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Research Article

EFFECT OF FK 506 ON SELECTED ENZYMATIC ACTIVITY LEVELS INVOLVED IN PROTEIN METABOLISM IN ALBINO RAT TISSUES

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ABSTRACT

Background: The plant Gmelina arborea has been traditionally used in India for several medicinal purposes like anthelmintic, diuretic, anti-inflammatory, antibacterial and antidiabetic. Aims: The aim of the present study is to explore the anthelmintic and antidiabetic activities of G. arborea bark extracts using ethanol, ethyl acetate, n-butanol and petroleum ether as solvents. Material and methods: The extracts were screened for phytochemical constituents and evaluated for their toxicity. The anthelmintic activity was evaluated on adult Indian earthworms, Pheretima posthuma. The antidiabatic activity of above extracts was evaluated in alloxan induced diabetic model of Wistar rats. Statistical analysis used: All data are verified for statistically significant by using one way ANOVA at 1 % level of significance ($p < 0.01$). Results and discussion: The tests for cardiac glycosides and steroids were positive for all the extracts. The ethanol and n-butanol extracts were containing most phytochemicals where as ethyl acetate extracts was containing least number of phytochemicals. All extracts were found to be non toxic to the living body. All extracts were able to show anthelmintic activity at 10 mg/ml concentration and well are comparable with the standard drugs such as piperazine citrate and albendazole. Among all the solvent extracts the n-butanol extract showed better anthelmintic activity even in comparison with both the standard drugs. All the extracts were able to reduce sugar level in blood. Ethanol extract was found to have good antidiabetic activity in comparison to other extracts. Conclusion: It can be concluded that the bark extracts of G. arborea possess anthelmintic and antidiabetic activities.

Keywords: Gmelina arborea, bark, anthelmintic, piperazine citrate, diabetes, alloxan.

INTRODUCTION

Proteins are the most abundant organic constituents and they are concerned with the structural organization and functional dynamics of the living organism [1]. The levels of tissue proteins are determined by their rates of synthesis and degradation resulting in continuous renewal which is known as protein turnover [2]. Free amino acids are essential and needed for protein synthesis and energy production purposes [3].

The proteinase enzymes function in the breakdown of proteins. Protein levels in animals are known to be regulated by proteinases and thus it is worth attempting in FK 506 treated rats to assess the physiological capabilities of these enzymes associated with protein catabolism (under FK 506 stress). The asparatate and alanine amino transferase reactions serve as strategic links between the carbohydrate and protein metabolism under altered physiological conditions and also under environmental stress. The aminotransferases are link enzymes that channel aminoacids for energy derivation [4]. Since ALAT gives rise to pyruvate, it can be used to represent the segment of carbohydrate metabolism. There are reports of derangement in cellular structure [5-6] histochemical and pathological changes, including impairment of normal functions of AAT and ALAT in immuno suppressive agents administered experimental models [7-8]. Hence the author measured the protease and aminotransferase activities in rat tissues exposed to FK 506 and the data obtained is presented under this paper.

Materials and methods:

Collection and growing Animals

Albino rats weighing 150 ± 10 gm 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 ± 5°C. 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.

Treatment of FK 506 with Rats:

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.

Assay of protease activity

Protease activity of the control and experimental tissues was estimated by the method of Moore and Stein (1954) [9]. Homogenates 5% (W/V) of individual tissues was prepared in ice-cold distilled water. The homogenates were centrifuged at 1000 xg for 15 min. The supernatants were used for enzyme assay. The mixture of 2 ml contained 100 µ moles of phosphate buffer (pH 6.8) 12mg of heat denatured hemoglobin as substrate and 0.5 ml of the homogenate supernatant. The contents were incubated at 37^{0}C for 15 min. The reaction was stopped by adding 2 ml of 10% TCA. The unincubated samples were treated with 2.0 ml of 10% TCA prior to the addition of the enzyme source. The contents of both incubated and unincubated samples were filtered and the free aminoacid content was determined in the filtrates. To 0.2 ml of the filtrate 2.0 ml of ninhydrin reagent was added and heated in boiling water both for 5 min and then cooled. The volume was made up to 10ml with distilled water. The colour absorbance was measured at 570 nm against a reagent blank in a spectrophotometer. All samples are corrected for zero time controls. The proteolytic activity is expressed as µ moles of tyrosine equivalents mg protein^{-1} h^{-1}.

Estimation of Asparate aminotransferase (AAT) (L-asparate-2-oxoglutarate aminotransferase; EC: 2.6.1.1)

L-asparate aminotransferase (AAT) was assayed by the method of Reitman and Frankel (1957) [10]. Tissue Homogenate 5%

(W/V) of each tissue was prepared in 0.25M sucrose solution and centrifuged at 1000xg for 15 min. The supernatants were used for assay. The reaction mixture of 2.0ml contained 100µ moles (1 ml) of potassium phosphate buffer (pH 7.4), 60µ moles of (0.3ml), L-aspartic acid (pH 7.4), 2µ moles (0.2ml) of α-ketoglutaric acid (pH 7.4), and 0.5 ml of the supernatant (enzyme source). After incubating for 15 minutes

at 37^0C the reaction was stopped by the addition of 1.0 ml of 2,4dinitrophenyl hydrazine solution and allowed to stand at room temperature for 20 minutes. Zero time controls were maintained for all samples by adding 1.0 ml of 2,4dinitrophenyl hydrazine solution prior to the addition of the supernatant. The colour was developed by adding 10ml of 0.4N NaoH and read at 546 nm in a spectrophotometer against a blank. The blank preparation is the same as that of experimental except the corresponding volume of distilled water was substituted for the supernatant. The AAT activity was expressed as μ moles of pyruvate formed mg protein^{-1} h^{-1}.

Estimation of Alanine Aminotransferase (ALAT) (DL – alanine 2–oxoglutarate aminotransferase EC:2.6.1.2

Tissue Homogenates 5% (W/V) were prepared in 0.25M cold sucrose solution and centrifuged at 1000 xg for 15 min. The supernatants were employed for the assay. The reaction mixture of 2.0 ml contained 100 μmoles of phosphate buffer, 60 μmoles of DL-alanine (pH 7.4), 2 μ mole of α-ketoglutarate (pH 7.4), and 0.5 ml of supernatant (enzyme source). After incubation for 15 minutes at 37^0C, the reaction was stopped by adding 1.0 ml of 2,4 dinitrophenyl hydrazine solution and allowed to stand for 20 min at room temperature. Zero time controls were maintained for all the samples by adding 1ml of 2,4 dinitrophenyl hydrazine solution prior to the addition of the supernatant 10ml of 0.4N NaoH was added to both experimental and zero time control tube and the colour was read at 546 nm in a spectrophotometer against a blank. The blank preparation is the same as that of experimental, except the corresponding volume of distilled water substitutes the homogenate. The ALAT activity was expressed as μ moles of pyruvate formed / mg protein^{-1}h^{-1}.

Table 1: Effect of FK506 on rat tissue protease (μM of tyrosine formed/mg protein/hr), AAT (μM of pyruvate formed/mg protein/hr) and ALAT(μM of pyruvate formed/mg protein/hr) activity levels *in vivo*.

Name of the enzyme	7 DAYS (1mg FK506/Kgwt)								28 days (1mg FK506/kg wt)							
	Brain		Heart		Liver		Kidney		Brain		Heart		Liver		Kidney	
	Control	Expt.	Control	Expt.	Control	Expt.	Control	Expt.	Control	Expt	Control	Expt.	Control	Expt.	Control	Expt.
Protease AV	0.544 ± 0.041	0.563 ± 0.023	0.371 ± .016	0.383 ± 0.024	0.786 ± 0.014	0.935 ± 0.011	0.283 ± 0.009	0.289 ± 0.012	0.501 ± 0.036	0.573 ± 0.043	0.382 ± 0.024	0.652 ± 0.075	0.81 ±0.052	1.86 ± 0.021	0.291 ± 0.024	0.642 ±0.018
SD PC		3.492%		3.23%*		18.95%*		2.07%		14.37% *		70.68% *		129.62% *		120.61% *
AAT AV	2.43 ± 0.021	2.99 ± 0.016	2.12 ± .162	2.84 ± 0.095	2.24 ± 0.096	5.10 ± 0.059	2.36 ± .046	2.91 ± 0.0630	2.44 ± .0832	4.14 ± 0.211	2.32 ± 0.072	4.61 ± 0.037	3.51 ± 0.096	6.41 ± 0.063	2.34 ± 0.37	3.05 ±0.021
SD PC		23.04% *		33.96% *		127.67% *		23.30%		69.67% *		98.70% *		82.62%*		30.34%*
ALAT AV	2.81 ± 0.044	3.04 ± 0.036	3.40 ± 0.030	4.91 ± 0.042	3.61 ± 0.066	5.08 ± 0.075	2.35 ± .021	2.96 ± 0.017	2.83 ± 0.013	4.44 ± 0.064	3.42 ± 0.052	5.03 ± 0.091	3.66 ±0.055	6.96 ± 0.06	2.33 ± 0.033	4.45 ± 0.071
SD PC		8.18%*		44.41% *		38.41%*		25.95% *		56.89% *		47.07% *		90.16%*		90.98%*

Each value is the mean ± SD of 7 samples ; AV: Average, SD: Standard Deviation, PC: Percent change over the control, *: p<0.01

RESULTS

Following FK 506 administration, both the 7 and 28 days FK 506 treated rat tissues exhibited elevated levels of their protease activity (Table 1, Fig 1 & 2) and the changes were found to be statistically significant ($p < 0.01$) over the control.The tissue specific protease activity in the control rat tissues was found to be more in liver, and was followed by brain > heart > kidney (Table 1).The percent elevation was greater for 28 days FK 506 treated group of rat tissues.

The data presented in Table 1 further shows that FK 506 treated rat tissues showed increased levels of AAT and ALAT levels over the controls (Table 1; Fig 3 & 4). Both the transaminase activities followed tissue specific trends in control group of rat tissue, where liver showed the highest activity of both AAT and ALAT and was followed by heart > brain > kidney. The enhanced transaminase activity levels in FK 506 administered tissues were found to be statistically significant ($p < 0.01$) over the control. For AAT and ALAT, the 28 days FK 506 treated rat tissues showed the highest percent increases in all rat tissues (Table 1).

DISCUSSION

The present study demonstrated that FK 506 treatment of rats not only alters rat tissues protein content but also changes the associated enzyme systems involved in catabolism of proteins.

Proteins are one of the most important constituents of tissues and their role in the regulation of cellular activities and cell membranes are well known [11]. Treatment of animals with environmental / pharmacologic agents is known to produce pathological lesions associated with increased proteolysis [12]. The decrease in tissue protein content upon FK 506 treatment could mean either inhibition in protein synthesis or an increase in proteolytic activity.

Lysosomal enzymes are released in free and active form liver during shock and hemorrhages. The increased proteolytic activity in FK 506 treated rat tissues may be due to stimulation of interecellular protease [13]. It has been shown that increase in protein content stimulate hydrolytic activity and inhibits oxidative enzyme activity, increased proteolytic activity may be due to leakage of hydrolase from the lysosomes due to FK-506 stress which in turn may enhance the proteolysis and these results are in agreement with the findings of earlier authors as reported for the agent CsA.

Increase in FAA content in FK 506 administered rat tissues can be reckoned as an indication of stress [14-15]. An elevation in the tissue FAA content upon FK 506 stress may favour glyconeogenetic pathways to meet extra energy demands to mitigate FK 506 stress. It has been shown that the microsomal enzyme systems namely the mixed function oxidases (MFO) increase in vertebrate tissues under xenobiotic toxicity [16].

FK 506 treatment of rat has induced elevation of rat tissue AAT and ALAT activities in the tissues of albino rat (Table 1). The asparate and alanine aminotransferase function as link between carbohydrate and protein metabolism by catalyzing in inter conversion of the strategic compounds like α-ketoglutaric acid to oxaloacetic acid to glutamic acid respectively [3] The increased transaminase level corresponds for the active transamination of amino acids to keto acids which in turn are fed into the TCA cycle through gluconeogenesis [17]. Like metabolic situation seems to prevail in rat tissues under FK 506 stress.

The liver is the major organ for all metabolic activities in the body and it contains a fuel component of all enzymes [1] and hence its high protease AAT and ALAT activity levels may be justified. In the brain tissue of the control rats the ALAT activity was found to be more than ALAT (Table 1). Since both AAT and ALAT are linked enzymes and both function in the TCA cycle, it is not important which predominates over the other because they function in gluconeogenesis and will change their activity depending on the physiological or stress conditions [18]. The elevated transaminase levels may be related to the elevated presser amine levels [19]. Impairment of AAT and ALAT activities with immunosuppressant administration was reported previously by few authors [7-8].

From the above, it is evident that FK 506 administration to albino rat tissues induces marked changes in enzyme systems that are associated with protein and amino acid metabolism.

CONCLUSION

The changes in enzymes involved in protein metabolism were presented in this dissertation. FK 506 treatment caused significant elevation of the protease AAT and ALAT activity levels of all the tissues studied *in vivo* (Table: 1; Fig: 1-6). The increased protease activity was attributed to increased tissue proteolytic activity of the rat tissues upon FK 506 stress and this is supported by a decrease in FK 506 treated tissue total

proteins and elevated FAA. In addition, increased AAT and ALAT levels in rat tissues treated with FK 506 was reported due to active transaminatation of amino acids to keto acids which in turn will be fed into the TCA cycle to overcome FK 506 stress by the rat tissues.

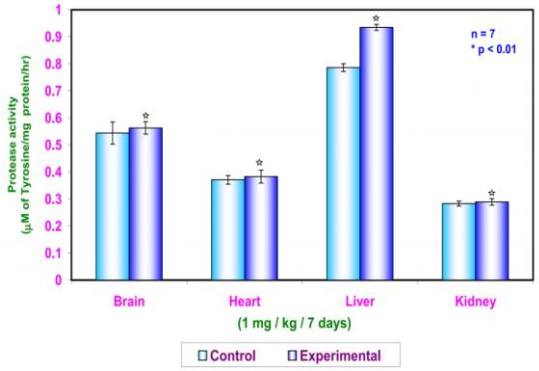

Fig 1: Effect of FK 506 on rat tissue Protease activity levels *in vivo*

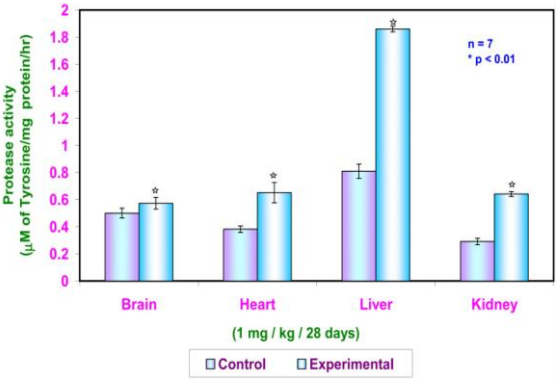

Fig 2: Effect of FK 506 on rat tissue Protease activity levels *in vivo*

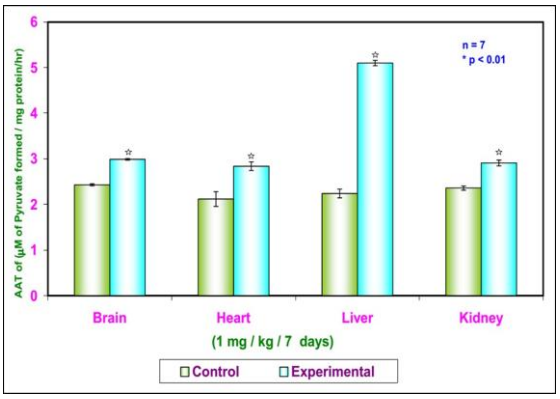

Fig 3: Effect of FK 506 on rat tissue AAT activity levels *in vivo*

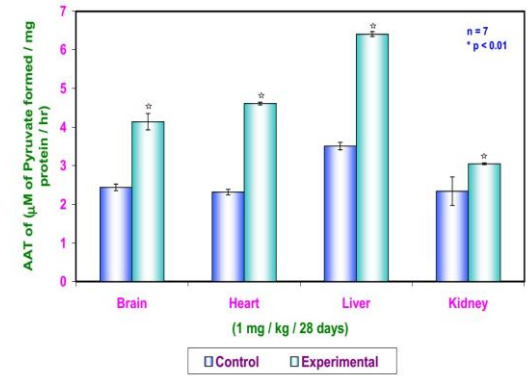

Fig 4: Effect of FK 506 on rat tissue AAT activity levels *in vivo*

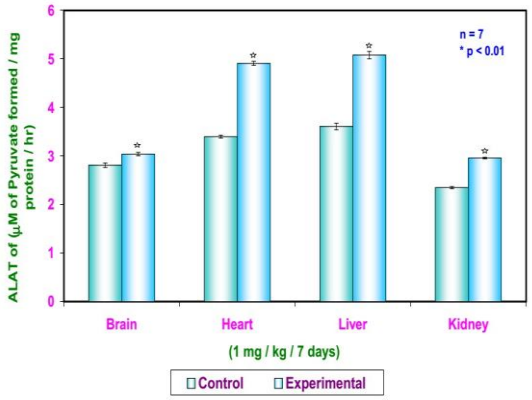

Fig 5: Effect of FK 506 on rat tissue ALAT activity levels *in vivo*

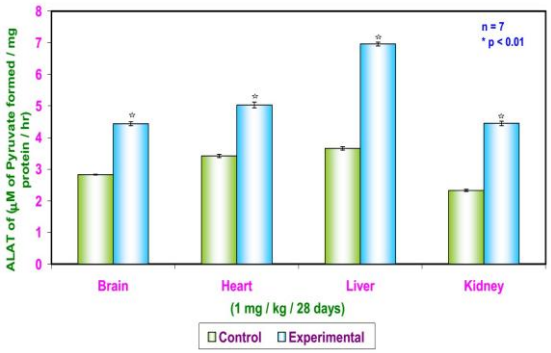

Fig 6: Effect of FK 506 on rat tissue ALAT activity levels *in vivo*

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