CHAPTER IV

RESULTS

Relative organ body weight ratioin rats after feeding with various concentrations of Trikatu

Analysis of organ weight in this study is an important endpoint for identification of potentially harmful effects of chemicals. Differences in organ weight among treatment groups are often accompanied with differences in body weight among these groups, making interpretation of organ weight differences more difficult. The relationship between organ weight and body weight was evaluated to determine which endpoint (organ weight and organ-to-body weight ratio) is likely to detect accurately drug target organ.

Tables 6 and 7 show the average daily body weights of rats in both experimental groups. In the acute group, 500 mg/kg Trikatu had no effect on the body weight but a significant decrease of body weight ($p<0.001$) was found in rats treated with 1,000 mg/kg Trikatu.In the sub-acute group, no change in body weight was observed in 50 and 150 mg/kg Trikatu treated male rats compared to controls.

The effect of Trikatu on weight of rat vital organs was shown in Tables 6 and 7. Liver weight was markedly increased inrats fed with high dose of Trikatu. The rats fed with 500 and 1,000 mg/kg Trikatu showed a significant increase in liver weight (14.75 and 35.1%, respectively) when compared to the control. A significant decrease of spleen weight was also found in 1,000 mg/kg Trikatu fed rats. In the sub-acute group, rats treated with 150 mg/kg Trikatu showed a significant increase in liver weight (24.22%), but rats treated with 50 mg/kg showed no change. Other organs weight in sub-acute group did not show any significant difference compared to the control.

Table 6 Organ weights and organ: body weight ratios of rats after treatment with Trikatu for 7 days

Groups	Number of rats	Initial Body weight (g)	Final Body weight (g)	Organ: Body weight ratios (g/100g B.W.)				
				Liver	Kidney	Lung	Spleen	Heart
Control	6	252.13 ± 1.97	289.23 ± 4.02	3.31 ± 0.09	0.83 ± 0.03	0.59 ± 0.03	0.32 ± 0.02	0.47 ± 0.05
500 mg/kg	6	245.03 ± 1.61	280.28 ± 4.20	3.97 ± 0.11*	0.90 ± 0.03	0.62 ± 0.05	0.35 ± 0.02	0.45 ± 0.06
1,000 mg/kg	6	235.60 ± 4.07	219.15 ± 5.48***	4.58 ± 0.36***	1.01 ± 0.15	0.68 ± 0.02	0.23 ± 0.02**	0.49 ± 0.03
				Organ weights (g)				
Control	6			9.58 ± 0.22	2.41 ± 0.08	1.71 ± 0.06	0.93 ± 0.04	1.37 ± 0.17
500 mg/kg	6			11.31 ± 0.30	2.58 ± 0.07	1.78 ± 0.14	1.01 ± 0.07	1.29 ± 0.12
1,000 mg/kg	6			9.91 ± 0.54	2.19 ± 0.29	1.48 ± 0.11	0.51 ± 0.08	1.06 ± 0.08

Values are expressed as mean ± S.E.M.

*: Significantly different from the control group at $p < 0.05$.

**: Significantly different from the control group at $p < 0.01$.

***: Significantly different from the control group at $p < 0.001$.

Table 7 Organ weights and organ: body weight ratios of rats after treatment with Trikatu for 30 days

Groups	Number of rats	Initial Body weight (g)	Final Body weight (g)	Organ: Body weight ratios (g/100g B.W.)				
				Liver	Kidney	Lung	Spleen	Heart
Control	6	239.63±1.78	363.7 ± 10.40	2.89 ± 0.06	0.68 ± 0.02	0.51 ± 0.03	0.26 ± 0.02	0.51 ± 0.04
50 mg/kg	6	222.37±0.97	347.5 ± 7.24	2.75 ± 0.04	0.69 ± 0.02	0.57 ± 0.04	0.29 ± 0.03	0.37 ± 0.05
150 mg/kg	6	233.50±6.34	363.46 ± 13.25	3.59 ± 0.11***	0.70 ± 0.01	0.59 ± 0.02	0.26 ± 0.02	0.40 ± 0.04
				Organ weights (g)				
Control	6			10.48±0.27	2.48±0.04	1.86±0.07	0.95±0.08	1.85±0.16
50 mg/kg	6			9.55±0.27	2.38±0.07	1.97±0.11	0.98±0.09	1.30±0.17
150 mg/kg	6			13.06±0.72	2.53±0.09	2.13±0.12	0.93±0.06	1.46±0.15

Values are expressed as mean ± S.E.M.

*: Significantly different from the control group at $p < 0.05$.

**: Significantly different from the control group at $p < 0.01$.

***: Significantly different from the control group at $p < 0.001$.

Histopathological studies of rat liver after feeding with various concentrations of Trikatu

Hepatocytes compose about 60% of the liver arranged in plates or cords that radiate from the central vein to the portal areas. In two-dimensional sections, they are typically one-layer thick and formed anastomoses (Miyai, 1991). On one surface they are separated from the sinusoidal wall by a peri-sinusoidal space, the space of Disse, where they are exposed to tissue fluids. Kupffer cells are a self-renewing fixed macrophage composing about 10% of all liver cells (Eustis, et al., 1990). Kupffer cells are phagocytic, secrete mediators of inflammation, and catabolize lipids and proteins (Figures 4 and 5).

In this experiment, daily exposure of high doses of (500 and 1,000 mg/kg B.W.) Trikatu for 7 days caused a significant pathomorphological changes in rat liver. Hepatocyte showed hydropic degeneration. The hydropic change in hepatocytes is a type of cytoplasmic clear areas alteration manifested on H&E-stained paraffin sections as clear spaces in the cytoplasm and a centrally located nucleus when compared to control. Because of disturbance of the cell membrane integrity, accumulation of intracytoplasmic fluid may occur. This change can be caused by xenobiotics with differing lobular localization and may be a precursor to hepatocyte necrosis (Gkretsi, et al., 2007; Wang, et al., 2007; Peichoto, et al., 2006; Matsumoto, et al., 2006; Chengelis, 1988). Pathomorphological changes in liver of male rats exposed to lower doses (150 mg/kg B.W.) of Trikatu for 30 days are similar to high dose (Figures 5C and F). On the other hand, liver tissue from rats treated with 50 mg/kg Trikatu did not exhibit any significant pathological changes (Figures 4A and D).

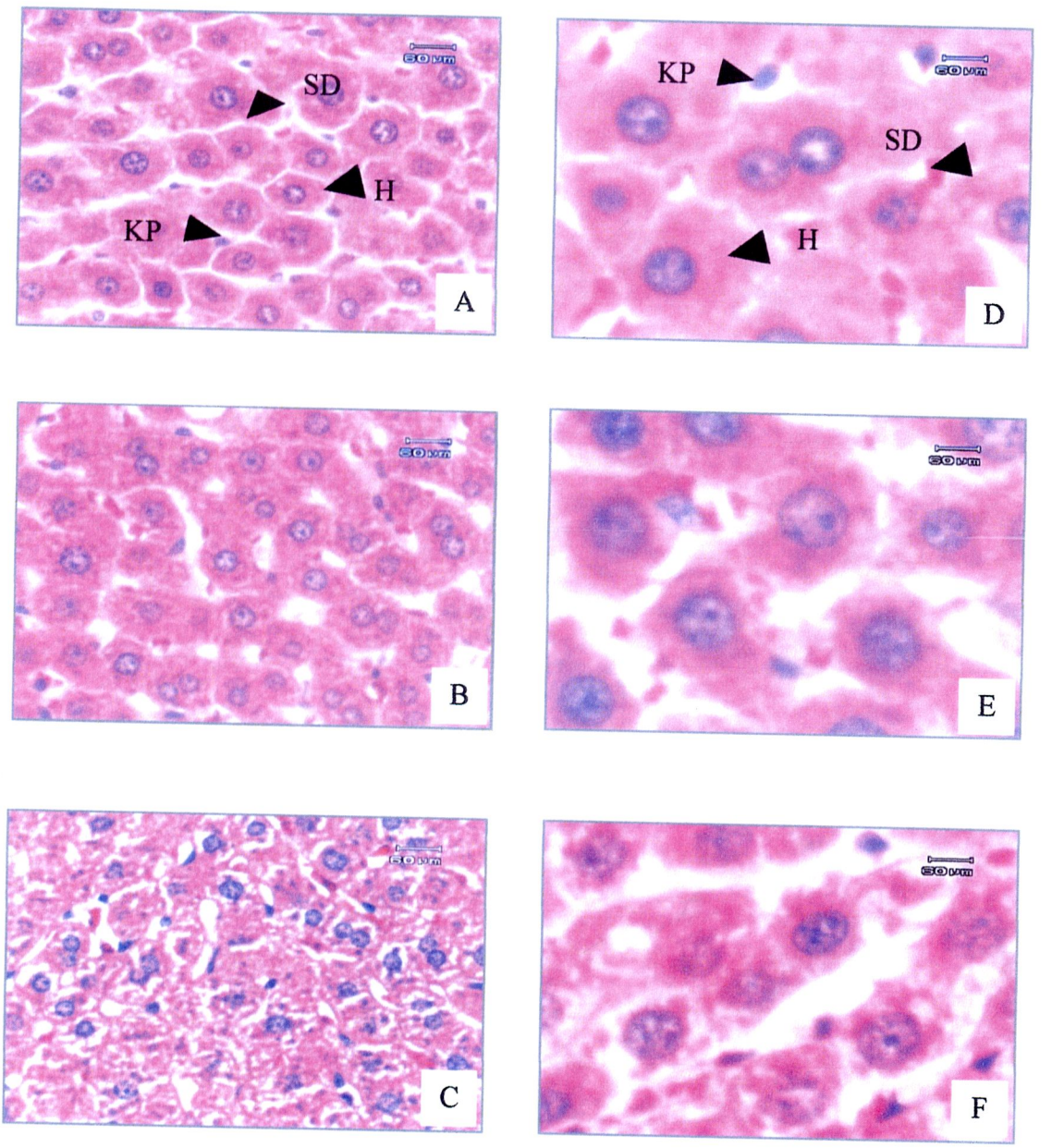

Figure 4 Acute effect of Liver histopathology

Representative liver tissue sections stained with hematoxylin and eosin, from male rats treated with Trikatu 500 mg/kg B.W. (B, E) and 1,000 mg/kg B.W.(C, F) for seven days as compared to controls (A, D). The arrows show Kupffer cells (KP) in the sinusoid (SD) that is slight space of the hepatocyte (H). The right column shows magnifications of the showed areas in the left column. Magnifications: left column, ×40; right column, ×100.

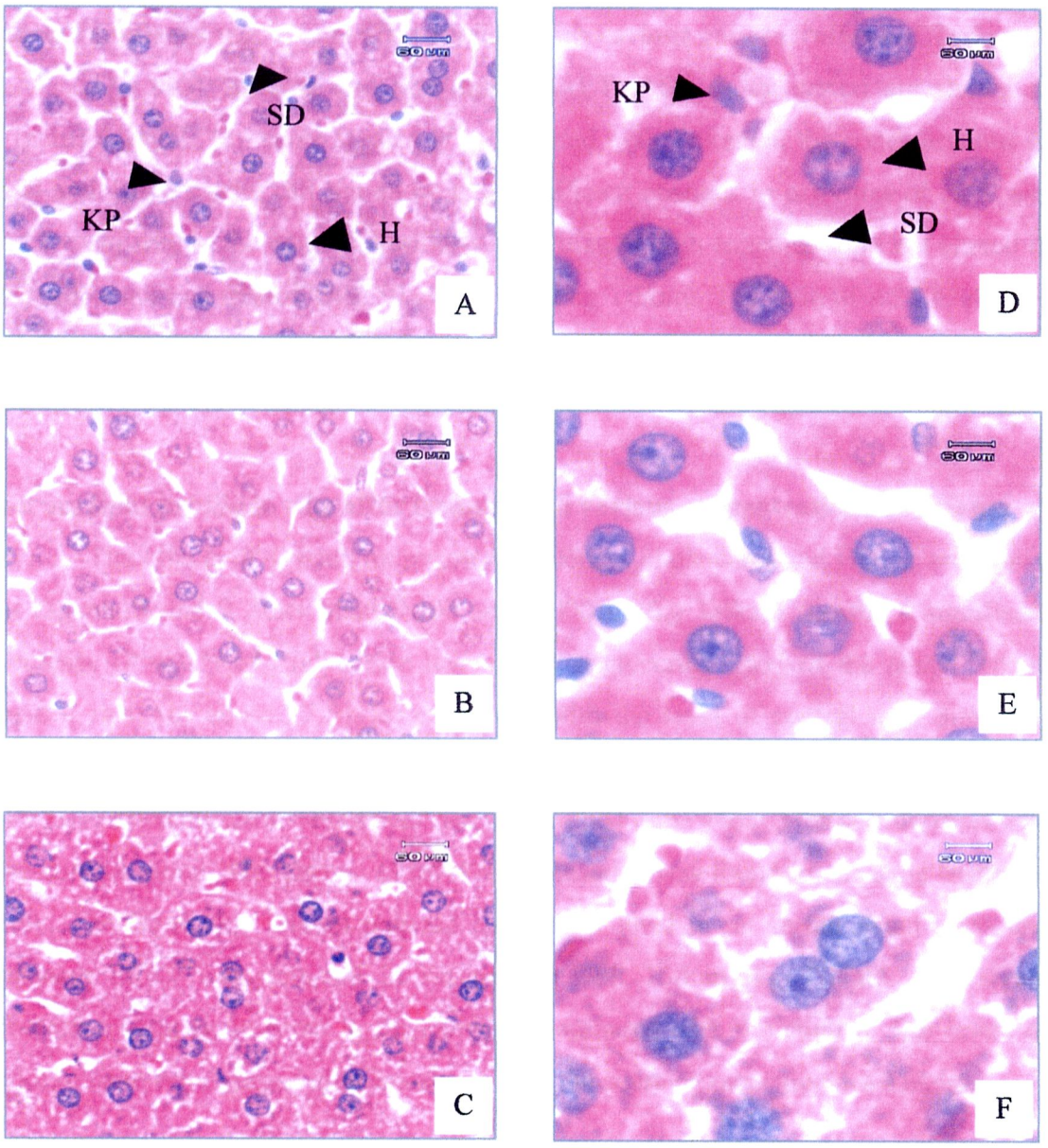

Figure 5 Subacute effect of Liver histopathology

Representative liver tissue sections stained with hematoxylin and eosin, from male rats treated with Trikatu 50 mg/kg B.W. (B, E) and 150 mg/kg B.W. (C, F) for 30 days as compared to controls (A, D). The arrows show Kupffer cells (KP) in the sinusoid (SD) that is slight space of the hepatocyte (H). The right column shows magnifications of the showed areas in the left column. Magnifications: left column, ×40; right column, ×100.

Liver functional test in rats after feeding with various concentrations of Trikatu

The total proteins and activities of ALT, AST in rat serum are shown in Figures 6 and 7. The acute studies of rats treated with Trikatu at the concentrations of 500 and 1,000 mg/kg did not show any significant changes in activities of AST and ALT compared to the control. Similarly, activities of both enzymes were not altered in the sub-acute rats treated with 50 and 150 mg/kg Trikatu. The ratio of AST to ALT is often used to diagnose toxicity upon liver. However, all rat treated groups showed no change in serum AST or ALT.

Serum lipid profile in rats after feeding with various concentrations of Trikatu

The effects of Trikatu on serum triglyceride, cholesterol and HDL-c are shown in Figures 8 and 9. Rats treated 500 and 1,000 mg/kg Trikatu showed a significant decrease ($P>0.05$) of triglyceride levels. The triglyceride level of the acute Trikatu treated rats was about 27.5% as compared with the level of control rats. Similarly, subacute oral administration of 50 and 150 mg/kg revealed a significant decrease ($P>0.01$) of cholesterol. This effect was determined to be about 37% as compared with the control group. The change in level of cholesterol was found in subacute treatment only. The 50 mg/kg Trikatu reduced cholesterol level in rat serum about 8.6% and stronger effect was detected in rat treated with 150 mg/kg Trikatu. The serum cholesterol can be lowered to 26.5% when compared with the control. The levels of HDL-c were not changed after treatment with the acute or subacute with Trikatu.

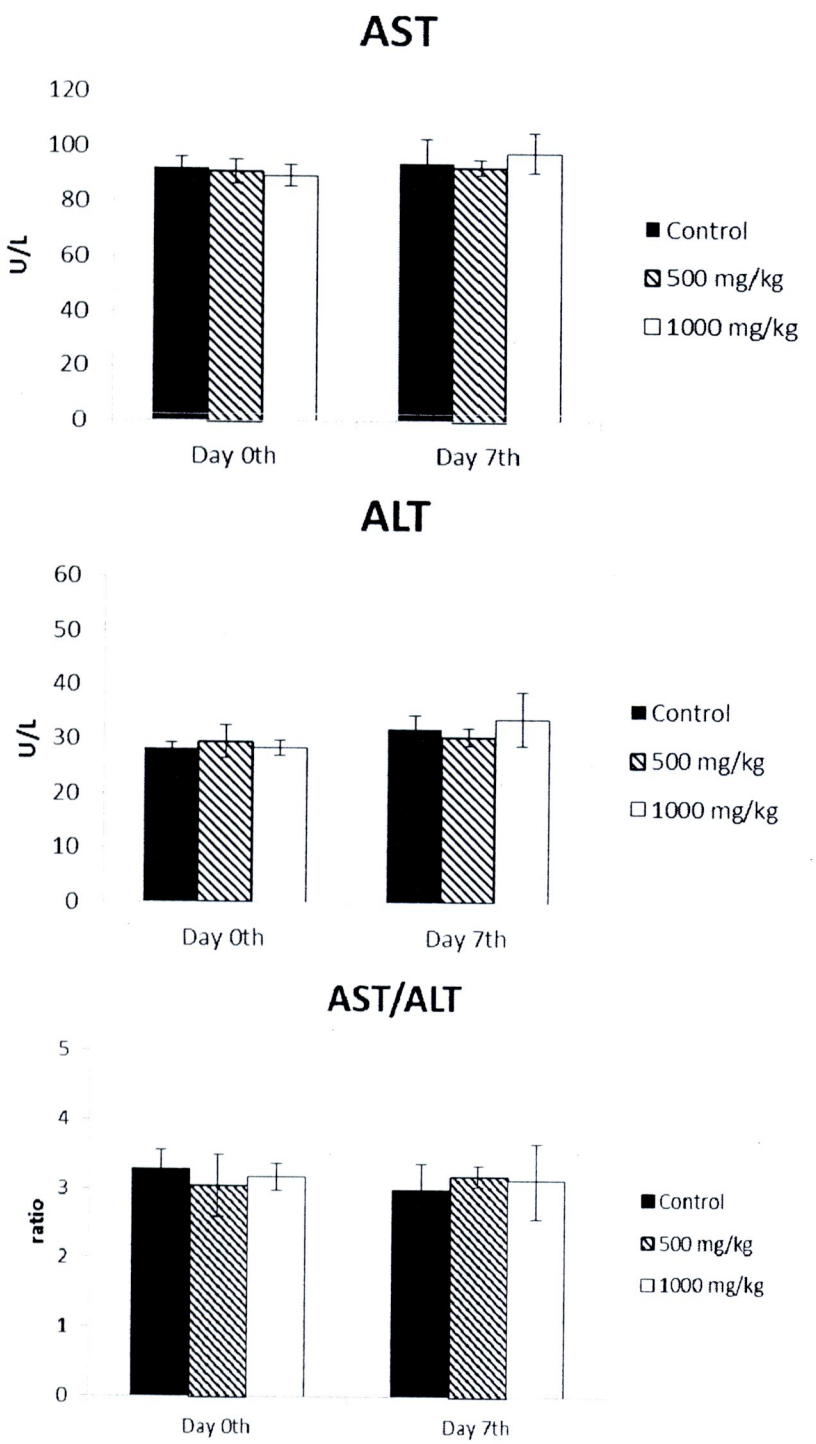

Figure 6 Serum transaminase activities in male Wistar rats after treatment with 500 mg/kg and 1,000 mg/kg Trikatu for 7 days.

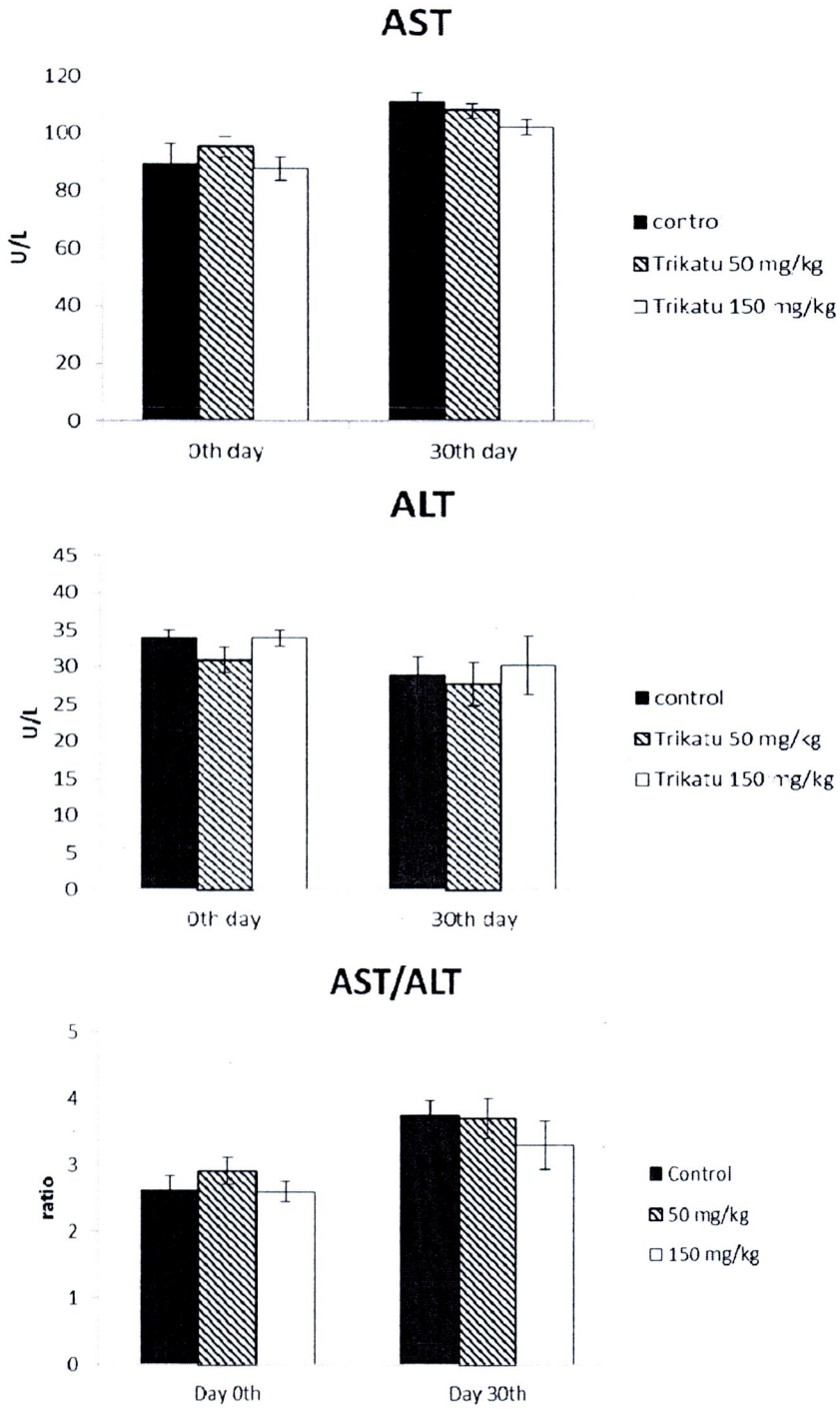

Figure 7 Serum transaminase activities in male Wistar rats after treatment with 50 mg/kg and 150 mg/kg Trikatu for 30 days.

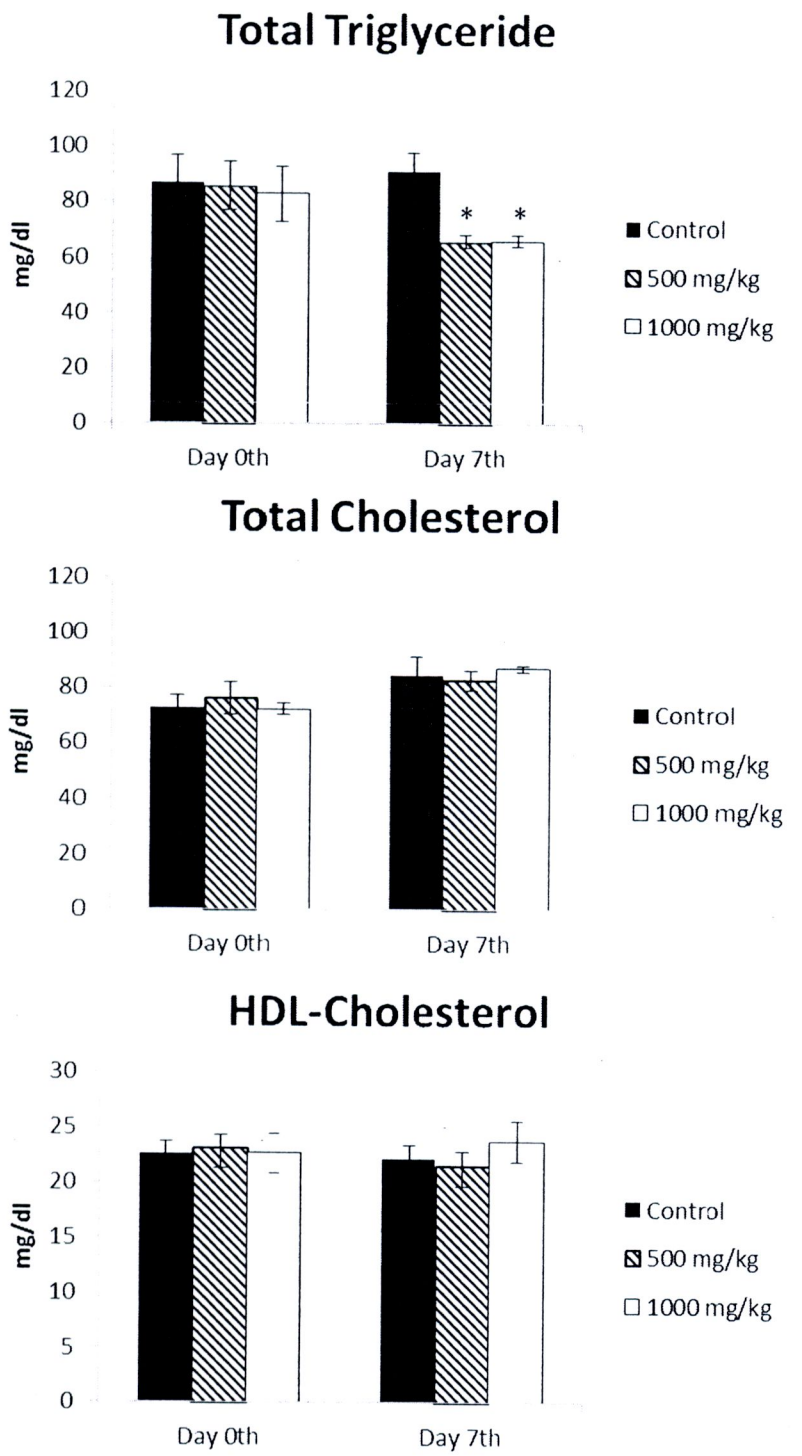

Figure 8 Serum lipid profiles in male Wistar rats after treatment with 500 mg/kg and 1,000 mg/kg Trikatu for 7 days.

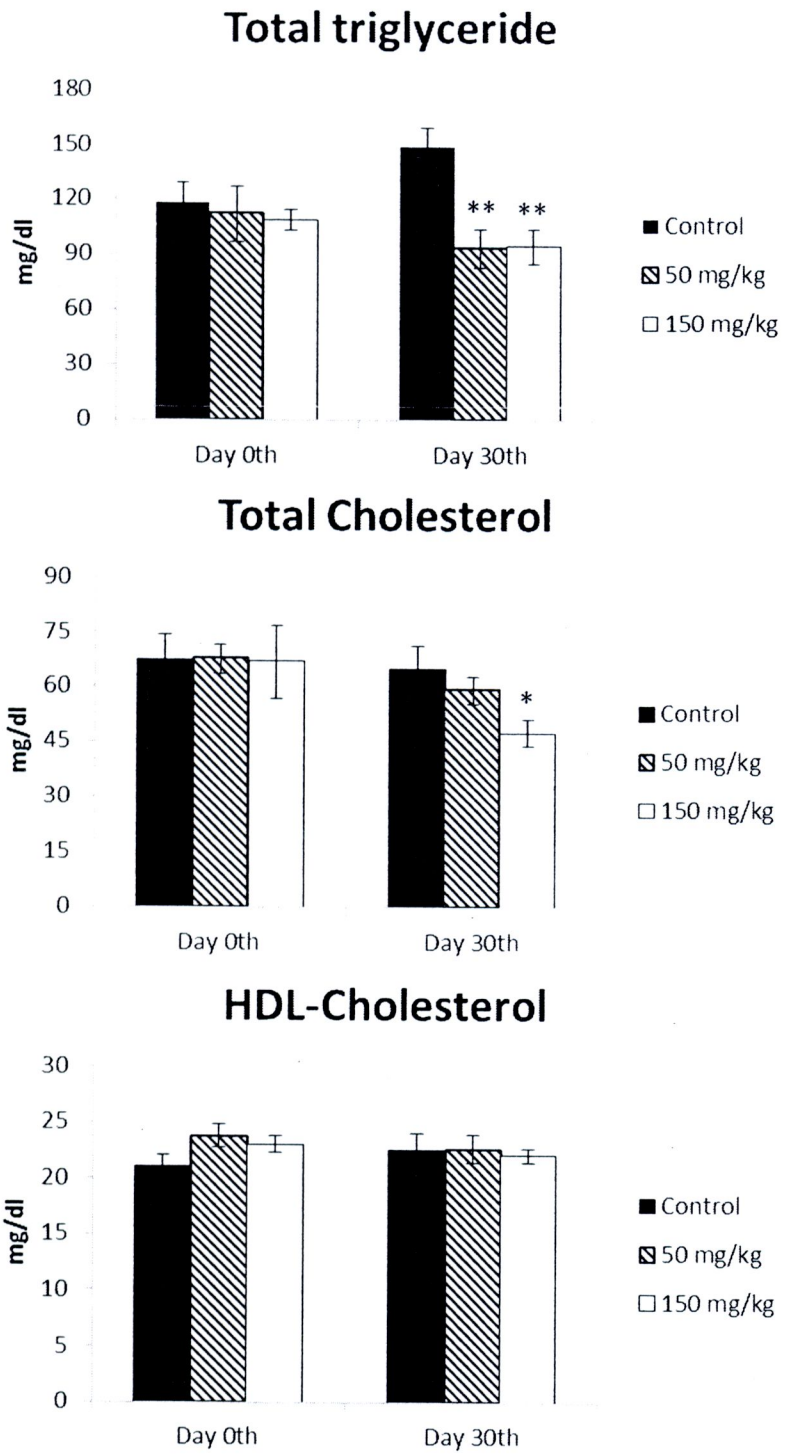

Figure 9 Serum lipid profiles in male Wistar rats after treatment with 50 mg/kg and 150 mg/kg Trikatu for 30 days.

GeLC-MS analysis of rat liver treated Trikatu

SDS-PAGE analysis of total proteins extracted from rat liver is shown in Figure 10. Few differences in protein pattern were observed between subacute and acute group. Metabolite or phenolic compound produced in subacute group may cause higher intensity of protein bands. However, theprotein samples on SDS-PAGE were cut into twelve fractions (A to L) before in-gel digestion. The obtained peptides were analyzed by nano-LC-MS/MS. The mass spectrum was normalized with an internal tryptic digested BSA and evaluated by DeCyder^{TM} MS. Protein abundance data were analyzed to filter out proteins with statistically significant ($p<0.05$). The result showed that more than 1,423 proteins were differentially expressed. The biological processes of these proteins were classified by STRAP software.

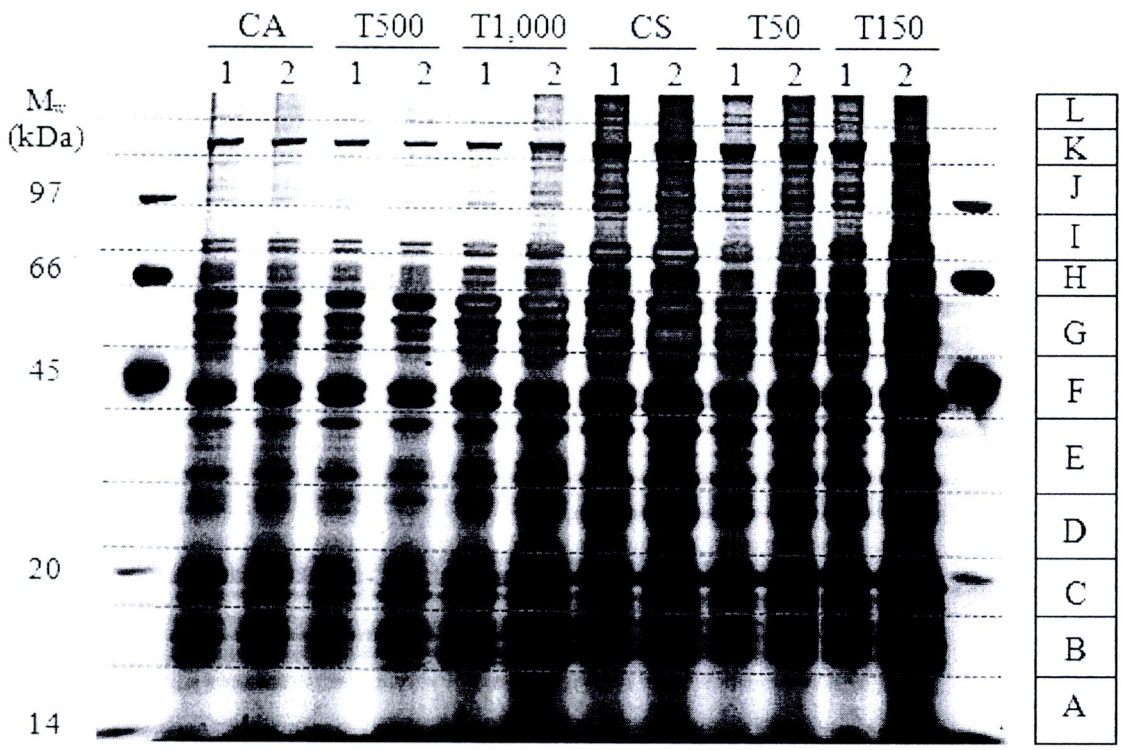

Figure 10 Fifty micrograms of total protein were separated by 1D-PAGE followed by slicing gel lanes into 12 fractions and analyzed the level of tryptic peptides in each gel plugs by LC-MS/MS.

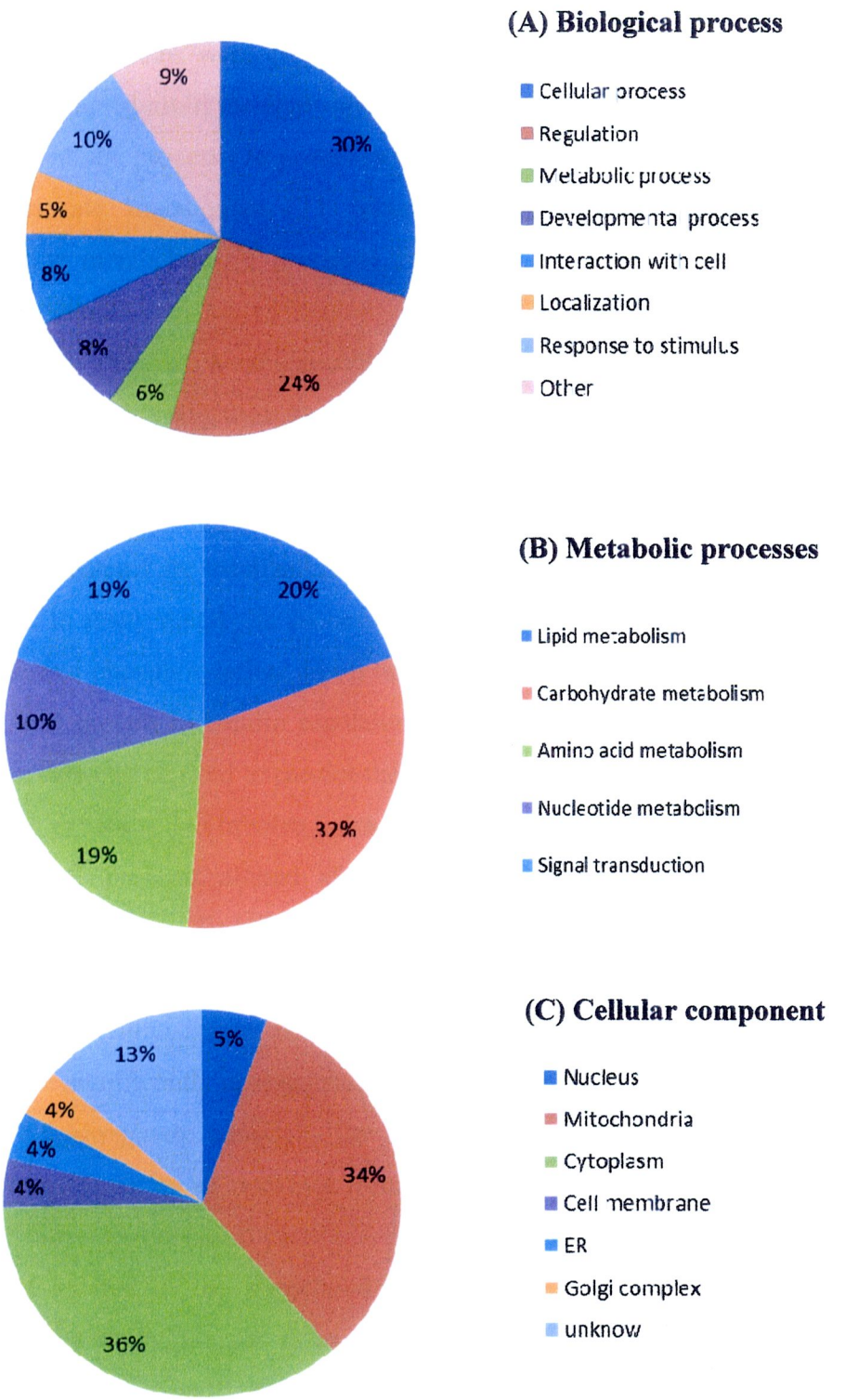

Figure 11 Protein classifications by STRAP software

The identified proteins were grouped according to their biological functions, derived from the annotated hit in the UniProtKB database and presented in Figure 11a. The proteins in metabolic process (76 proteins) were involved in lipid metabolism (14 proteins), carbohydrate metabolism (23 proteins), amino acid metabolism (14 proteins), nucleotide metabolism (7 proteins) and signal transduction (18 proteins) (Figures 11b and Table 8).

The metabolic proteins were subjected to a network interaction analysis by STRING database, which integrates protein-protein interaction data from various sources, including experimental repositories, structural computational analysis, previous knowledge and differentially expression proteins in many organelles. Interestingly, many proteins are localized in mitochondria and cytoplasm (Figure 11c). They are involved in many metabolic processes such as glycolysis, gluconeogenesis, TCA cycle, oxidative phosphorylation, lipolysis, and protein degradation. Moreover, insulin receptor and protein in insulin regulation process are linked to this network (Figure12). These data can be used to predict the effect of Trikatu on lipid and glucose metabolism, which is believed to involve in lowering triglyceride level in rat serum.

The candidate proteins altered after Trikatu treatments were statistically analyzed by Mev software. Significance proteins were determined by one-way ANOVA and p-value cut-off of 0.05 were adjusted-alpha mode testing correction. Dihydrolipoamide acetyltransferase (Dlat) and methylcrotonoyl-CoA carboxylase alpha (Mccc1) were significantly up-regulated ($p<0.05$). Dlat is an enzyme component of the pyruvate dehydrogenase complex. The increase of this protein may cause an increase in the transformation of pyruvate from glycolysis into acetyl-CoA which is then used in the citric acid cycle to carry out cellular respiration. Mccc1 is a biotin-requiring enzyme located in the mitochondria. The increase of this protein may accelerate the breakdown of leucine to eventually yield acetyl CoA. Whereas liver glycogen phosphorylase (Pygl) and carnitine palmitoyltransferase 1 (Cpt1a) significantly down-regulated ($p<0.01$). Pygl catalyzes the rate-limiting step in the degradation of glycogen into glucose. The inhibition of glycogen phosphorylase has been proposed the release of glucose as one method for treating type 2 diabetes. Cpt1a is associated with the outer mitochondrial membrane and mediates the transport of long-chain fatty acids across the membrane. This inhibition is a good target for future

attempts to regulate Cpt1a for the treatment of metabolic disorders such as diabetes. Our candidate proteins are important to describe the toxicity and pharmacological action of Trikatu *in vivo*. However, the expression level of these proteins remained to be confirmed.

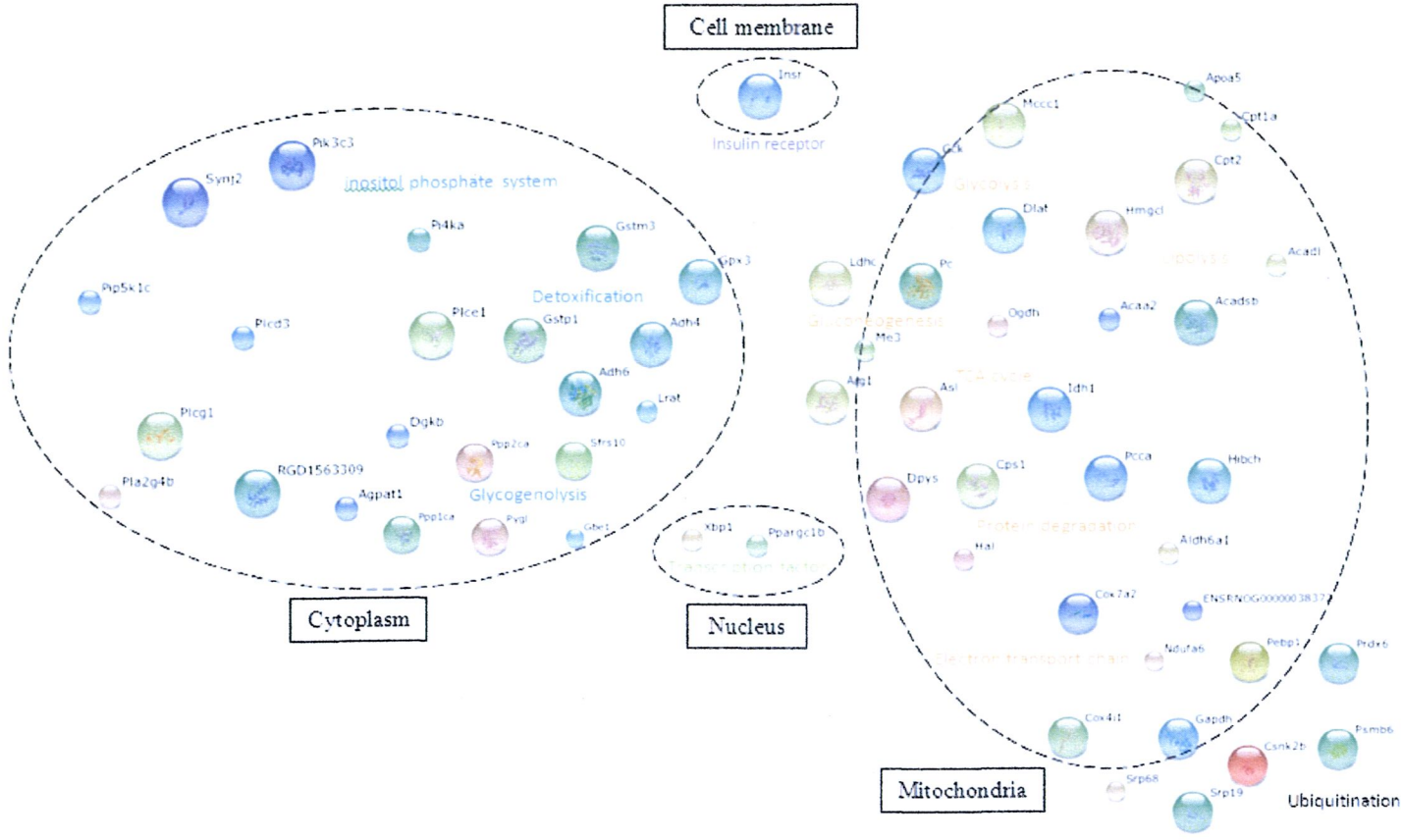

Figure 12 High-confidence protein-protein interaction network of the differentially expressed proteins derived from STRING database.

Table 8 Relative ratio of metabolic processes proteins altered after Trikatu treatment

Protein name	Accession number	Gene name	Biological processes[a]	Localization[a]	Acute effect[b]		Subacute effect[b]	
					T500/C	T1,000/C	T50/C	T150/C
Metabolism								
Lipid metabolism								
1-acylglycerol-3-phosphate O-acyltransferase 1	gi|149027956	Agpat1	Glycerolipid metabolism	Endoplasmic reticulum	0.82	0.93	0.87	0.89
lecithin retinol acyltransferase	gi|11560109	Lrat	Glycerophospholipid metabolism	Cytoplasm	0.58*	0.91	0.67*	0.69*
phosphatidylethanolamine binding protein 1	gi|406294	Pebp1	Glycerophospholipid metabolism	Cytoplasm	14.63	18.73*	0.84*	0.94
sphingomyelinphosphodiesterase 3	gi|16758394	Smpd3	Lipid signaling	Golgi apparatus	1.57**	0*	1.25*	1.24*
Acetyl-Coenzyme A carboxylase beta	gi|16758804	Acc2	Fatty acid synthesis	Mitochondria	0.99	0.81	0	0.84
short-chain-acyl-CoA dehydrogenase	gi|111334	Acads	Fatty acid oxidation	Mitochondria	0.94	0.84	0.92	0.98
hydroxymethylglutaryl-CoA lyase	gi|13242293	Hmgcl	ketone bodies	Mitochondria	0.91	0.92	0.90	0.98
enoyl-CoA hydratase	gi|155369702	Echd	Fatty acid oxidation	Mitochondria	1.13	1.43	0.97	0.99
long-chain specific acyl-CoA dehydrogenase	gi|6978431	Acadl	Fatty acid oxidation	Mitochondria	0.93	0	0.94	0.91
acetyl-Coenzyme A acyltransferase 2	gi|149027156	Acaa2	Fatty acid oxidation	Mitochondria	0.97	0	0.87	0.81

Table 8 (Cont.)

Protein name	Accession number	Gene name	Biological processes[a]	Localization[a]	Acute effect[b]		Subacute effect[b]	
					T500/C	T1,000/C	T50/C	T150/C
microsomal triglyceride transfer protein large subunit	gi|157819691	Mttp	Lipid transport	Golgi apparatus	0	1.15	1.13	0
apolipoprotein E	gi|162287337	Apoe	Lipid transport	Cytoplasm	0.88	0.92	0.98	0.96
diacylglycerol kinase beta	gi|9506535	Dgkb	Phosphatidylinositol signaling system	Cytoplasm	1.28	0	1.25	1.21
diacylglycerol kinase, delta	gi|109486333	Dgkd	Phosphatidylinositol signaling system	Cytoplasm	0	0	0	17.06
Sugar metabolism								
hexokinase-1	gi|6981022	Hk1	Glycolysis and gluconeogenesis	Cytoplasm	1.09	0	1.26	1.09
glyceraldehyde-3-phosphate dehydrogenase	gi|62657298	Gapdh	Glycolysis and gluconeogenesis	Cytoplasm	1.00	1.14	1.16	1.03
alcohol dehydrogenase 4	gi|253735823	Adh4	Glycolysis and gluconeogenesis	Cytoplasm	0*	0	0	0.97
alcohol dehydrogenase 6	gi|58865738	Adh6	Glycolysis and gluconeogenesis	Cytoplasm	0.86*	0.86*	0.92	1.01
glucokinase	gi|204380	Gck	Glycolysis and gluconeogenesis	Cytoplasm	0.79	0.70	0.82	0.80
dihydrolipoamideacetyltransferase	gi|220838	Dlat	Pyruvate metabolism	Mitochondria	0*	1.34*	1.11*	1.21*
pyruvate carboxylase	gi|929988	Pc	Pyruvate metabolism	Mitochondria	11.02*	0	0*	0

Table 8 (Cont.)

Protein name	Accession number	Gene name	Biological processes[a]	Localization[a]	Acute effect[b]		Subacute effect[b]	
					T500/C	T1,000/C	T50/C	T150/C
malic enzyme 3, NADP(+)dependent	gi|149069021	Me3	Pyruvate metabolism	Mitochondria	0.79	0.89	1.00	0.86
L-lactate dehydrogenase C chain	gi|149055819	Ldhc	Pyruvate metabolism	Cytoplasm	0.73	0	0.97	1.05
isocitrate dehydrogenase 1	gi|89573967	Idh1	TCA cycle	Mitochondria	0.83*	0	0.88*	0.91
2-oxoglutarate dehydrogenase	gi|62945278	Ogdh	TCA cycle	Mitochondria	13.95	11.46	1.28	1.15
cytochrome c oxidase subunit 4	gi|8393180	COX4i1	Oxidative phosphorylation	Mitochondria	0.99	1.14	1.49	1.42
NADH dehydrogenase [ubiquinone] 1 alpha	gi|194473636	Ndufa6	Oxidative phosphorylation	Mitochondria	0.85	0	0.98	0
NADH dehydrogenase [ubiquinone] iron-sulfur protein 2	gi|58865384	Ndufs2	Oxidative phosphorylation	Mitochondria	1.14	1.08	2.21	0
cytochrome c oxidase assembly protein 11	gi|157818671	COX11	Oxidative phosphorylation	Mitochondria	0.90	0.93	1.33	1.22
ATPase, H+ transporting, lysosomal V0 subunit A1	gi|149054256	Atp6v0a1	Oxidative phosphorylation	Mitochondria	0.75	0.73	1.31	1.61
cytochrome c oxidase 7A2	gi|11968072	COX7a2	Oxidative phosphorylation	Mitochondria	1.28	1.49	1.16	1.16
3-hydroxyisobutyryl-CoA hydrolase	gi|61556993	Hibch	Propanoate metabolism	Mitochondria	0.81	0.87	0.63*	0.66*

Table 8 (Cont.)

Protein name	Accession number	Gene name	Biological processes[a]	Localization[a]	Acute effect[b]		Subacute effect[b]	
					T500/C	T1,000/C	T50/C	T150/C
aldehyde dehydrogenase 6,A1	gi|149025145	Aldh6a1	Propanoate metabolism	Mitochondria	0.87*	0.95	0.85*	0.97
protein phosphatase 1, catalytic subunit, alpha	gi|149061963	Ppp1ca	Glycogen metabolism	Cytoplasm	0	0	0	0
liver glycogen phosphorylase	gi|288457	Pygl	Glycogen metabolism	Cytoplasm	0.68*	0*	0**	0.69*
alpha-propionyl-CoA carboxylase	gi|206050	PCCA	Propanoate metabolism	Mitochondria	0.99	0.86	1.27	1.12
Asparagine-linked glycosylation 3	gi|56788800	Alg3	N-Glycan biosynthesis	Endoplasmic reticulum	0	0.83	0	0
Amino acid Metabolism								
methylcrotonoyl-CoA carboxylase subunit alpha	gi|57528264	Mccc1	Leucine degradation	Mitochondria	1.55**	1.59**	1.48*	1.31*
argininosuccinatelyase	gi|31377525	Asl	Alanine, aspartate and glutamate metabolism	Cytoplasm	0.64	0.82	1.01	0.82
carbamoyl-phosphate synthase	gi|8393186	Cps1	Alanine, aspartate and glutamate metabolism	Mitochondria	0.80	0.78*	0.78*	0
serine hydroxymethyltransferase 2	gi|149066589	Shmt2	Glycine, serine and threonine metabolism	Mitochondria	0.87	0.92	0.96	0.97
succinyl-CoA:3-ketoacid-coenzyme A transferase 2A	gi|58866010	Oxct2b	Valine, leucine and isoleucine degradation	Mitochondria	1.08	0.81*	0.96	0.82*
S-adenosylmethionine decarboxylase 1	gi|149033003	Amd1	Arginine and proline metabolism	Unkown	0.83	0	0.93	0.95
pyrroline-5-carboxylate reductase 2	gi|58865992	Pycr2	proline metabolism	Cytoplasm	0.92	0.84	0.90	1.01

Table 8 (Cont.)

Protein name	Accession number	Gene name	Biological processes[a]	Localization[a]	Acute effect[b]		Subacute effect[b]	
					T500/C	T1,000/C	T50/C	T150/C
proline dehydrogenase (oxidase) 2	gi|149056325	Prodh2	Proline metabolism	Mitochondria	0.86	0	0	0
glutathione S-transferase Mu 3	gi|10120486	Gstm3	Glutathione metabolism	Cytoplasm	0	0	17.28	13.62
glutathione S-transferase P	gi|2143764	Gstp1	Glutathione metabolism	Cytoplasm	0.92	0	0.86	0
histidine ammonia lyase	gi|149067179	Hal	Histidine metabolism	Cytoplasm	0.86	0.80*	1.05	0
peroxiredoxin-6	gi|16758348	Prdx6	Phenylalanine metabolism	Cytoplasm	0.83	0.97	0.92	0.90
arginase 1	gi|149032925	Arg1	Arginine metabolism	Cytoplasm	0.76*	0	0.76*	0.88
glutathione peroxidase 3	gi|149052630	Gpx3	Glutathione metabolism	Cytoplasm	1.15	1.18	0	1.13
Nucleotide metabolism								
adenylatecyclase 9	gi|149042667	Adcy9	purine metabolism	Unkown	1.06	1.18	1.25	1.13
adenylatecyclase 10	gi|11067413	Adcy10	purine metabolism	Unkown	0.92	0	14.02	12.87
3'-phosphoadenosine 5'-phosphosulfate synthase 1	gi|157823805	Papss1	purine metabolism	Unkown	13.31	13.40	1.20	1.23
uridine 5'-monophosphate synthase	gi|70794780	Umps	pyrimidine metabolism	Nucleus	0.72	0.84	1.22	1.26

Table 8 (Cont.)

Protein name	Accession number	Gene name	Biological processes[a]	Localization[a]	Acute effect[b]		Subacute effect[b]	
					T500/C	T1,000/C	T50/C	T150/C
cytosolic 5'-nucleotidase 1B	gi|58865540	Nt5c1b	pyrimidine metabolism	Cytoplasm	13.74	12.11	1.09	1.10
dihydropyrimidinase	gi|13928984	Dpys	pyrimidine metabolism	Cytoplasm	0	0	16.77	16.62
thymidylate kinase	gi|157817466	Dtymk	pyrimidine metabolism	Nucleus	0	1.25*	0**	1.11
Signal Transduction								
solute carrier family 27 (fatty acid transporter), member 4	gi|291084711	Slc27a4	PPAR signaling pathway	Endoplasmic reticulum	0.85	0.93	0.72	0.94
peroxisome proliferator-activated receptor gamma coactivator 1-alpha	gi|13786188	PCG-1a	PPAR signaling pathway	Nucleus	0.91	0.85	0.82	0.91
apolipoprotein A-V	gi|18034777	Apoa5	PPAR signaling pathway	Lipoprotein	0.94	0.89	0.90	0.88
nuclear receptor subfamily 1, group H, member 2	gi|149056042	Nr1h2	PPAR signaling pathway	Nucleus	0	0.86	0	0
carnitinepalmitoyltransferase 1	gi|294521	Cpt1a	PPAR signaling pathway	Mitochondria	0	0.95	0**	0.91
insulin receptor	gi|1737455	Insr	Insulin signaling pathway	Cell membrane	1.06	1.36	1.23	1.12
Rap guanine nucleotide exchange factor (GEF) 1	gi|149039159	Rapgef1	Insulin signaling pathway	Unkown	0.83	0.81	0.98	0
protein kinase, AMP-activated, gamma 2 non-catalytic subunit	gi|149031390	Prkag2	Insulin signaling pathway	Unkown	0.85	0.84	0.91	0.83

Table 8 (Cont.)

Protein name	Accession number	Gene name	Biological processes[a]	Localization[a]	Acute effect[b]		Subacute effect[b]	
					T500/C	T1,000/C	T50/C	T150/C
mitogen-activated protein kinase 9	gi|8394233	Mapk9	Insulin signaling pathway	Cytoplasm	0.92	0.89	0.92	0.88
phosphatidylinositol 3-kinase catalytic subunit type 3	gi|12621140	Pik3c3	Phosphatidylinositol signaling system	Unkown	0.96	0.80*	0.94	0.93
phosphatidylinositol 4-kinase alpha	gi|25742825	Pi4ka	Phosphatidylinositol signaling system	Unkown	0.94	0	0.91	0
phosphatidylinositol-4-phosphate 5-kinase type-1 gamma	gi|111185932	Pip5k1c	Phosphatidylinositol signaling system	Cell membrane	0.87	0.77	14.64	14.65
1-phosphatidylinositol-4,5-bisphosphate phosphodiesterase epsilon-1	gi|16758594	Plce1	Phosphatidylinositol signaling system	Golgi apparatus	1.28	0*	0**	1.12
1-phosphatidylinositol-4,5-bisphosphate phosphodiesterase eta-1	gi|300794799	Plch1	Phosphatidylinositol signaling system	Cytoplasm	0.97	0.91	0.95	0
1-phosphatidylinositol-4,5-bisphosphate phosphodiesterase gamma-1	gi|6981370	Plcg1	Phosphatidylinositol signaling system	Cytoplasm	1.14	1.00	1.16	1.08
synaptojanin-2 isoform 2	gi|164565437	Synj2	Phosphatidylinositol signaling system	Cytoplasm	0.89	0.92	13.12	0
phospholipase C, delta 3	gi|149054434	Plcd3	Phosphatidylinositol signaling system	Cytoplasm	1.01	0.85	0.84	0

[a]Data depicted from RGD database (gene ontology biological process and protein location annotation).

[b]The protein relative abundance ratio was calculated from the averages of more three biological replicates from each sample group.

*A value of expression ratio with a statistically significant difference ($p<0.05$) between treated group and control.

**A value with a statistically significant difference ($p<0.01$) between treated group and control.