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Research Article

FK 506 INDUCED CHANGES OF CERTAIN KEY ENZYMATIC ACTIVITIES INVOLVED IN CARBOHYDRATE METABOLISM IN THE TISSUES OF ALBINO RATS

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ABSTRACT

The present investigation demonstrated significant variations in enzyme activities involved in carbohydrate metabolism in different tissues of albino rats, upon FK 506 treatment. The biochemical and physiological properties of the tissues are essentially dependent on several adaptive and stress conditions. After treatment of immunosuppressive agent (FK 506); phosphorylase '*a*', aldolase, Succinate dehydrogenase, Malate dehydrogenase and lactate dehydrogenase activities were analyzed in control rat tissues. phosphorylase 'a' activity was maximum in the liver tissue followed by brain > heart > kidney.

Key words: Albino rat, FK 506

INTRODUCTION

A biological system with a balanced metabolic pattern is characterized by a dynamic equilibrium between enzymatic activities [1]. Any abnormality in the enzyme system and there in enzyme may lead to an inhibition or hyper functions of the organ concerned, which ultimately manifests as disease [2]. Carbohydrates, lipids and proteins can serve as energy sources for animals. The TCA cycle is the main pathway for the oxidation of carbohydrates, lipids and proteins [3]. The major function of carbohydrates in metabolism is to provide energy for various cellular activities. A variety of enzyme systems are associated either directly or indirectly with the metabolism of carbohydrates in many pathological conditions [4, 5]. All metabolic activities are under the control of enzymes. The myriad chemical reactions going on continually in living matter would not be possible without enzymes, which are important tools of the living cell [6].

Immuno suppressive agent like CsA has been shown to interact with carbohydrates, glucose [7], phosphorylation events [8] and with certain enzymatic activities like LDH in various experimental models [9]. Thus FK 506 administration interacts in some way the normal functioning of carbohydrate metabolism, but there is little or no experimental data concerning to the effect of FK 506 on the key enzymatic activities involved in carbohydrate metabolism. Hence, the author tried to bridge this gap and studied the fate of certain key enzymatic activities that are involved in carbohydrate metabolism in FK 506 treated rat tissues. Such as phosphorylase 'a', phosphorylase 'ab', aldolase, SDH, MDH and LDH.

MATERIALS AND METHODS

Experimental material (Animal)

Albino rats weighing 150 ± 10g were selected for the present study. Animals were Fed *ad libitum* with commercial rat diet supplied by Kamadhenu Agencies, Bangalore, India and were housed at constant room temperature of 15 ± 5ºC. They were allowed to acclimate to laboratory conditions for at least ten days after arrival before use. Prior to experiment, they were fasted for 24 hr with free access to water. They were divided into 4 groups of 7 each.

Chemicals

FK 506 (Tacrolimus) is a product of Sigma and was a gift from Dept. of Medicine, University of Mississippi Medical Centre, Jackson, USA. All other chemicals used were of technical grade.

Treatment of Animals

Group I & II rats acted as controls, received only saline (Oral) over 7 or 28 days respectively (daily doses). Group III rats were gavaged daily with 1 mg/kg body wt. of FK 506 in 0.5ml of saline / 7 days, (short term) and group IV were gavaged daily with 1 mg / kg body wt. of FK 506 in 0.5ml of saline over 28 days (long term).

After 7 or 28 days of FK 506 treatment of rats, they were anaesthetized with pentobarbitone 5 mg / kg and were sacrificed. Major tissues, like brain, heart, liver and kidney, were isolated, quickly blotted on a filter paper, weight frozen in liquid nitrogen and were stored at -80^{0}C till used. Whenever needed, blood samples were collected by cardiac puncture and were allowed to clot at room temperature, and were centrifuged at 2000 xg for 10 min. The separated serum samples were kept for several hours / several days in a refrigerator and were subsequently used for experimentation.

Estimation of Glycogen Phosphorylase

Phosphorylase activity in control and experimental sample was assayed by the method of Cori *et al*., (1955) [10] in the direction of glycogen synthesis by determining the amount of inorganic phosphate formed from glucose-1-phosphate. Homogenates 2% (W/V) of tissues were prepared in the medium containing 0.01M sodium fluoride (NaF) and 0.037 ethylene diamine tetraacetic acid (EDTA) (PH 6.0) as recommended by Guillory and Mommaerts (1962) [11] to avoid interconversion of phosphorylase.

The enzyme was diluted (1:3) with cysteine (0.03M) β-glycerophosphate (0.015M) buffer (PH 6.0) 0.1 ml of the diluted enzyme was added to 0.2 ml of 1% glycogen. For each sample, two tubes were maintained. The reaction was started by the addition of 0.2 ml of glucose-1-phosphate (0.06M) to one of the tubes and 0.2 ml of mixture of 0.06M glucose-1-phosphate and 0.004M adenosine-5-monophosphate (AMP) to the other for estimating the active phosphorylase (a) & total phosphorylase (ab), respectively. After 15 min incubation for total phosphorylase and 30 min. for active phosphorylase (at 37°C), the reaction was stopped with 2.0 ml of 10% trichloroacetic acid (TCA). The inorganic phosphate formed was estimated by the method of Fiske and Subba Row (1921) [12]. The above contents were centrifuged. To the filtrate 1.0 ml of ammonium molybdate solution and 0.4 ml of aminonaphthasulfonic acid (ANSA) were added. The colour was read at 660 nm against a blank prepared with 2.0ml of trichloroacetic acid 1.0 ml of ammonium molybdate solution and 0.4 ml of ANSA. Phosphorylase activity was expressed as μ moles of inorganic phosphate formed/mg protein^{-1}h^{-1}.

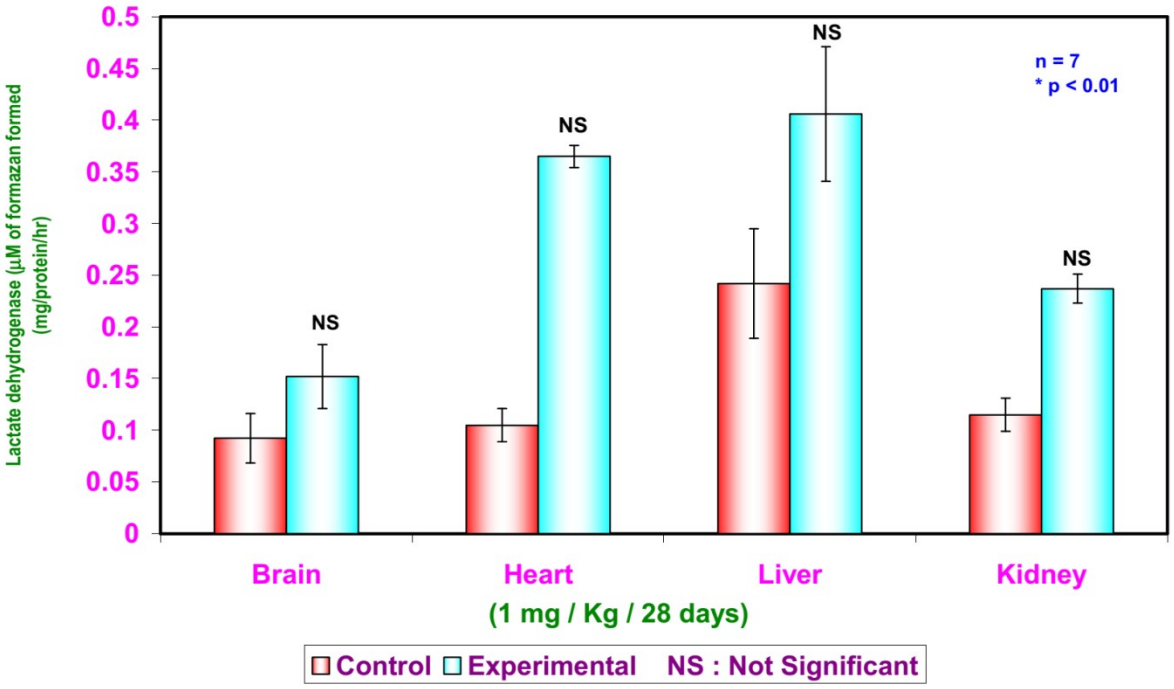
Estimation of Aldolase activity

The aldolase activity in control and experimental rat tissues was estimated by the method of Bruns and Bergmayer (1963) [13] where in the triosephosphates formed were estimated by using 2,4-dinitrophenyl hydrazine. Homogenates 2% (w/v) of tissues were prepared in cold distilled water and centrifuged at 1000xg for 5 min. The reaction mixture of 3 ml contained 1.75ml of collidine hydrazine buffer (PH 7.4) 0.25 ml of fructose-1-6 diphosphate (0.02M, PH 7.4) 0.5 ml of distilled water and 0.5 ml of homogenate supernatant. The reaction mixture was incubated for 15 min at 37°C and the reaction was arrested by adding 3.0ml of 10% TCA was read at 540 nm a spectrophotometer. Then the contents were filtered 1.0 ml of the filtrate and allowed to standard for 10 min at room temperature. Then 1.0 ml of 2,4-dinitrophenylphyodrazine was added and the contents were incubated at 37°C for 10 min. After incubation, 8ml of 0.75N NaoH was added. The reddish brown colour developed against a zero time control. The aldolase activity was calculated according to [14] and the values were expressed as μ moles of fructose 1, 6 diphosphate cleaved per mg protgein^{-1}h^{-1} .

Statistical Analysis

For each parameter, the mean of individual observations (for both control and experimental groups) were taken into consideration statistical significance of the data was analyzed through one way ANOVA.

Fig 1: Effect of FK 506 on rat tissue phosphorylase 'a' activity levels *in vivo*



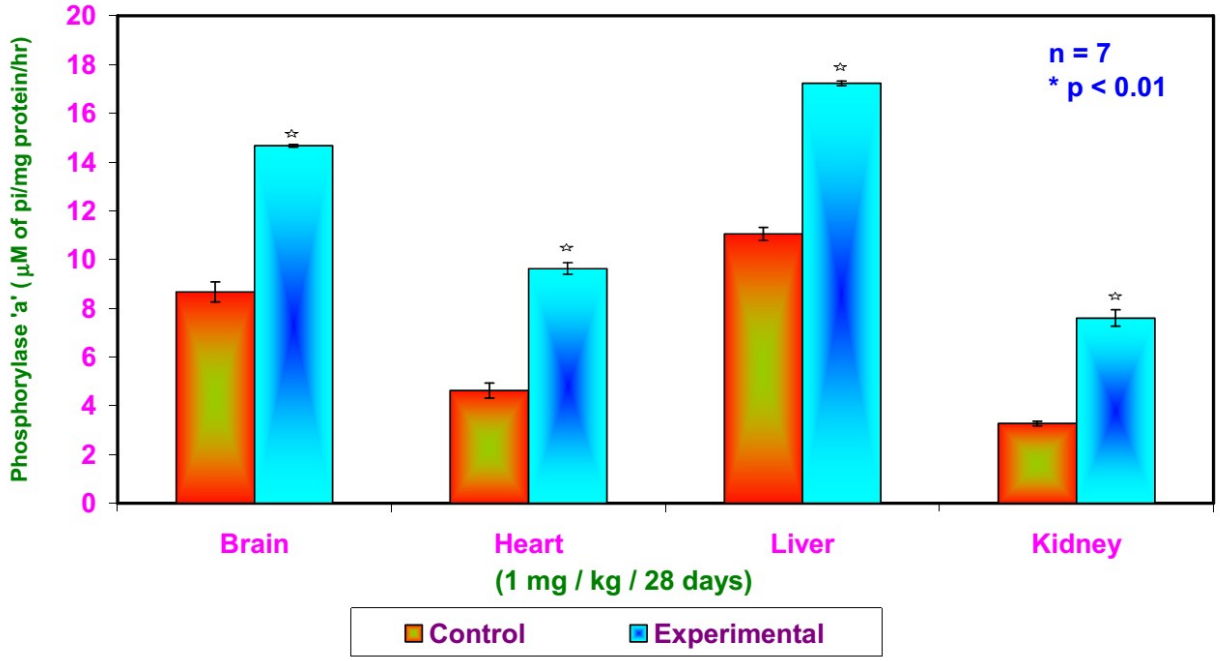


Fig. 2: Effect of FK 506 on rat tissue Phosphorylase 'a' activity levels in vivo

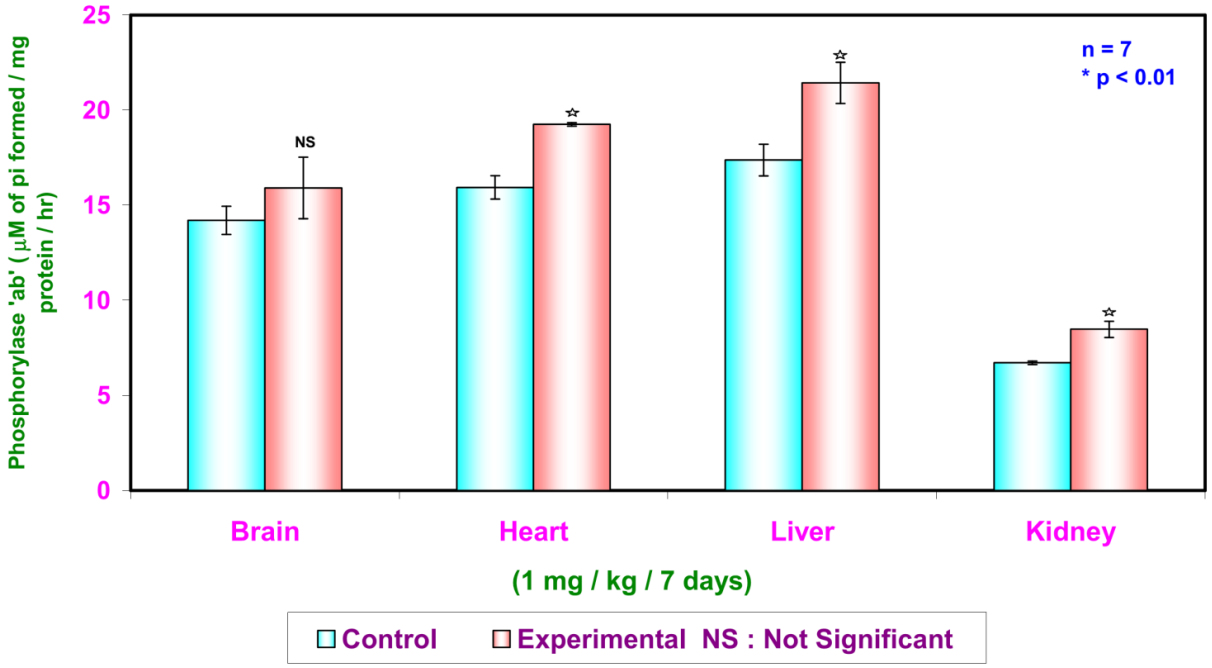


Fig. 3: Effect of FK 506 on rat tissue Phosphorylase'ab'activity levels in vivo

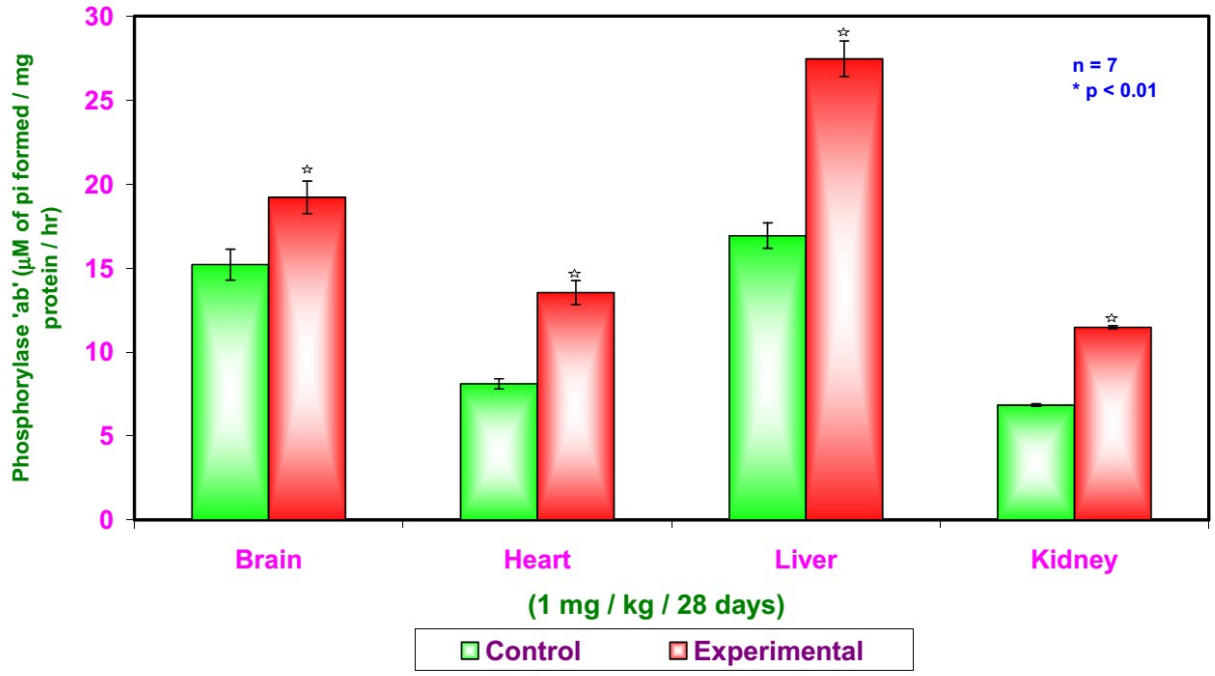


Fig. 4: Effect of FK 506 on rat tissue Phosphorylase'ab'activity levels in vivo

RESULTS

In control rat tissues phosphorylase 'a' activity was maximum in the liver tissue followed by brain > heart > kidney (Table 1; Fig 1 & 2). FK 506 increased the phosphorylase 'a' activity in both 7 and 28 days treated groups of rat tissues and the changes were found to be time and dose in a dependent and further the changes were found to be statistically significant ($p < 0.01$) over the control. The total phosphorylase activity was found to be enhanced in rat tissues by FK 506 administration (Table 1; Fig 3 & Fig 4) and in control tissues the phosphorylase 'ab' activity was highest in liver and was followed by brain > heart > kidney. All the changes were found to be statistically significant over their control groups ($p < 0.01$). The alterations in aldolase activity of selected tissues from FK 506 treated rats in shown in Table 1; Fig 5 and 6. Statistically significant ($P < 0.01$) increases in rat tissue aldolase activities were observed in both 7 and 28 days FK 506 treated rat tissues over the corresponding control tissues

(Table 1; Fig 5 Fig 6). In control tissues, liver exhibited the highest aldolase activity followed by brain > heart > kidney (Table 1).

Succinate dehydrogenase (SDH) levels were measured in control and FK 506 treated groups of rat tissues, the data shown in Table 2; Fig 7 and 8. The FK 506 treated rat tissues exhibited lowered levels of SDH activity. The tissue specific trend in the control tissues was higher for brain followed by liver > heart > kidney. All the changes were found to be statistically significant ($P < 0.01$) over their controls. The percent changes observed were greater for 28 days FK 506 treated group of rats compared to 7 days FK 506 treated ones for brain 51.64%, for heart 47.03%, for liver 64.48% and for kidney 60.22% (Table 2). Malate dehydrogenase (MDH) and lactate dehydrogenase (LDH) activity levels in control and experimental rat tissues are also measured. Both of the enzymatic activities were affected in FK 506 treated rat tissues. Both 7 and 28 days of FK 506 treatment produced increased levels of MDH and LDH activities compared to their corresponding controls and the changes were found to be statistically significant ($P < 0.01$) (Table 2, Fig. 9-12). With regard to percent depletion it was greater for 28 days FK 506 treated rat tissues compared to 7 days FK 506 treated ones. The tissue specific trend of MDH in the control groups was brain > heart > kidney, and for LDH followed by liver > kidney> brain (Table 2).

DISCUSSION

The present study further demonstrated that FK 506 treatment of rat tissues exerted levels of increased in the LDH activity (Table 2). The results are in agreement with the report of [15]. Similar trends were also reported by Loreal *et al*., (1991) [9]. The reports of earlier authors show an increased serum glucose levels upon immuno suppressive agents treatment [16]. This has been attributed due to altered insulin secretion due to FK 506 treatment. In the present study, glycogen content decreased in all tissues of albino rats treated with FK 506. The percent reduction is much higher in the liver compared to other tissues [16]. Catecholamines can also elevate the concentrations of plasma glucose and lactate. Thus Hyperglycemia induced by catecholamines is attributable in part to activation via cyclic AMP of hepatic glycogen phosphorylase. This enzyme converts glycogen to glucose phosphate, the rate limiting step in glycogenolysis [5]. The observed depletion of phosphorylases (Table 1) may be due to the role of cyclic AMP which acts as both an activator of phosphorylases (Table 1) and an inhibitor of glycogen synthetase.

Two phosphorylases are responsible for the breakdown of glycogen. These phosphorylases are known to exist in two forms, namely phosphorylase ‘a’ and phosphorylase ‘b’. The former being the active form while the later is inactive [17]. Agreeing with the decrease in the tissue glycogen content in FK 506 treated rat tissues, the tissue phosphorylase activities increased in all FK 506 rat tissues. Phosphorylase ‘a’ is known to be active in the

absence of AMP while phosphorylase 'b' is active only in the presence of AMP. Both these phosphorylases are isolated in crystalline form from various sources [18]. Phosphorylase activity in different tissues increased following FK 506 administration (Table 1). It is well known that the increase in phosphorylase activity is dependent on cAMP synthesis, the enzyme responsible for the formation of cAMP namely adenylcyclase should increase in activity level during the early stages. The rapid depletion of the glycogen content and a significant increase of aldolase in the FK 506 treated tissues (Table 1) indicates effective enhancement of glycolysis in the tissues. The increase in the aldolase activity suggests not only the enhancement of the glycolytic pathway but also the operation of HMP pathway, possibly, like an intermediary type of metabolism to cope with excess energy demands due to FK 506 toxic stress. The enzyme aldolase is well studied in connection with its localization, structure and mechanism of action [19]. Aldolase activity is increased in a variety of disease conditions like haemorrhagic pancreatitis [20] muscular dystrophy [21]. Few experimental results were available with regard to FK 506 affecting normal functions of the pancrease [22, 23]. The present observed trend of FK 506 altering the activity of rat tissue aldolase activity, however, is not merely restricted to pathological conditions induced upon the rat tissues by FK 506; but further is also known to be influenced by FK 506 intoxifications as evidenced by disturbances in the carbohydrate metabolism [24]. Under toxic stress the shift in equilibrium is more towards the right [25] resulting in more formation of dihydroxyacetone phosphate, which is later converted to glyceraldehyde-3-phosphate.

SDH is a marker enzyme of Krebs cycle, is tightly bound to the inner membrane of mitochondria, and catalyses the removal of a protein from succinate reducing FAD [26]. This too may be one of the reasons for decreased SDH levels in FK 506 treated rat tissues. In the present investigation MDH activity was found to be inhibited in rat tissues due to FK 506 treatment. Since the decrease in SDH activity is known to be associated with the reduction in other oxidative enzymes like MDH. The decrease in SDH activity is consistent with the decrease in MDH activity (Table 2). Nevertheless, the general decrease in the MDH activity in the rat tissues under FK 506 stress the oxidative metabolic status, perhaps due to the decrease in the rate of O_2 consumption probably that results as a consequence of mitochondrial damage under FK 506 stress [27] MDH functions in the bidirectional transport of reducing equivalents from extra mitochondrial NADH into the mitochondrial respiratory chain, operating through the malate asparatase shuttle. FK 506 treated rat tissues may experience a similar situation. LDH has been reported to be as good as an indicator of myocardial infraction as in the serum glutamine oxaloacetic acid transaminase. LDH also increase in acute myocardial in fraction [28]. Defective carbohydrate metabolism severely limits the ability for high intensity and ischemic exercise [29]. Increased LDH activity indicates pyruvate conversion to lactate. LDH plays an important role in carbohydrate metabolism and catalyzes the Inter coversion of lactate and pyruvate [30]. This is dependent on a heterogenous group of components, called as isoenzymes, whose quantitative changes are significant during certain pathological conditions [31, 32]. In the present investigation, the LDH profile strongly

suggests the prevalence of anaerobic oxidation. The LDH activity increases during conditions favouring anaerobic respiration to meet the energy demands.

CONCLUSION

Phosphorylase, a, ab and aldolase activities in rat brain, heart, liver and kidney tissues were measured in short and long term FK 506 treated rat tissues. The enzyme activities were found to be elevated in all tissues treated with FK 506 (Table 1; Fig:1-2). The increased level of cAMP might be responsible for increased Phosphorylase activities. The increase in the aldolase activity (Fable 1; Fig 3 & 4) in FK 506 administered rat tissues suggests increased glycolysis and operation of HMP pathway. The decreased SDH activity levels of FK 506 administered rat tissues indicate the depression of oxidative metabolism. The data on enzymes of carbohydrate metabolism in control and FK 506 treated tissues of rat was presented in Table 2; Fig: 5 & 6.

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