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EFFECTS OF EXTRACTED PLANT EXTRACTS AGAINST FRESHWATER SNAIL *LYMNAEA ACUMINATA* BODY TISSUES

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ABSTRACT
Exposure to the sub-lethal doses of (40% and 80% of LC_{50} of 24h) *Jatropha gossypifolia* latex over caused significant alteration in carbohydrate and nitrogenous metabolism in nervous, hepatopancreas and ovotestis tissues of freshwater snail *Lymnaea acuminata*. The freshwater snail *Lymnaea acuminata* also exposed to sub-lethal doses of 40% and 80% of LC_{50} of 24h of *Jatropha gossypifolia* latex+taraxerol, *Jatropha gossypifolia* latex+ellagic acids and *Jatropha gossypifolia* latex+rutin in binary combinations (1:1) exposed to the sub-lethal doses to freshwater *Lymnaea acuminata* to measure the carbohydrate and nitrogenous metabolism in snail's body tissues i.e nervous, hepatopancreas and ovotestis in time and as well as dose dependent.

KEYWORDS: Snail, metabolism, enzyme activity and compound.

INTRODUCTION
The use of pesticides has been recognized as part of agricultural practices throughout the World. Unfortunately the indiscriminate use of these pesticides to improve agricultural production and yield may have impacts on non-target organism, especially aquatic lives. The World Health Organisation (WHO, 1992).[1] reported that roughly 3 million cases of pesticides poisoning occur annually, resulting in 220,000 deaths World Wide. Many of these chemicals are mutagenic (Garaj-Vrhovac and Zaljezic, 2000;[2] Kumar et al., 2009;[3] Nwani et al., 2010).[4] linked to the development of cancers (Leiss and Savitz, 1995),[5] or may lead to the development deficits (Arbuckel and Server 1998).[6]

To the hazardous nature has prompted the scientists, to find out non-disruptive, suitable, and newer options for the control of weed and aquatic harmful pest. In recent time, the use of plant/natural pesticides has gained more popularity all over the world. These plant products are a focus of attention as a suitable alternative to synthetic pesticides due to some ideal properties such as low cost, easy availability, and biodegradability in nature (Marston and Hostettmann, 1985;[7] Verma and Dubey, 1999).[8]

The aim of this study is to measure the effects of sub-lethal doses exposure to the *Jatropha gossypifolia* latex and active compound taraxerol, rutin, and ellagic acid on different biochemical parameters of freshwater snail *Lymnaea acuminata*, which is the intermediate host of *Fasciola hepatica* and *Fasciola gigantica*, which causes endemic fascioliasis in cattle and livestock in northern part of India.

MATERIAL AND METHODS
The freshwater snails *Lymnaea acuminata* (2.6±0.3 cm in shell length) was collected from the local freshwater bodies of Gorakhpur district. The snail was found attached either to the under surface of leaves or moving around the green vegetation near the bank; they were also found floating on the surface of water.

The collected animals were stored in plastic tank containing de-chlorinated tap water for acclimatization to laboratory conditions. Experimental conditions of water were determined in the beginning of the experiments by the method of APHA (1992),[9] Water analysis for the experiment various physico-chemical parameters viz. temperature, pH, dissolved O_2, free CO_2 and total alkalinity. Water temperature ranged from 27.4°C-28.6°C. The other parameters were within the following ranges; total alkalinity 43-62 ppm, pH 6.8-7.7, dissolved oxygen 7.8-10.3 mg/L.

1.1 collection of plant materials
The plants *Jatropha gossypiifolia* is belongs to family-Euphorbiaceae was collected locally from Gorakhpur, Uttar Pradesh and specimen was identified by the plant identification laboratory, Department of Botany, DDU, Gorakhpur University Gorakhpur, India, where a voucher specimen is deposited.

1.2 Extraction of active compound taraxerol

Taraxerol was isolated from the stem bark of *Codiaeum variegatum* by the method of Chtterjee and Banerjee, 1977.[10] The stem-bark of *C. variegatum* was dried in an incubator at 37^0C and dried stem bark was powdered with the help of a mechanical device. The dried powdered stem bark (2 kg) of *C. variegatum* was extracted in soxhlet apparatus with petrol for about 70 hours and a little amount of concentrated solution was obtained. After evaporation of the solvent by vacuum pump, the isolated compound in dried form was obtained. The organic constituents present in stem bark i.e. taraxerone-2, taraxerol, taraxeryl acetate-4 and sitosterol were extracted with petrol. Taraxerol is soluble in organic solvents such as $CHCl_3$ and $CHCl_3$-MeOH. Identification of the isolated compound was further confirmed with an authentic sample of taraxerol ($C_{32}H_{48}O_9$), supplied by sigma chemical Co. U.S.A. The extracted compound was stored in an airtight desicator and used for biochemical experiments.

Rutin ($C_{27}H_{30}O_{16}$) (EC NO-205-814-1), ellagic acid ($C_{14}O_6O_8$) (4,4,5,5,6,6-Hexahydroxydiphenic acid, 2,6,2,6-dilactone) (EC NO-207-508-3), betulin ($C_{30}H_{50}O_2$) (Lup-20 (2a)-ene-3β-28-diol) (EC NO-207-475-6) supplied by Sigma Chemical Co. P.O. Box 14508 St. Louis. Mo 63178 USA 314-771-5750. Rutin is obtained from the leaf of *Croton tiglium*, Ellagic acid is found in the flower of *Euphorbia hirta* and Betulin is found in the stem bark of *Euphorbia lathyrus*, taraxerol extracted from the stem bark of *Codiaeum variegatum* and latex of *Jatropha gossypifolia* were prepared using the method of Yadav and Singh, 2006.[11] Singly and binary (1:1) combinations were prepared with lyophilized powder of *Jatropha gossypifolia* with Rutin, Ellagic acid, Taraxerol and Betulin.

1.3 Treatment protocol for Dose-Response relationship

Adult snail *Lymnaea acuminata* was collected from freshwater natural ponds and stored in glass aquaria containing de-chlorinated tap water for acclimatization in laboratory condition each aquarium contain 30 experimental animals. Group of 30 snails in each aquarium were exposed to sub-lethal doses (40% and 80% of 24h LC_{50}) of *Jatropha gossypifolia* latex and binary combinations (1:1) of *J. gossypifolia* latex+taraxerol, *J. gossypifolia* latex+ellagic acid and *J. gossypifolia* latex+rutin active compounds for 24h exposure periods. The control group snails were not exposed to any treatment. After completion of treatment the test animals were removed from aquaria, and washed with freshwater. The nervous, hepatopancreas and ovotestis tissue of snail *L. acuminata* was quickly dissected out in an ice tray and used for biochemical analysis. Since *Lymnaea acuminata* is an active grazer the possibility existed that animals would not be able to eat during the treatment period because of immobility or incapacitation of redula musculature. No food was therefore given to the control or experimental snails.

Each experiment was replicated at least six times and the values have been expressed as mean ±SE of six replicates. Student's't' test and analysis of variance were applied to locate significant changes (Sokal and Rohlf, 1973).[12]

Biochemical Estimation

Protein- Protein levels were estimated according to the method of Lowry et al., (1951),[13] using bovine serum albumin as standard. Homogenates (5 mg/mL, w/v) were prepared in 10% TCA.

Total Free Amino acids- Estimation of total free amino acid was made according to the method of Spies (1957).[14] Homogenates (10 mg/mL, w/v) were prepared in 95% ethanol, centrifuged at 6000xg and used for amino acid estimation.

Nucleic acids-Estimation of DNA and RNA was performed by method of Schneider (1957),[15] using diphenylamine and orcinol reagents, respectively. Homogenates (1 mg/mL, w/v) were prepared in 5% TCA at 90^0C, centrifuged at 5000xg for 20 min and supernatant was prepared used for estimation.

Glycogen- Glycogen was estimated by the Anthrone method of Van Der Vies (1954),[16] as modified by Mahendru and Agarwal (1982),[17] for snails. In present experiment 50 mg of tissue was homogenized with 5 mL of cold 5% TCA. The homogenate were filtered and 1.0 mL of filtrate was used for assay.

Pyruvate- Pyruvate level was measured according to Friedemann and Haugen (1943).[18] Homogenate (50 mg/mL, w/v) was prepared in 10% TCA. Sodium pyruvate was taken as standard.

Lactate-Lactate was estimated according to Barker and Summerson (1941),[19] modified by Huckabee (1961),[20] Homogenate (50 mg/ml, w/v) was prepared in 10% cold TCA. Sodium lactate was taken as standard.

Protease- Protease activity was estimated by the method of Moore and Stein (1954),[21] Homogenate (50 mg/mL, w/v) was prepared in cold distilled water.

Acid and Alkaline phosphatase- Activities of acid and alkaline phosphatase were measured by the method of Andersch and Szcypinski, (1947),[22] as modified by Bergmeyer, (1967),[23] and Singh and Agarwal, (1983),[24] Tissue homogenate (2% w/v) were prepared in ice cold 0.9% saline and centrifuged at 5000xg at 0^0C for 15 min.

Lactic dehydrogenase- Lactic dehydrogenase activity was measured according to the method of Anon (1984),[25] Homogenates (50 mg/mL, w/v) were prepared in 1 mL of 0.1M phosphate buffer, pH 7.5 for 5 min in an ice bath.

Succinic dehydrogenase- Succinic dehydrogenase activity was measured by the method of Arrigoni and Singer (1962).[26] Homogenate (50 mg/mL, w/v) was prepared in 1 mL of 0.5M potassium phosphate buffer pH 7.6 for 5 min in an ice bath.

Cytochrome oxidase- Cytochrome oxidase activity was measured according to the method of Cooperstein and Lazarow (1951).[27] Homogenates (50 mg/mL, w/v) were prepared in 1 ml of 0.33M phosphate buffer (pH 7.4) for 5 min in ice bath.

Acetylcholinesterase- Acetylcholinesterase was estimated by the method of Ellman et al. (1961),[28] as adapted by Singh and Agarwal (1982),[29] for snail tissue. Homogenates (50 mg/mL, w/v) were prepared in 0.1M phosphate buffer in ice bath.

RESULTS

Effects on nitrogenous metabolism:

Data of sub-lethal doses of 40% and 80% LC_{50} of 24h of *Jatropha gossypifolia* latex exposure to freshwater snail *L. acuminata* are given in table 1 and 2. Exposure of snails to sub-lethal doses of *Jatropha gossypifolia* latex for 24h caused significant alterations in nitrogenous and carbohydrate metabolism in different body tissues of the freshwater snail *L. acuminata*. Total protein and nucleic acids (DNA and RNA) levels were significantly reduced, while free amino acid level was significantly enhanced after the exposure to sub-lethal doses in all the body tissues. Acid and alkaline phosphatase activities were significantly reduced, while protease activity was increased after the exposure.

Total protein levels were reduced to 27%, 32% and 23% of controls after exposure to sub-lethal doses of 80% of LC_{50} 24h of *Jatropha gossypifolia* latex respectively in the nervous, hepatopancreas and ovotestis tissue of *L. acuminata*, respectively. The DNA level was reduced to 38%, 30% and 26% of controls after treatment with 80% of LC_{50} 24h of *Jatropha gossypifolia* latex in nervous, hepatopancreas and ovotestis tissue of *L. acuminata*, respectively. The RNA level was reduced to 32%, 35% and 24% of controls after treatment with sub-lethal doses of 80% of LC_{50} 24h of *Jatropha gossypifolia* latex respectively in nervous, hepatopancreas and ovotestis of *L. acuminata*. Total free amino acid levels were induced to 168%, 148% and 175% of controls after treatment with sub-lethal doses of 80% of LC_{50} 24h of *Jatropha gossypifolia* latex respectively in nervous, hepatopancreas and ovotestis of *L. acuminata* (Table 1).

Activity of acid phosphatase was inhibited to 71%, 78% and 73% of controls after treatment with sub-lethal doses of 80% of 24h LC_{50} of *Jatropha gossypifolia* latex respectively in nervous, hepatopancreas and ovotestis. The activity of alkaline phosphatase was reduced to 62%, 66% and 68% of controls after treatment with sub-lethal doses of 80% of 24h LC_{50} of *Jatropha gossypifolia* latex respectively in nervous, hepatopancreas and ovotestis. The protease activity was increased to treatment with sub-lethal doses of 80% of 24h LC_{50} of *Jatropha gossypifolia* latex respectively in the nervous, hepatopancreas and ovotestis of snail *L. acuminata* (Table 1).

Effects of carbohydrate metabolism

Glycogen and pyruvate levels were significantly reduced, while lactate level was significantly enhanced after the exposure to sub-lethal doses in all the body tissues (Nervous, hepatopancreas and ovotestis). Lactic dehydrogenase (LDH), cytochrome oxidase and acetylcholinesterase (AChE) activities were significantly reduced, while succinic dehydrogenase (SDH) activity was increased after the exposure.

Glycogen level was reduced to 31%, 36% and 30% of controls after treatment with sub-lethal doses of 80% of 24h LC_{50} *Jatropha gossypifolia* latex respectively in nervous, hepatopancreas and ovotestis tissues of *L. acuminata*. Pyruvate level was reduced to 28%, 38% and 26% of controls after treatment with sub-lethal doses of 80% of 24h LC_{50} *Jatropha gossypifolia* latex respectively in nervous, hepatopancreas and ovotestis tissues of *L. acuminata*. Lactate level was increased to 171%, 185% and 173% of controls after treatment with sub-lethal doses of 80% of 24h LC_{50} of *Jatropha gossypifolia* latex respectively in nervous, hepatopancreas and ovotestis tissues of snail *L. acuminata* (Table 2).

Lactic dehydrogenase activity was reduced up to 50%, 57% and 49% of controls after treatment with sub-lethal doses of 80% of 24h LC_{50} of *Jatropha gossypifolia* latex respectively in nervous, hepatpancreas and ovotestis tissue of snail *L. acuminata*. Activity of cytochrome oxidase was reduced to 52%, 59% and 55% of controls after treatment with sub-lethal doses of 80% of 24h LC_{50} of *Jatropha gossypifolia* latex respectively in nervous, hepatopancreas and ovotestis of snail *L. acuminata*. Acetylcholinesterase activity was reduced to 58%, 41% and 43% of controls after treatment with sub-lethal doses of 80% of 24h LC_{50} of *Jatropha gossypifolia* latex respectively in nervous, hepatopancreas and ovotestis of snail *Lymnaea acuminata*. The succinic dehydrogenase (SDH) activity was increased to 163%, 158% and 160% of controls after treatment with sub-lethal doses 0f 80% of 24h LC_{50} of *Jatropha gossypofolia* latex respectively in nervous, hepatopancreas and ovotestis tissues of freshwater snail *Lymnaea acuminata* (Table 2).

Data of sub-lethal doses of 40% and 80% of LC_{50} of 24h of binary combination of *J. gossypifolia* latex+taraxerol, *J. gossypifolia* latex+ellagic acid and *J. gossypifolia* latex+rutin exposed to snail *Lymnaea acuminata* are given in Fig 1-6. Exposure of snail to sub-lethal doses of *J. gosssypifolia* latex+taraxerol, *J. gosssypifolia* latex+ellagic acid and *J. gossypifolia* latex+rutin for 24h caused significant alteration in nitrogenous and carbohydrate metabolism in different body tissue of the

freshwater snail *L. acuminata*. Total protein and nucleic acid (DNA & RNA) levels were significantly reduced while free amino acid was significantly enhanced after the exposure to sub-lethal doses in all the body tissues. Acid and alkaline phosphatase activities were significantly reduced, while protease activity was increased after the exposure (Fig 1-6).

Table 1: Changes in total protein, total free amino acids, nucleic acid (DNA & RNA) (µg/mg), protease (µmole of tyrosine equivalents/mg protein/h) and acid and alkaline phosphatase (µmole substrate hydrolysed/30 min/mg protein) level in different tissues of *Lymnaea acuminata* after exposure to 40% and 80% of LC_{50} of *Jatropha gossypifolia* latex extracts after 24h.

	Tissues	Control	40% of LC_{50}	80% of LC_{50}
Protein	Nervous	62.50±0.470 (100)	31.87±0.21^{+} (51)	16.87±0.02^{+} (27)
	Hepatopancreas	63.16±0.660 (100)	31.58±0.81^{+} (50)	20.21±0.76^{+} (32)
	Ovotestis	64.00±1.290 (100)	29.44±0.86^{+} (46)	14.72±0.81^{+} (23)
Amino acids	Nervous	27.83±0.052 (100)	44.52±1.01^{+} (160)	43.39±0.21^{+} (168)
	Hepatopancreas	25.83±1.485 (100)	33.06±0.34^{+} (128)	38.22±1.04^{+} (148)
	Ovotestis	38.83±0.772 (100)	63.29±0.13^{+} (163)	67.95±0.12^{+} (175)
DNA	Nervous	74.83±0.660 (100)	42.63±0.26^{+} (57)	28.43±0.16^{+} (38)
	Hepatopancreas	71.66±0.123 (100)	45.18±0.28^{+} (63)	21.49±0.23^{+} (30)
	Ovotestis	71.50±0.524 (100)	40.64±0.16^{+} (52)	20.32±0.46^{+} (26)
RNA	Nervous	52.16±0.660 (100)	23.99±0.21^{+} (46)	16.69±0.29^{+} (32)
	Hepatopancreas	49.83±0.718 (100)	27.40±0.75^{+} (55)	17.44±0.21^{+} (35)
	Ovotestis	53.01±0.401 (100)	20.18±2.21^{+} (38)	12.72±0.56^{+} (24)
Protease	Nervous	0.394±0.001 (100)	0.539±0.046^{+} (137)	0.579±0.047^{+} (147)
	Hepatopancreas	0.397±0.001 (100)	0.535±0.001^{+} (135)	0.563±0.001^{+} (142)
	Ovotestis	0.459±0.002 (100)	0.638±0.003^{+} (139)	0.688±0.003^{+} (150)
Acid phosphatase	Nervous	0.182±0.001 (100)	0.160±0.005^{+} (88)	0.129±0.002^{+} (71)
	Hepatopancreas	0.249±0.002 (100)	0.229±0.007^{+} (92)	0.194±0.006^{+} (78)
	Ovotestis	0.255±0.001 (100)	0.230±0.002^{+} (90)	0.186±0.004^{+} (73)
Alkaline phosphatase	Nervous	0.389±0.001 (100)	0.295±0.001^{+} (76)	0.158±0.002^{+} (62)
	Hepatopancreas	0.392±0.002 (100)	0.290±0.002^{+} (74)	0.258±0.001^{+} (66)
	Ovotestis	0.475±0.003 (100)	0.375±0.003^{+} (79)	0.323±0.001^{+} (68)

- Values are mean ±SE of six replicates.
- Values in parenthesis are % change with control taken as 100%.
- Data were analysed through student's 't' test.
- +, P<0.05, when treated groups were compared with controls

Table 2: Changes in glycogen (mg/g), pyruvate (µmole/g), lactate (mg/g), LDH (µmole/mg protein/h), SDH (µmole of dye reduced/min/mg protein), cytochrome oxidase (arbitrary unit/min/mg protein) and AChE (µmole 'SH' hydrolysed/min/mg protein) after exposure to 40% and 80% of LC_{50} of *Jatropha gossypifolia* latex extracts in different tissues of *Lymnaea acuminata* after 24h.

	Tissues	Control	40% of LC_{50}	80% of LC_{50}
Glycogen	Nervous	6.00±0.204 (100)	2.70±0.23^{+} (45)	1.86±0.01^{+} (31)
	Hepatopancreas	6.73±0.191 (100)	3.49±0.78^{+} (52)	2.42±0.76^{+} (36)
	Ovotestis	8.20±0.111 (100)	3.61±0.66^{+} (44)	2.46±0.80^{+} (30)
Pyruvate	Nervous	0.512±0.004 (100)	0.163±0.05^{+} (32)	0.143±0.23^{+} (28)
	Hepatopancreas	0.557±0.013 (100)	0.222±0.21^{+} (40)	0.211±0.09^{+} (38)
	Ovotestis	0.532±0.011 (100)	0.154±0.13^{+} (29)	0.138±0.11^{+} (26)
Lactate	Nervous	2.995±0.028 (100)	4.732±0.17^{+} (158)	5.121±0.14^{+} (171)
	Hepatopancreas	3.618±0.057 (100)	6.150±0.03^{+} (170)	6.693±0.01^{+} (185)
	Ovotestis	3.858±0.026 (100)	6.170±0.71^{+} (160)	6.674±0.06^{+} (173)
LDH	Nervous	0.082±0.008 (100)	0.063±0.001^{+} (78)	0.041±0.001^{+} (50)
	Hepatopancreas	0.085±0.001 (100)	0.069±0.004^{+} (82)	0.048±0.005^{+} (57)
	Ovotestis	0.083±0.006 (100)	0.067±0.001^{+} (81)	0.040±0.003^{+} (49)
SDH	Nervous	29.17±0.440 (100)	36.17±0.17^{+} (124)	47.54±0.34^{+} (163)
	Hepatopancreas	40.52±0.443 (100)	48 .62±0.16^{+} (120)	64.02±0.17^{+} (158)
	Ovotestis	26.32±0.567 (100)	32.90±0.26^{+} (125)	42.11±0.28^{+} (160)
Cytochrome oxidase	Nervous	18.43±0.263 (100)	11.24±0.128^{+} (61)	9.58±0.004^{+} (52)
	Hepatopancreas	15.93±0.014 (100)	11.78±0.007^{+} (66)	10.53±0.006^{+} (59)

	Ovotestis	17.02±0.016 (100)	10.89±0.006^{+} (64)	9.36±0.004^{+} (55)
AChE	Nervous	0.062±0.005 (100)	0.037±0.002^{+} (61)	0.035±0.003^{+} (58)
	Hepatopancreas	0.082±0.005 (100)	0.053±0.001^{+} (65)	0.033±0.001^{+} (41)
	Ovotestis	0.068±0.003 (100)	0.042±0.002^{+} (62)	0.029±0.004^{+} (43)

- Values are mean ±SE of six replicates.
- Values in parenthesis are % change with control taken as 100%.
- Data were analysed through student's 't' test.
- +, P<0.05, when treated groups were compared with controls

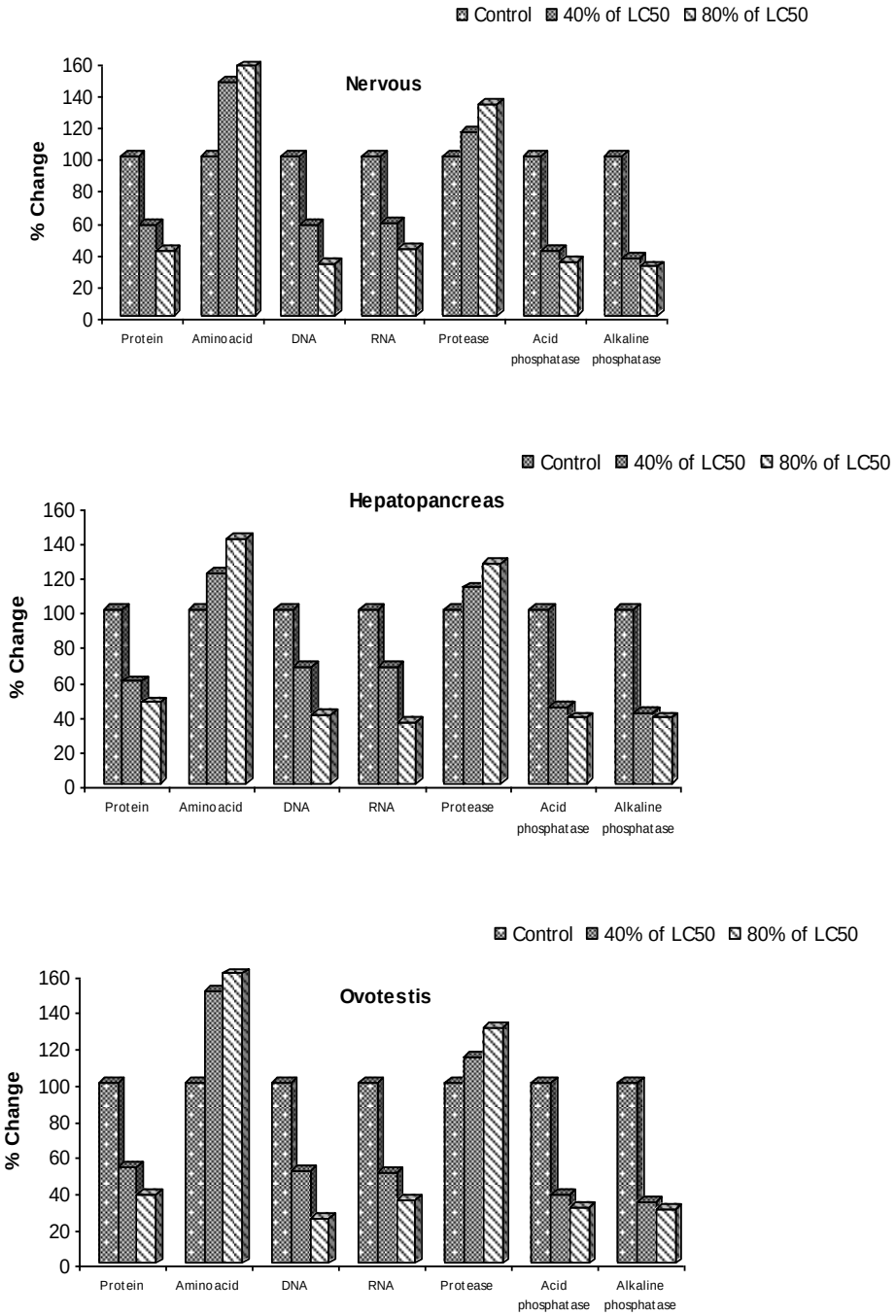

Fig. 1: Bar diagram showing percent change in the level of protein, free amino acids, nucleic acids (DNA & RNA), protease, acid and alkaline phosphatase in the nervous, hepatopancreas and ovotestis tissues of the freshwater snail *Lymnaea acuminata* after treatment of 40% and 80% of LC_{50} (24h) of extracts of *Jatropha gossypifolia* latex+taraxerol.

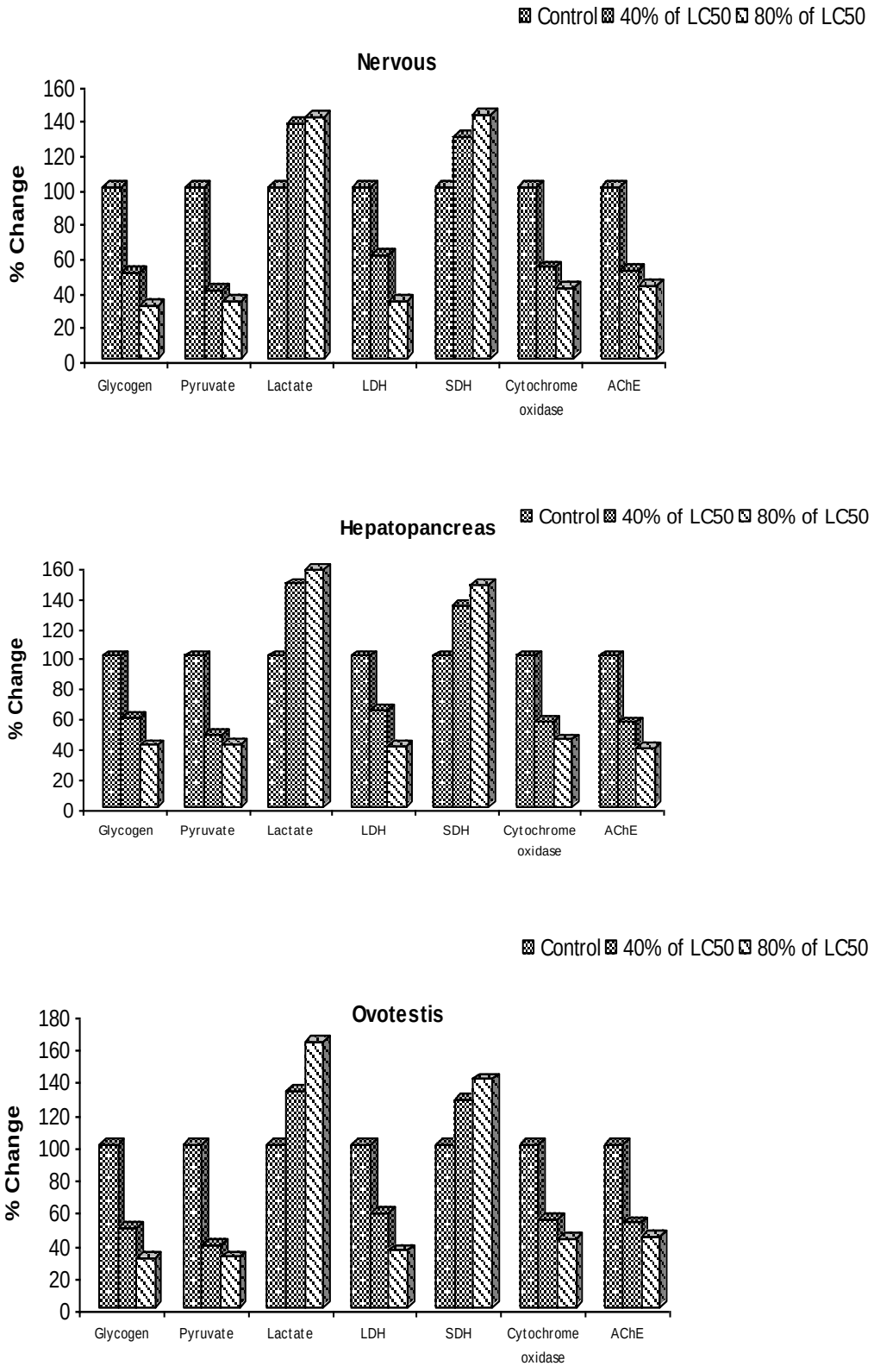

Fig 2: Bar diagram showing percent change in the level of glycogen, pyruvate, lactate, lactic dehydrogenase (LDH), succinic dehydrogenase (SDH), cytochrome oxidase and AChE in the nervous, hepatopancreas and ovotestis tissues of the freshwater snail *Lymnaea acuminata* after treatment of 40% and 80% of LC_{50} (24h) of *Jatropha gossypifolia* latex+taraxerol.

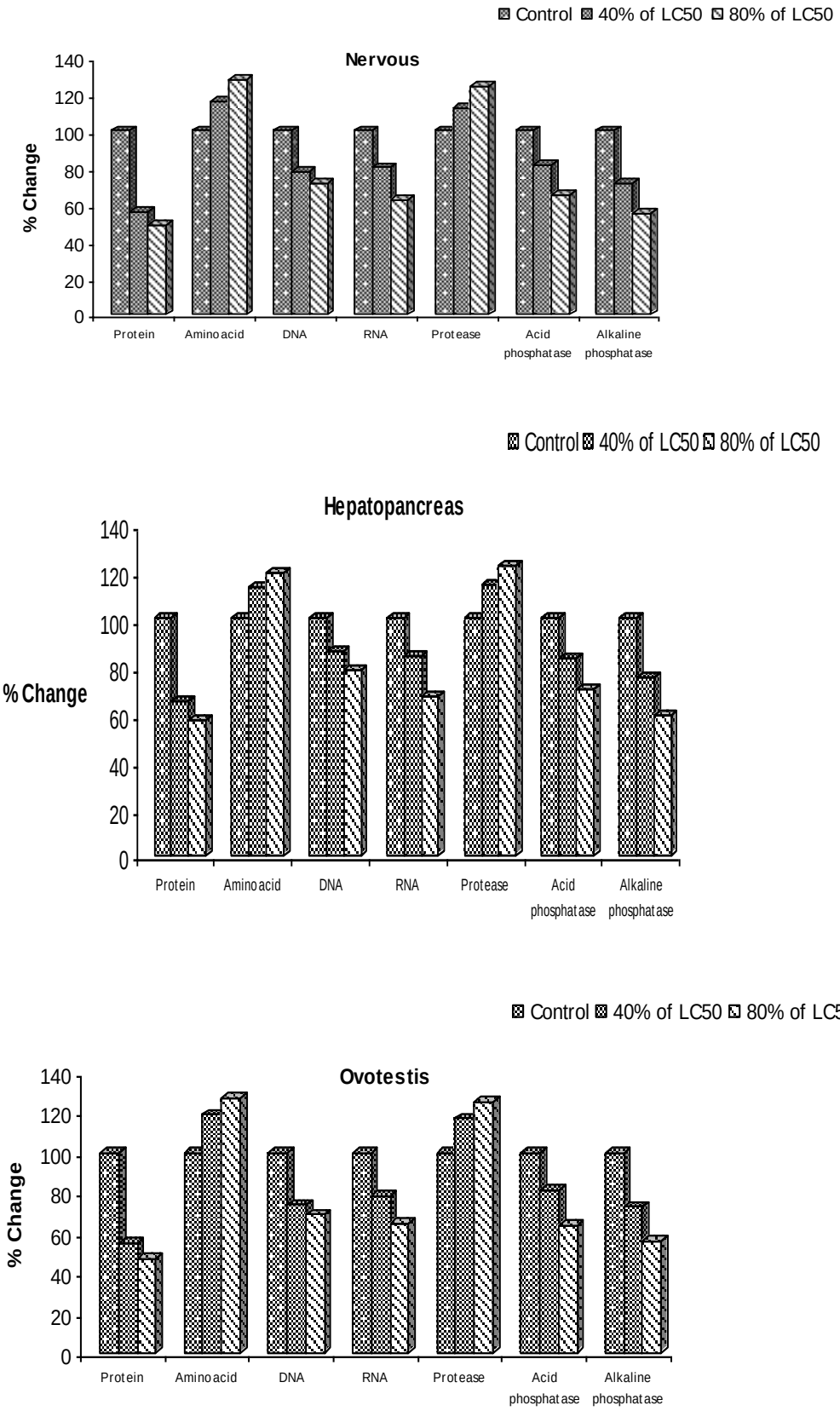

Fig. 3: Bar diagram showing percent change in the level of protein, free amino acids, nucleic acids (DNA & RNA), protease, acid and alkaline phosphatase in the nervous, hepatopancreas and ovotestis tissues of the freshwater snail *Lymnaea acuminata* after treatment of 40% and 80% of LC_{50} (24h) of extracts of *Jatropha gossypifolia* Latex+ellagic acid.

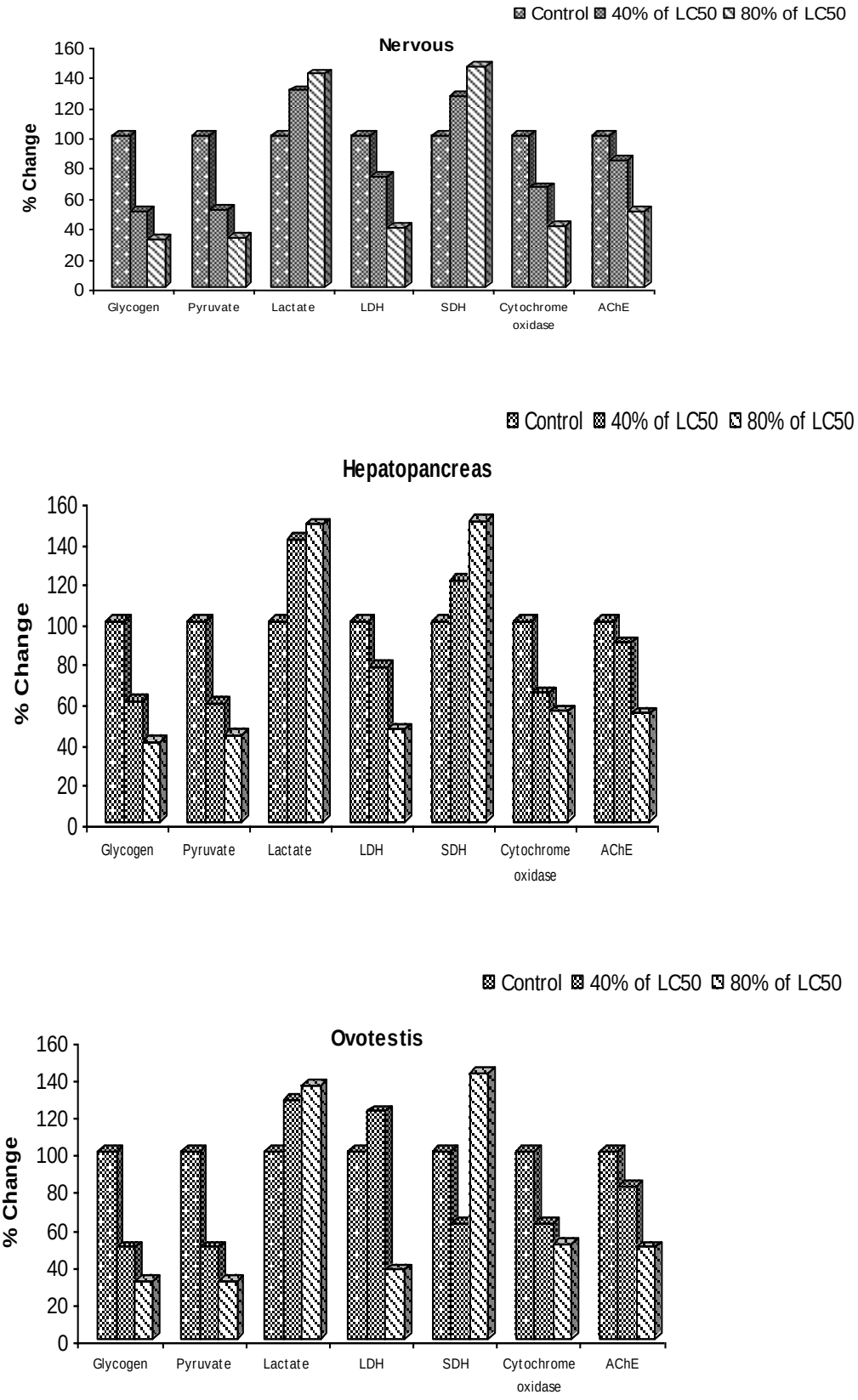

Fig. 4: Bar diagram showing percent change in the level of glycogen, pyruvate, lactate, lactic dehydrogenase (LDH), succinic dehydrogenase (SDH), cytochrome oxidase and AChE in the nervous, hepatopancreas and ovotestis tissues of the freshwater snail *Lymnaea acuminata* after treatment of 40% and 80% of LC_{50} (24h) of *Jatropha gossypifolia* latex+ellagic acid.

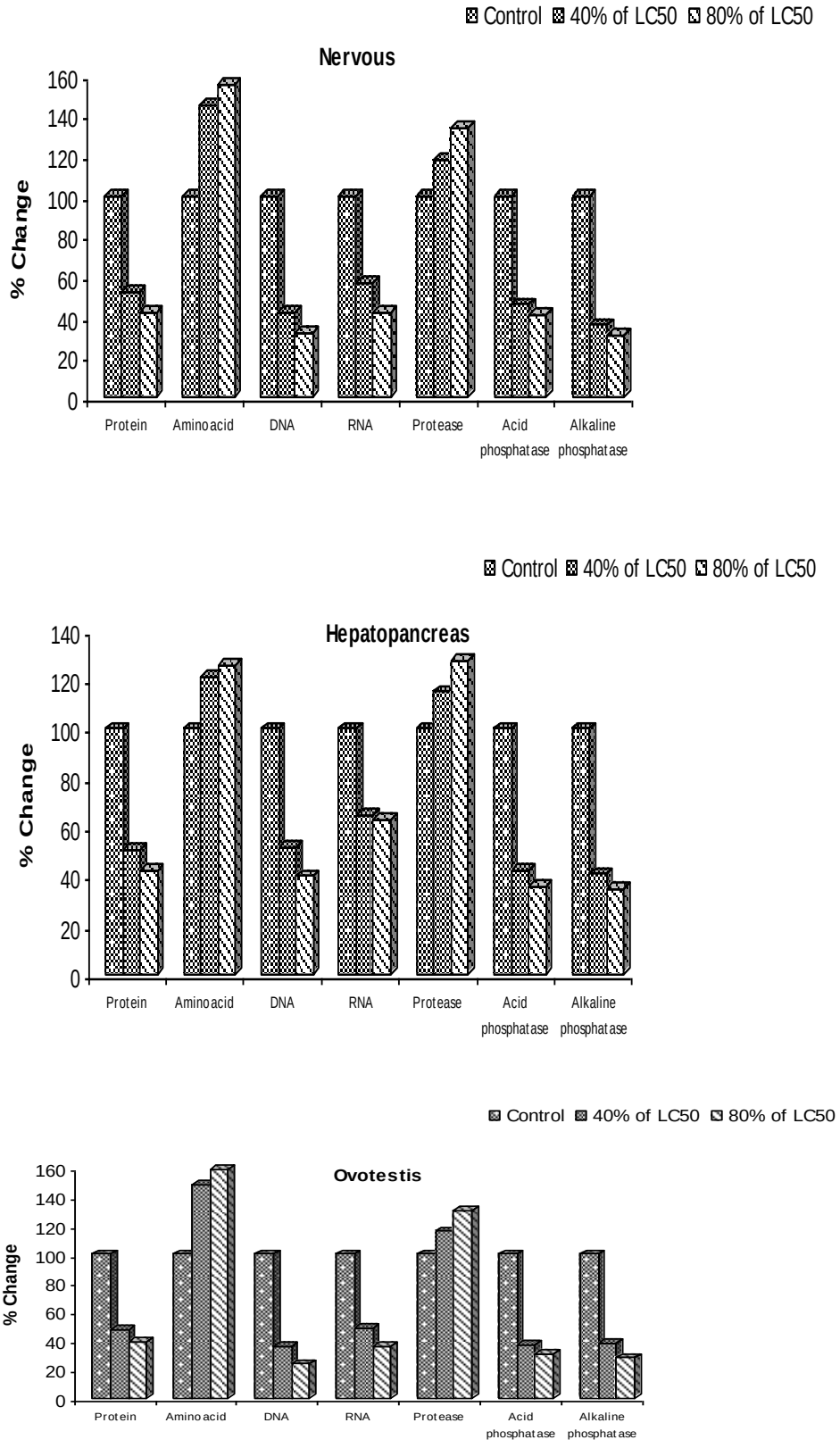

Fig. 5: Bar diagram showing percent change in the level of protein, free amino acids, nucleic acids (DNA & RNA), protease, acid and alkaline phosphatase in the nervous, hepatopancreas and ovotestis tissues of the freshwater snail *Lymnaea acuminata* after treatment of 40% and and 80% of LC_{50} (24h) of extracts of *Jatropha gossypifolia* latex+rutin.

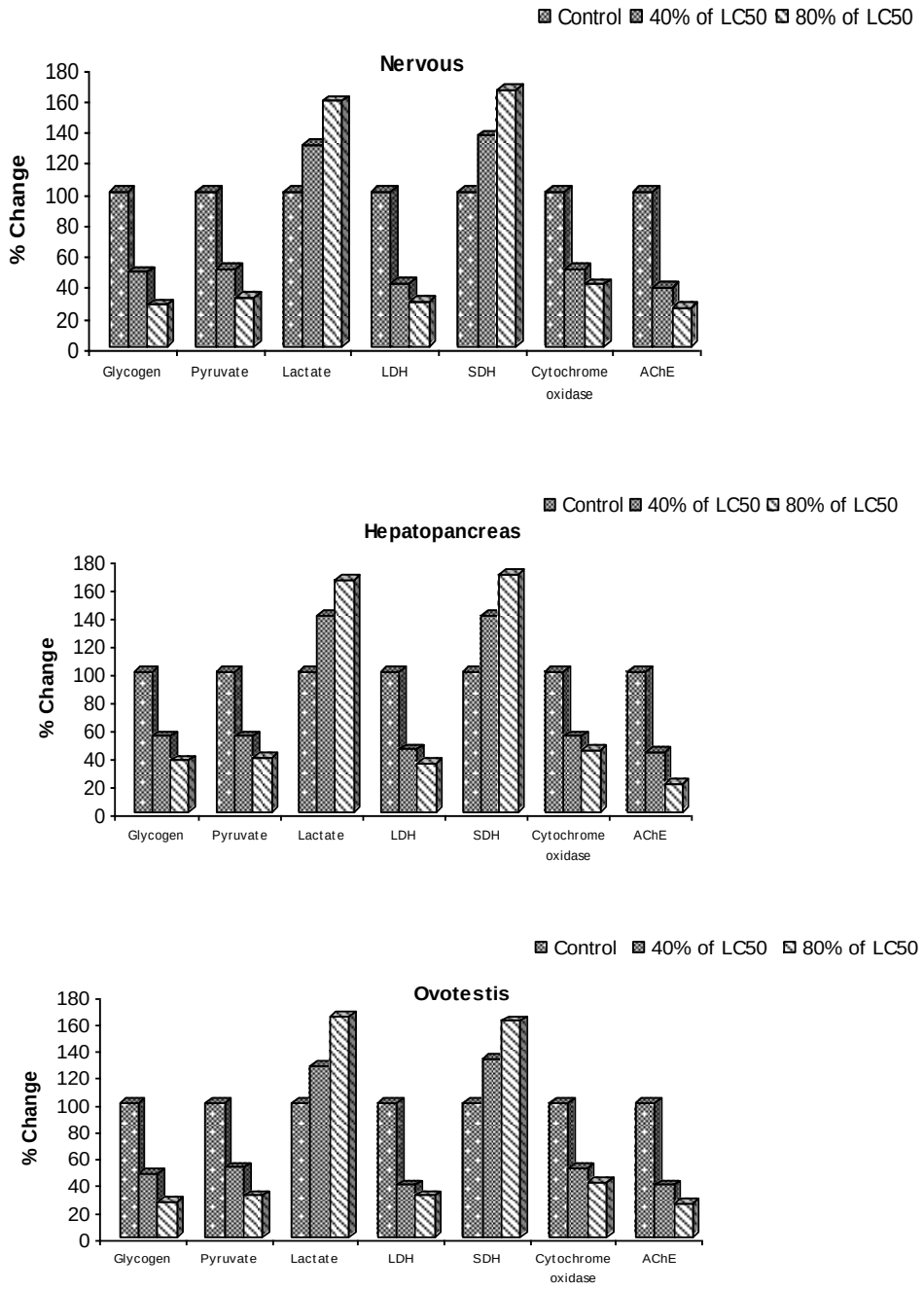

Fig. 6: Bar diagram showing percent change in the level of glycogen, pyruvate, lactate, lactic dehydrogenase (LDH), succinic dehydrogenase (SDH), cytochrome oxidase and AChE in the nervous, hepatopancreas and ovotestis tissues of the freshwater snail *Lymnaea acuminata* after treatment of 40% and 80% of LC_{50} (24h) of *Jatropha gossypifolia* latex+rutin.

DISCUSSION

It is clear from the result section, exposure to sub-lethal doses of *Jatropha gossypifolia* latex and binary combination of *J. gossypifolia* latex+taraxerol, *J. gossypifolia* latex+ellagic acid and *J. gossypifolia* latex+rutin against snail *Lymnaea acuminata* significantly altered the nitrogenous metabolism (protein, amino acid, DNA, RNA, protease and acid and alkaline phosphatase) and carbohydrate metabolism (glycogen, pyruvate, lactate, LDH, cytochrome oxidase and AChE) in nervous tissues, hepatopancreas and ovotestis of the snail *Lymnaea acuminata*. The rate of alteration in all the cases was significantly ($P \leq 0.05$) time and dose dependent

Gastropods exposed to stressful conditions generally use glycogen as their principal and immediate energy source (Goddard and Martin 1966).[30] Protein is a stored energy source during chronic period of stress. Animals exposed to sub-lethal concentrations of toxicant experience stress

during the process of detoxification. The metabolic rates of the fish *Mystus vittatus* (Bloch, 1797) reared at different concentration of toxicant can be greater than that of animals reared in freshwater (Aranachalem *et al.* 1980).[31] The depletion of protein fraction in nervous tissue, hepatopancreas, and ovotestis of *Lymnaea acuminata* may have been due to their degradation and possible utilization of degraded products for metabolic purposes. The increase in the level of free amino acids was probably the result of the breakdown of protein for energy requirements and impaired incorporation of amino acids in protein synthesis, but it also could be attributed to the reduced use of amino acids and their involvement in the maintenance of an acid-base balance. Stress conditions induce the transamination pathway. Inhibition of DNA synthesis might affect levels of protein and amino acids by decreasing the level of RNA in the machinery for protein synthesis (Nordenkjold *et al*. 1979).[32] Euphorbiales are potential inhibitors (Singh *et al*. 1996).[33] of DNA synthesis, causing in a reduction of RNA levels and consequently affecting protein synthesis and amino acid levels, as shown by our results.

The enzyme protease functions in hydrolysing proteins to free amino acids and small peptides. The increase in the protease activity corroborates with the enhancement in the level of free amino acids in the three tissues, the formation of which might be the result of protein hydrolysis, suggesting stimulation during toxic stress. A similar trend in protease activity has been reported by several workers in different animals (*Tilapia mossambica* (Peters), *Pila globosa* (Swaimson) including mammal (Millward, 1970;[34] Siva Prasada Rao, 1980;[35] Sivaiah, 1980;[36] Kabeer Ahammad Sahib *et al*. 1984).[37] The enzyme aminotransaminase provides a link between carbohydrate and protein metabolism because they interconvert metabolites such as α-ketogluterate, pyruvate, and oxaloacetate on the one hand and alanine, aspartate and glutamate on the other hand. Aminotransaminase plays an important role in the utilization of amino acids for the oxidation and/or for gluconeogenesis. An increase in aminotransaminase activity indicated the utilization of amino acids for the formation of oxaloacetate, α-ketogluterate, and pyruvate to meet energy demands. This is supported by the observation of Malla Reddy and Bashamohiden (1995),[38] in the metabolism of selected tissues of the fish *Cyprinus caprio* exposed to sub-lethal concentrations of the toxicant cypermethrin. Because the plant used in this study may have some antiphosphatase activity, the reduction in protein level may be due to the inhibition of alkaline phosphatase activity as the latter plays an important role in protein synthesis (Pilo *et al.* 1972).[39]

Anticholinesterase compounds are known to inhibit mitochondrial cytochrome oxidase, which is a terminal enzyme of the electron transport chain. Inhibition of cytochrome oxidase by plant moieties indicates that euphorbs might have a profound impact on oxidative metabolism. Decrease in cytochrome oxidase might either be the result of reduced availability of oxygen, which in turn reduces the capacity of the electron transport system to produce ATP or could be due to the direct impact of the active moiety, such as the function of cytochrome oxidase in the electron transport system (Sambasiva Rao 1999).[40] Because euphorbs are anticholinesterase inhibitors, they may affect the Kreb's cycle by diminishing the rate of the electron transport system and oxidative phosphorylation, reducing the synthesis of ATP.

Reduction of glycogen level is believed to result from the greater stress the organs experienced during the detoxification of active moieties and their metabolites. Euphorbiales inhibit acetylcholinesterase activity, which results in an increase of acetylcholinesterase convert (Singh *et al.* 1996).[33] An increased level of acetylcholine has been shown to enhance the secretion of catacholine (Nilsson *et al*. 1976),[41] which may bring about glucogenolysis. Thus glycogenolysis seems to be the result of increased secretion of catecholine due to stress (Singh and Agarwal 1993).[42]

The increase in lactate also suggests a shift towards anaerobiosis as a consequence of hypoxia leading to respiratory distress (Siva Prasada Rao 1980).[35] Development of such internal hypoxic conditions may ultimately responsible for the shift to the less efficient anaerobic metabolism, evidenced by the change in lactate content observed during this study.

This may encourage the use of active compound molluscicides. Bioactive products of plant origin have become the focus of attention because they are less expensive and less hazardous to the environment than the synthetic counterparts. The need to find methods of control which are both effective and environmentally safe should be considered.

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