 (65)	27.56±0.175 (90)
	Gonad	39.18± 0.003 (100)	28.63±0.010 (75)	25.14±0.100 (66)	35.51±0.008^{+} (93)
RNA	Muscle	33.31± 0.220 (100)	20.98±0.280 (63)	18.65±0.061 (56)	31.65±0.008^{+} (95)
	Liver	29.62± 0.180 (100)	19.84±0.260 (67)	15.71±0.071 (59)	27.25±0.007^{+} (92)
	Gonad	35.48± 0.130 (100)	21.64±0.310 (61)	20.57±0.008^{+} (58)	33.71±0.081 (95)
Protease	Muscle	1.61± 0.021 (100)	1.86±0.008^{+} (116)	1.93±0.004 (120)	1.62±0.008^{+} (101)
	Liver	1.10± 0.002 (100)	1.29±0.006 $^{+}$ (118)	1.34±0.018 (122)	1.16±0.008^{+} (106)
	Gonad	1.00± 0.016 (100)	1.20±0.004^{+} (120)	1.24±0.002^{+} (124)	1.07±0.003 (107)
Acid phosphatase	Muscle	1.62± 0.11 (100)	1.10±0.081 (68)	0.90±0.006^{+} (56)	1.51±0.016 (93)
	Liver	1.12± 0.019 (100)	0.79±0.093 (71)	0.76±0.070 (68)	1.08±0.018 (97)
	Gonad	1.89± 0.15 (100)	1.30±0.046 (69)	1.13 ±0.008^{+} (60)	1.85±0.029 (98)
Alkaline phosphatase	Muscle	2.02± 0.008 (100)	1.39±0.008^{+} (69)	1.17±0.003 (58)	1.94±0.063 (96)
	Liver	1.41± 0.003 (100)	0.96±0.004^{+} (68)	0.81±0.031 (57)	1.33±0.071 (94)
	Gonad	1.98± 0.004 (100)	1.33±0.002^{+} (67)	1.11±0.160 (56)	1.92± 0.081 (97)

Significant (P<0.05) Student's 't' test was applied between treated groups and withdrawal group. Values are mean ± SE of six replicas. Values in parenthesis are percent change with control taken as 100%.

Table 2. Changes in glycogen (mg g^{-1}), pyruvate (µmol g^{-1}), lactate (mg g^{-1}), activities of LDH (µmol pyruvate reduced min^{-1} mg $protein^{-1}$) and SDH (µmol of dye reduced min^{-1} mg $protein^{-1}$), cytochrome oxidase (arbitrary units min^{-1} mg $protein^{-1}$), AChE (µmol of sulfohydryl min^{-1} mg $protein^{-1}$) in muscle, liver and gonad tissues of *Channa punctatus* after exposure to sublethal doses of (40)% and (80)% of LC_{50} of aqueous stem bark extract of *Euphorbia tirucalli* for 24h and recovery after 7th day of withdrawal experiment of treatment.

Parameter	Tissue	Control (100%)	40% of LC_{50} (24h)	80% of LC_{50} (24h)	7th day of withdrawal
Glycogen	Muscle	10.54±0.156 (100)	7.80±0.150 (74)	7.37±0.621 (70)	9.49±0.161 (90)
	Liver	9.40± 0.120 (100)	7.14±0.760 (76)	6.21±0.530 (69)	8.84±0.100 (94)
	Gonad	9.56± 0.008 (100)	7.55±0.340 (79)	6.50±0.120 (68)	9.17±0.090 (96)
Pyruvate	Muscle	4.97± 0.120 (100)	2.98±0.640 (60)	2.59±0.780 (52)	4.72±0.98 (95)
	Liver	4.60 ± 0.012 (100)	2.89±0.320 (63)	2.53±0.120 (55)	4.51±0.150 (98)
	Gonad	3.98± 0.015 (100)	2.34±0.620 (59)	2.02±0.310 (51)	3.72±0.162 (93)
Lactate	Muscle	3.60± 0.710 (100)	3.92±0.180 (109)	3.67±0.161 (102)	3.38±0.100 (94)
	Liver	2.97± 0.010 (100)	3.38±0.190 (114)	3.44±0.180 (116)	2.92±0.009+ (98)
	Gonad	3.12± 0.016 (100)	3.43±0.100 (110)	3.71±0.560 (119)	2.99±0.004+ (93)
LDH	Muscle	46.50± 0.360 (100)	37.20±0.610 (80)	35.81±0.004+ (77)	43.25±0.863 (93)
	Liver	41.89± 0.810 (100)	33.93±0.310 (81)	30.57±0.003+ (73)	40.21±0.511 (96)
	Gonad	55.62± 0.750 (100)	43.93±0.320 (79)	37.26±0.007+ (67)	53.95±0.418 (97)
SDH	Muscle	61.00± 0.120 (100)	67.71±0.812 (111)	70.15±0.064 (115)	61.61±0.141 (101)
	Liver	65.18± 0.130 (100)	70.39±0.801 (108)	76.91±0.032 (118)	67.78±0.103 (104)
	Gonad	58.12± 1.61 (100)	62.18±0.197 (107)	66.83±0.018 (115)	61.03±0.810 (105)
Cytochrome oxidase	Muscle	14.21± 0.140 (100)	9.23±0.181 (65)	8.38±0.008+ (59)	13.64±0.160 (96)
	Liver	12.01± 0.120 (100)	8.04±0.096 (67)	6.97±0.010 (58)	11.65±0.153 (97)
	Gonad	16.11± 0.006 (100)	10.31±0.011 (64)	8.86±0.016 (55)	15.14±0.216 (94)

AChE	Muscle	0.98±0.072 (100)	0.66±0.016 (68)	0.58±0.063 (60)	0.92±0.321 (94)
	Liver	0.88± 0.028 (100)	0.54±0.078 (62)	0.51±0.017 (58)	0.81±0.501 (93)
	Gonad	0.81± 0.020 (100)	0.52±0.032 (65)	0.41±0.054 (51)	0.74±0.360 (91)

Significant (P<0.05) Student's 't' test was applied between treated groups and withdrawal group. Values are mean ± SE of six replicas. Values in parenthesis are percent change with control taken as 100%.

The activity of acid phosphatase was inhibited to 51%, 54%, and 53% of controls after treatment with sublethal doses of 191.35 µM of aqueous stem extract, respectively, in the nervous, hepatopancreas, and ovotestis tissue. The activity of alkaline phosphatase was reduced to 41%, 47%, and 42% of controls after treatment with sublethal doses 191.35 µM of aqueous stem extract, respectively, in the nervous, hepatopancreas and ovotestis tissue. The protease activity was increased to 117%, 112%, and 118% of controls after treatment with sublethal doses of of 191.35 µM of aqueous stem extract, respectively, in the nervous, hepatopancreas, and ovotestis tissue of *L. acuminata*. Glycogen and pyruvate levels were signifi cantly reduced, while the lactate level was significantly enhanced after exposure to sublethal doses in all body tissues. Lactic dehydrogenase (LDH), cytochrome oxidase, and acetylcholinesterase (AChE) activities were significantl reduced, while the succinic dehydrogenase (SDH) activity was increased after exposure. The glycogen level was reduced to 52%, 59%, and 61% of controls after treatment with sub-lethal doses 191.35 µM of aqueous stem – leaves extract, respectively, in the nervous, hepatopancreas, and ovotestis tissue of *L. acuminata*.

The pyruvate level was reduced to 44%, 52%, and 44% of controls after treatment with sublethal doses 214.312 µM of aqueous stem extract of respectively, in the nervous, hepatopancreas, and ovotestis tissue of *L. acuminata*. The lactate level was increased to 110%, 112%, and 109% of controls after treatment with sublethal doses of 191.35 µM of aqueous stem extract, respectively, in the nervous, hepatopancreas, and ovotestis tissue of *L. acuminata*. The lactic dehydrogenase activity was reduced to 41%, 43%, and 45% of controls after treatment with sublethal doses of 191.35 µM of aqueous stem extract, respectively, in the nervous, hepatopancreas, and ovotestis tissue of *L. acuminata*. The activity of cytochrome oxidase was reduced to 52%, 46%, and 49% of controls after treatment with sublethal doses of 191.35 µM of aqueous stem extract, respectively, in the nervous, hepatopancreas, and ovotestis tissue of *L. acuminata*.

The acetylcholinesterase activity was reduced to 51%, 56%, and 58% of controls after treatment with sublethal doses of 191.35 µM of aqueous stem extract, respectively, in the nervous, hepatopancreas, and ovotestis tissue of *L. acuminata*. The succinic dehydrogenase activity was increased to 122%, 126%, and 122% of controls after treatment with sublethal doses of 191.35 µM of aqueous stem extract, respectively, in the nervous, hepatopancreas, and ovotestis tissue of *L. acuminata* (Table 1 and 2).

Withdrawal effect

Following recovery was found in the tissues of snail on exposure to 80%of LC_{50} for 96h, as the levels of total protein increased (94% in nervous tissue, 95% in hepatopancreas and 96% in ovotestis), while recovery in total free amino acid (101%, 102%, 101%), DNA (93%, 93%,

94%), RNA (96%. 95%, 97%), glycogen (94%, 95%, 93%), pyruvate (97%, 95%, 93%), lactate (102%, 101%, 103%), AChE (91%, 91%, 95%), Cytochrome oxidase (96%, 94%, 95%), Protease (102%, 103%, 103%), Acid phosphatase (94%, 95%, 94%), Alkaline phosphatase (91%, 97%, 94%), LDH (91%, 93%, 91%) and SDH (102%, 103%, 104%), were found in nervous tissue, hepatopancreas and ovotestis respectively, in comparison to the control.(Table I and II).

5. DISCUSSION

The reduction of the protein fraction in the various tissues of the snails may have resulted from the breakdown of those proteins and potential metabolic use of the degraded products. According to Mommensen and Walsh (1992) [19], proteins, which are the primary source of the nitrogenous metabolism, are primarily engaged in the architecture of the cell and also serve as a source of energy during extended periods of stress. Increment in the free amino acids level was the result of breakdown of protein for energy requirement and impaired incorporation of amino acids in protein synthesis.

Inhibition of DNA synthesis might affect protein as well as amino acid levels by decreasing the level of RNA in the protein synthesis machinery [20] However, in any tissue total depletion of glycogen will not occur, because it would result in the disruption of enzyme systems associated with the carbohydrate metabolism [21] (Heilmeyer et al., 1970), since the enzyme systems are associated with glycolysis and TCA cycle from a constitutive enzyme system.

Carbohydrates are the primary and immediate source of the metabolism (Arasta et al., 1996) [22]. Suggesting that, in stress conditions, carbohydrate reserves deplete to meet energy demand, thus depletion of glycogen may be due to direct utilization for energy generation, a demand caused by active moiety-induced hypoxia. The glycogenolysis seems to be the result of increased secretion of catecholamine due to stress. Higher energy demands during exposure result in a fall in pyruvate levels, which raises the probability of a switch to anaerobic dependency due to a striking decrease in oxygen consumption. The level of tissue lactic acid is known to act as an index of anaerobiosis which might be beneficial to the animal to tolerate hypoxic conditions [23] (Thoye, 1971).

The increase in lactate also suggests a shift towards anaerobiosis because of hypoxia leading to respiratory distress. Lactic dehydrogenase catalyzes the interconversions of lactic acid and pyruvic acid during anaerobic conditions. Inhibition of lactic dehydrogenase and cytochrome oxidase activity shows that aqueous extracts of stem bark of *E. tirucalli* significantly inhibits the aerobic as well as anaerobic metabolism in exposed animals.

Succinic dehydrogenase is one of the active regulatory enzymes of the TCA cycle, while inhibition of cytochrome oxidase activity supports that Euphorbiales show a profound impact on the oxidative metabolism.

Withdrawal experiments were performed to see whether biochemical alteration caused by aqueous extracts of stem bark of *Euphorbia tirucalli* would return to normal, if the treatment ends. In the various body tissues of the freshwater snail *L. acuminata*, there was a nearly complete recovery of the total protein, total free amino acid, lactate, nucleic acid (DNA and RNA), and pyruvate level. There was also a partial recovery of the glycogen level.

6. CONCLUSION

It is believed that the aqueous extracts of stem bark of *Euphorbia tirucalli* may be used as a potent source of molluscicides; being less expensive, easily available, easily soluble in water, and more safe for the non-target animals than synthetic molluscicides.

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