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Diclofenac induced toxic manifestations on adjuvant induced arthritic rats pheripheral and reproductive organ of male wistar rats rattus norvegicus

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Non-Steroidal Anti-inflammatory Drugs (NSAIDs) are the most frequently prescribed therapeutic agents, used for the treatment of rheumatic diseases, because they have analgesic, antipyretic and anti-inflammatory actions. Diclofenac (Voltaren) is a well-known member of the acetic acid family of NSAIDs, used to reduce inflammation and pain associated with arthritis osteoarthritis, and ankylosing spondylitis. There is considerable interest in the toxicity of diclofenac because of its clinical use and for the study of the mechanisms of nephrotoxicity, renal dysfunction, hematotoxicity and hypersensitivity reactions. In the present study we have reported the basic metabolites and activities of enzymes were studied in different tissues against adjuvant and diclofenac sodium treatment. The change due to the adjuvant treatment was attributed to the inflammation associated changes and the further alteration in diclofenac sodium was implicated to its toxic manifestation, besides the drug side effect is caused by metabolic idiosyncrasy.

Key words: Inflammation, adjuvant arthritis, metabolites, enzymes.

INTRODUCTION

Non-Steroidal Anti-inflammatory Drugs (NSAIDs) are the most frequently prescribed therapeutic agents, used for the treatment of rheumatic diseases, because they have analgesic, antipyretic and anti- inflammatory actions, which are mediated by inhibition of the biosynthesis of prostaglandins. The NSAIDs are heterogeneous group of compounds, often chemically unrelated, but mostly organic acids (Brooks and Day, 1991; Boelsterli et al., 1995). The precise mechanisms by which NSAIDs exert their chemo-preventive effects are not fully explained, but likely involve inhibition of cyclo-oxygenase, the enzyme that converts arachidonic acid to prostaglandins. By inhibiting prostaglandins, NSAIDs may indirectly enhance immune responses. In addition, they may up regulate expression of major histo-compatibility complex antigens.

Diclofenac (Voltaren) is a well-known member of the acetic acid family of NSAIDs, which includes seven others, such as etodolac (Lodine), indomethacin (Indocin), ketorolac (Toradol), nabumetone (Relafen), sulindac (Clinoril), and tolmetin (Tolectin) and used to reduce inflammation and pain associated with arthritis, osteoarthritis, and ankylosing spondylitis, this rapidly metabolized mainly by hepatic hydroxylation and subsequent conjugation (Hardman et al., 1995).

There is considerable interest in the toxicity of diclofenac because of its clinical use and for the study of the mechanisms of nephrotoxicity, renal dysfunction, hematotoxicity and hypersensitivity reactions. This non-steroidal drug has been a prime suspect in causing cell injury due to its ability to covalently bind to macromolecules in situations where intracellular levels of NADH, NADPH, GSH, and other reducing agents are very low. Covalently bound protein adducts of diclofenac have been detected in hepatic tissues of mice and rats, as well as in human hepatocytes. Diclofenac is eliminated following conjugation by sulfate and glucuronic acid. Excretion and accumulation of conjugates have been correlated to renal function and end-stage renal disease. Usually after detoxification pathways are impaired, and if metabolite(s) are formed in excessive amounts, they can cause cell injury leading to cell death. Concomitantly, diclofenac causes potentially severe liver injury, which may be due to its

Table 1. Estimation of pentose sugar level in male rat peripheral and reproductive organ.

	Control	FCA	FCA+FIA	DS
	Mean ± S.D	Mean ± S.D	Mean ± S.D	Mean ± S.D
µg of pentose sugar/100 mg of wet tissue of *Rattus norvegicus*				
Liver	0.492 ± 0.060	0.817 ± 0.183	0.270 ± 0.150	1.901 ± 0.595
Kidney	0.055 ± 0.008	0.128 ± 0.020	0.075 ± 0.018	0.119 ± 0.031
Testis	0.066 ± 0.013	0.060 ± 0.019	0.095 ± 0.011	0.053 ± 0.023*

bio-activation leading to the formation of reactive oxygen species, such as, O_2, HO, and H_2O_2 and NO.

Studies also investigated the potential involvement of these free radicals and their after-effects during diclofenac-induced nephrotoxicity in vivo. Although researchers have made some progress in understanding diclofenac-induced mechanisms of organ toxicity, the precise pathways of nephrotoxicity still remain obscure. Many clinical studies have reveal that a variety of immune and non-immune mechanisms contribute towards development of toxicity. In the present study adjuvant induced arthritic alteration and diclofenac sodium treatment associated changes in some tissues of the Wistar rats were determined in understanding the inflammation related metabolic changes in those organs.

MATERIALS AND METHODS

Male Wistar rats weighing between 180 - 220 g were purchased from the animal house, King Institute, Chennai. Animals without any symptoms of wound or lesion in the skin or legs were selected for the study. They were housed one per cage with 12 h light: 12 h dark normal photo period regime in the laboratory.

Pellet feed was provided *ad libitum* to the rats. *Mycobacterium tuberculin* suspension was prepared in the concentration of 2 mg/ml, were supplied by the Division of Microbiology, Tuberculosis Research Centre – Indian council of Medical Research (TRC-ICMR), Chennai. The Freund's incomplete adjuvant (FIA) was supplied by the Department of Antitoxin, King Institute and Chennai.

To make the Freund's Complete Adjuvant (FCA) 10mg/ml heat killed *M. tuberculin* suspension was mixed with 5 ml of FIA to get 5 ml of FCA. Four groups of six animals, one representing the control and another three test groups were selected for the study. The sham control animals were injected with 0.2 ml of double distilled water at the right leg footpad on day '0' and kept for 21 days.

To the test group rats 0.2 ml of FCA on day '0'was injected was taken as complete adjuvant treated rats (FCA), and same dose of IFA was injected on day '7' at the same site as booster dose, was taken as complete with incomplete adjuvant (FCA+FIA) treated rats, in the same FCA+IFA treated rats 10µl of diclofenac sodium given intramuscularly for every five days, totally four doses starts from day "0" up to the day 15, in the interval of five days was taken as Diclofenac sodium (DS) treated rats, both FCA, FCA+FIA and DS treated rats were kept under constant observation from day 1 up to 21 days. On day 22 both control and test rats were mildly anesthetized by using chloroform inhalation liver, kidney and testes were removed for the biochemical analysis.

The total carbohydrate content viz., pentose and hexose, Protein bound sugars viz., pentose and hexose were estimated by the Dobosis et al. (1957) method. The Ketose sugar content was estimated by the phenol-boric acid- sulphuric acid Borotinski (1984) method. Lactic acid content was estimated by Barker and Summerson (1941) method. Friedmann and Haugen (1943) method was followed for the estimation of Pyruvic acid. Uric acid levels were estimated using spectrophotometry method coined by Mayne (1994). The total lipid levels were estimated by using the method of Folch et al., (1957). Free fatty acid level was estimated using the method given by Doncomb (1963). The water content was estimated using the method coined by Ramalingam et al. (1980). Acid and Alkaline phosphotase enzymes activities were assayed following the procedure adopted by Tenniswood et al. (1976). The LDH activity was determined following the procedure of King (1965). GOT and GPT was estimated by using Rietman and Frankel (1957) method Cited In: Witter and Grubbs (1966). The results were calculated for mean, standard deviation (SD) and analysis of variance (ANOVA) was performed by using Zar (1974) method.

RESULTS

Elevated free sugars (pentose and hexose) in both FCA and FCA+FIA treated rats of all regions found invariably. Diclofenac sodium treatment showed elevated levels of the free sugar metabolites only with liver and kidney regions (Tables 1 and 3). The protein bound metabolites levels found decreased in all regions except in FCA+FIA of kidney and testis regions. Besides to the Diclofenac treatment the level was found further decreased when compared to the control (Tables 4 and 5).

Elevated pyruvic level noted in both adjuvant treated groups, but diclofenac sodium treatment showed decreased content. The lactic acid level found increased with FCA group liver, FCA+FIA liver and kidney and with diclofenac sodium treatment in the testis. The lactate/pyruvate ratio found elevated in liver and kidney and decreased in Kidney region of FCA group (Tables 6 - 8).

Total lipid level found decreased invariably in all the regions of in both adjuvant groups. Diclofenac sodium treatment showed increase in kidney and testis and further to control (Table 9). Free fatty acid level increased in liver and kidney and testis of FCA treatment, but FCA+FIA treatment showed decrease. Diclofenac sodium treatment showed decrease in the liver, kidney and testis (Table 10). FCA, FCA+FIA treatment showed increased uric acid level. DS treatment showed decreased level in liver but increased with kidney and testis (Table 11).

ACP activity showed increase in FCA, FCA+FIA and decreased with DS except increased with testis of DS treatment (Table 12). ALP activity was found decreased in the liver of FCA group, but showed elevation in other

Table 2. Estimation of hexose sugar level in male rat peripheral and reproductive organ.

	Control	FCA	FCA+FIA	DS
	Mean ± S.D	Mean ± S.D	Mean ± S.D	Mean ± S.D
µg of hexose of /100 mg of wet tissue of *Rattus norvegicus*				
Liver	0.840 ± 0.140	1.465 ± 0.228	0.402 ± 0.297	1.853 ± 0.578
Kidney	0.055 ± 0.008	0.117 ± 0.020	0.069 ± 0.017	0.121 ± 0.033
Testis	0.018 ± 0.012	0.048 ± 0.009	0.035 ± 0.014	0.069 ± 0.023*

Table 3. Estimation of ketose sugar level in male rat peripheral and reproductive organ.

	Control	FCA	FCA+FIA	DS
	Mean ± S.D	Mean ± S.D	Mean ± S.D	Mean ± S.D
µg ketose sugar/100 mg of wet tissue of *Rattus norvegicus*				
Liver	0.125 ± 0.047	0.045 ± 0.008	0.237 ± 0.048	0.151 ± 0.085
Kidney	0.013 ± 0.008	0.033 ± 0.007	0.012 ± 0.006	0.019 ± 0.010
Testis	0.009 ± 0.004	0.025 ± 0.009	0.007 ± 0.001	0.008 ± 0.04*

Table 4. Estimation of protein bound pentose sugar level in male rat peripheral and reproductive organ.

	Control	FCA	FCA+FIA	DS
	Mean ± S.D	Mean ± S.D	Mean ± S.D	Mean ± S.D
µg of protein bound pentose sugar/100 mg of wet tissue of *Rattus norvegicus*				
Liver	1.140 ± 0.118	0.567 ± 0.368	1.082 ± 0.069	0.969 ± 0.044
Kidney	0.379 ± 0.035	0.323 ± 0.029	0.445 ± 0.016	0.293 ± 0.050*
Testis	0.442 ± 0.019	0.323 ± 0.029	0.543 ± 0.020	0.283 ± 0.039*

FCA-Freund's complete adjuvant; FIA-Freund's incomplete adjuvant; DS-Diclofenac sodium.
* Significance at $P < 0.05$ level and all values are mean ± S.D of original six observations.

Table 5. Estimation of protein bound hexose sugar level in male rat peripheral and reproductive organ.

	Control	FCA	FCA+FIA	DS
	Mean ± S.D	Mean ± S.D	Mean ± S.D	Mean ± S.D
µg of protein bound hexose sugar/100 mg of wet tissue of *Rattus norvegicus*				
Liver	1.048 ± 0.003	0.485 ± 0.042	0.708 ± 0.074	0.715 ± 0.054
Kidney	0.317 ± 0.053	0.249 ± 0.021	0.402 ± 0.017	0.202 ± 0.036*
Testis	0.344 ± 0.073	0.230 ± 0.022	0.484 ± 0021	0.219 ± 0.031*

Table 6. Estimation of pyruvic acid level in male rat peripheral and reproductive organ.

	Control	FCA	FCA+FIA	DS
	Mean ± S.D	Mean ± S.D	Mean ± S.D	Mean ± S.D
µg of pyruvic acid/100 mg of wet tissue of *Rattus norvegicus*				
Liver	172.3 ± 69.3	202.0 ± 77.05	190.15 ± 43.91	35.56 ± 19.42
Kidney	112 ± 52.5	243 ± 82	190 ± 43	35.56 ± 03.88*
Testis	130 ± 20.6	207 ± 10.4	326 ± 10	35.56 ± 9.96*

Table 7. Estimation of lactic acid level in male rat peripheral and reproductive organ.

	Control	FCA	FCA+FIA	DS
	Mean ± S.D	**Mean ± S.D**	**Mean ± S.D**	**Mean ± S.D**
µg of lactic acid/100 mg of wet tissue of *Rattus norvegicus*				
Liver	03.88 ± 1.35	11.66 ± 6.23	11.10 ± 4.39	4.40 ± 1.17
Kidney	8.33 ± 1.81	7.21 ± 3.89	12.77 ± 5.74	3.88 ± 1.35
Testis	4.99 ± 2.78	4.40 ± 1.70	4.99 ± 0.78	19.42 ± 4.75

Table 8. Estimation of percentage of lactate/pyruvate ratio in male rat peripheral and reproductive organ.

	Control	FCA	FCA+FIA	DS
	Mean ± S.D	**Mean ± S.D**	**Mean ± S.D**	**Mean ± S.D**
% of lactate/pyruvate ratio/100 mg of wet tissue of Rattus norvegicus				
Liver	0.022 ± 0.009	0.057 ± 0.011	0.058 ± 0.009	0.123 ± 0.019
Kidney	0.073 ± 0.016	0.029 ± 0.017	0.067 ± 0.021	0.109 ± 0.026
Testis	0.038 ± 0.009	0.021 ± 0.007	0.015 ± 0.007	0.028 ± 0.007

FCA-Freund's complete adjuvant; FIA-Freund's incomplete adjuvant; DS-Diclofenac sodium.
* Significance at $P < 0.05$ level and all values are mean ± S.D of original six observations.

Table 9. Estimation of total lipids level in male rat peripheral and reproductive organ.

	Control	FCA	FCA+FIA	DS
	Mean ± S.D	**Mean ± S.D**	**Mean ± S.D**	**Mean ± S.D**
µg of total lipids/100 mg of wet tissue of Rattus norvegicus				
Liver	0.538 ± 0.114	0.383 ± 0.040	0.359 ± 0.033	0.459 ± 0.058
Kidney	0.194 ± 0.032	0.154 ± 0.037	0.160 ± 0.020	0.273 ± 0.026*
Testis	0.194 ± 0.032	0.154 ± 0.037	0.160 ± 0.020	0.273 ± 0.019*

Table 10. Estimation of free fatty acid level in male rat peripheral and reproductive organ.

	Control	FCA	FCA+FIA	DS
	Mean ± S.D	**Mean ± S.D**	**Mean ± S.D**	**Mean ± S.D**
µg of free fatty acids/100 mg of wet tissue of Rattus norvegicus				
Liver	0.618 ± 0.032	2.328 ± 0.974	0.523 ± 0.037	0.342 ± 0.034
Kidney	0.630 ± 0.038	1.410 ± 0.420	0.325 ± 0.047	0.094 ± 0.036*
Testis	0.568 ± 0.011	1.164 ± 0.636	0.361 ± 0.026	0.501 ± 0.036*

Table 11. Estimation of uric acid level in male rat peripheral and reproductive organ.

	Control	FCA	FCA+FIA	DS
	Mean ± S.D	**Mean ± S.D**	**Mean ± S.D**	**Mean ± S.D**
µg of uric acids/100 mg of wet tissue of Rattus norvegicus				
Liver	0.213 ± 0.078	0.547 ± 0.054	1.231 ± 0.057	0.142 ± 0.090
Kidney	0.189 ± 0.073	0.261 ± 0.017	0.782 ± 0.063	0.285 ± 0.039
Testis	0.189 ± 0.073	0.308 ± 0.016	0.403 ± 0.041	0.213 ± 0.075*

Table 12. Activity of acid phosphatase enzyme in male rat peripheral and reproductive organ.

	Control	FCA	FCA+FIA	DS
	Mean ± S.D	**Mean ± S.D**	**Mean ± S.D**	**Mean ± S.D**
µg of ACP/PNPP to PNP/protein/100 mg of wet tissue of Rattus norvegicus				
Liver	0.494 ± 0.098	0.690 ± 0.072	1.326 ± 0.108	0.428 ± 0.040
Kidney	0.318 ± 0.098	1.089 ± 0.085	1.131 ± 0.101	0.305 ± 0.002*
Testis	0.316 ± 0.071	0.515 ± 0.085	0.446 ± 0.094	0.365 ± 0.033*

FCA-Freund's complete adjuvant; FIA-Freund's incomplete adjuvant; DS-Diclofenac sodium.
* Significance at $P < 0.05$ level and all values are mean ± S.D of original six observations.

Table 13. Activity of alkaline phosphatase enzyme in male rat peripheral and reproductive organ.

	Control	FCA	FCA+FIA	DS
	Mean ± S.D	**Mean ± S.D**	**` Mean ± S.D**	**Mean ± S.D**
µg of ALP/PNPP to PNP/protein/100 mg of wet tissue of Rattus norvegicus				
Liver	1.407 ± 0.035	0.571 ± 0.036	2.202 ± 0.249	0.529 ± 0.033
Kidney	0.935 ± 0.099	2.203 ± 0.424	3.598 ± 0.421*	4.828 ± 0.784
Testis	0.935 ± 0.099	2.915 ± 0.063	3.818 ± 0.045*	1.880 ± 0.290

Table 14. Activity of lactate dehydrogenase enzyme in male rat peripheral and reproductive organ.

	Control	FCA	FCA+FIA	DS
	Mean ± S.D	**Mean ± S.D**	**Mean ± S.D**	**Mean ± S.D**
µg of LDH/protein/100 mg of wet tissue of Rattus norvegicus				
Liver	49.37 ± 08.63	63.01 ± 34.95	59.62 ± 20.14	16.21 ± 04.09
Kidney	104 ± 7.048	56.3 ± 14.5	98.7 ± 0.14*	20.01 ± 06.91
Testis	95 ± 13	73 ± 4.38	79 ± 4.28*	12.42 ± 2.64

regions of both adjuvant treated rats. Diclofenac sodium showed elevation in kidney and testis (Table 13). LDH activity was found increased in the liver region of both test groups. Bit all other groups showed overall decreeased activity (Table 14).

FCA of liver and testis regions showed decreased GOT activity, but kidney showed increase. FCA+FIA treatment showed increased activity in the kidney and testis regions. But in the liver the activity showed decrease. Diclofenac sodium treatment showed elevated activity in kidney and testis (Table 15). GPT activity showed decrease in all the regions tested of FCA, FCA+FIA treatment, however in the DS treatment the activity was found elevated in the liver region compared to control and FCA+FIA treatment. But the kidney and testis showed further decrease (Table 16).

DISCUSSION

Free and bound sugars

The hepatotoxicity and renal toxicity are considered as a common characteristic of the NSAIDs. There is no clear statement whether this side effect is caused by a "metabolic idiosyncrasy" or "immunological idiosyncrasy. "As to diclofenac, one of the commonly used NSAIDs, a recent clinical evaluation of the cases reported to the US Food and Drug Administration suggested that the patients with hepatotoxicity by the drug had very few sign of "immunological idiosyncrasy" (Banks et al., 1995), I*n vitro* studies with hepatocytes prepared from the experimental animals have directly shown that some NSAIDs are cytotoxic to the hepatocytes at a concentration close to the respective

Table 15. Activity of GOT enzyme in male rat peripheral and reproductive organ.

	Control	FCA	FCA+FIA	DS
	Mean ± S.D	**Mean ± S.D**	**Mean ± S.D**	**Mean ± S.D**
Units of GOT/protein/100 mg of wet tissue of Rattus norvegicus				
Liver	108.61 ± 21.72	88.26 ± 07.57	98.00 ± 01.65	105.7 ± 08.99
Kidney	97.50 ± 07.40	101 ± 81.9	113.20 ± 08.47	113.2 ± 16.73
Testis	48.40 ± 05.91	40 ± 6.54	55.00 ± 08.88	60.93 ± 11.41

Table 16. Activity of GPT enzyme in male rat peripheral and reproductive organ.

	Control	FCA	FCA+FIA	DS
	Mean ± S.D	**Mean ± S.D**	**Mean ± S.D**	**Mean ± S.D**
Units of GPT/protein/100 mg of wet tissue of Rattus norvegicus				
Liver	253.50 ± 54.60	191.33 ± 63.34	222.85 ± 10.55	288.10 ± 11.73
Kidney	235.0 ± 16.40	81.00 ± 06.36	128.00 ± 13.50	86.83 ± 07.15
Testis	62.50 ± 24.20	49.50 ± 14.20	69.60 ± 08.44*	36.10 ± 12.80

FCA-Freund's complete adjuvant; FIA-Freund's incomplete adjuvant; DS-diclofenac sodium.
* Significance at $P < 0.05$ level and all values are mean ± S.D of original six observations.

therapeutic ranges (Akesson and Akesson, 1984; Sorensen and Acosta, 1985; Castell et al., 1988; Jurima-Romet et al., 1994; Helfgott et al., 1990; Sallie, 1990; Ouellette et al., 1991). The mechanism by which diclofenac causes alteration in liver metabolism in individuals is not yet fully understood. Both the formation of a toxic metabolite and covalent binding of the drug to hepatic proteins have been invoked to explain its toxicity.

Studies with cultured hepatocytes suggested that diclofenac hepatotoxicity depended on its metabolism, that is, a reactive metabolite and intermediate were responsible for the cytotoxicity (Jurima-Romet et al., 1994). Kretz-Rommel and Boelsterli (1993) proposed oxidative metabolism rather than reactive metabolite of diclofenac (Hargus et al., 1994; Kretz and Boelsterli, 1994) was involved in the cytotoxicity of diclofenac. This indicates that the drug itself caused the cytotoxicity, but not by its reactive metabolite, although the possibility remains that the concentrations of the inhibitors were insufficient to inhibit diclofenac metabolism, because they were limited due to their own cytotoxicity. These findings are proposed mechanism that the NSAIDs trigger as the uncoupler of mitochondrial oxidative phosphorylation and reduce cellular ATP contents indicate that the hepatotoxicity of the NSAIDs is "structure dependent" rather than "metabolism dependent (Cannon et al., 1991). Although in some case reports, the adverse hepatic effects of diclofenac showed features compatible with a drug hypersensitivity reaction (Breen et al., 1986; Schapira et al., 1986; Salama et al., 1991), and direct toxic effect of the drug metabolite (Helfgott et al., 1990; Sallie, 1990; Iveson et al., 1990). Changes in ATP turnover rate was supported by increases in the rate of anaerobic glycolysis support the view that pro-inflammatory mediators impose a metabolic demand; these cells may be more susceptible to dysfunction on the basis of diminished oxygen delivery and/or mitochondrial dysfunction (Berg et al., 2003).

The involvement of oxidative enzymes and non-oxidative enzymes in the granulocytes in human rheumatoid arthritis revealed that the ratio of activities of oxidative and non-oxidative enzymes of Pentose-phosphate pathway of carbohydrate metabolism was altered in the plasma cell and the cells of the patients with systemic impairment of connective tissue disorders (Shishinken et al., 1988). Gluconeogenesis, glycolysis and glycogenolysis were studied in rat liver following the infusion of two non-steroidal anti-inflammatory drugs (NSAIDs), diclofenac, and aspirin, they strongly stimulating glycogenolysis and glycolysis (GGL/GL) and increased oxygen consumption by the liver tissue. Such response can be attributed to the uncoupling effects of the two drugs on oxidative phosphorylation. Henderson et al. (1978) implicated elevated Pentose-shunt activity in the synoviocytes of human rheumatoid arthritis to the enhanced potential of glucose-6-phosphate utilization. Moreover, hyperglycemia is associated with increased collagenase and protease activity (Theule, 1996). The supply of energy is through appropriate nutrients to site of injury seemed to be necessary means for restoring the proper cellular metabolism, wound healing, immune competence and proper organ function (Lin et al., 1998; Rasschart et al., 1990). In view of the elevated levels of the carbohydrate intermediates like pentose, hexose and ketose and decreased protein bound carbohydrates in peripheral and reproductive organs of diclofenac treatment against control and adjuvant treatment infer proinflammatory mediators impose meta-

bolic demand response of increased in anaerobic glycollysis and also to restoration of normal cellular function and immediate energy source to meet the antigen-induced stress. It suggests that diclofenac sodium induced oxidative phosphorylation and thereby reduce oxidative content causing demand for energy metabolites. It implicate the depletion of macromolecules of by diclofenac sodium toxicity in the present study such that cytotoxicity of diclofenac sodium treatment. Further it may be understood that continuous depletion of ATP thereby increased chance of anaerobic glycolysis.

Pyruvic and lactic acid

The aerobic and anaerobic scope of an animal depends upon the residual levels of pyruvate and lactate content in the body tissue and consequent clearance of accumulated lactate anions after short burst of activity. Tissue exhibits differential ability to accumulate as well as to eliminate the lactate anions through specific pathways during stress (Stetson and Good, 1951). Hyperlactemia is observed commonly in patients with severe inflammation, it is released in large quantity from the sites of inflammation. The high rate of production of lactate anions suggests that the lactate output from different tissue may be due to inflammation and by the different cell population activities. However studies support the hypothesis that lactates may be more a product of inflammation than a marker of tissue hypoxia (Haji et al., 1999). Some soluble mediators in the extra vascular tissues attributed to the role as permeability factor, however arthritic reaction is associated with permeability response and leukocyte activation.

Gobelet and Gerster (1984) noticed high lactate content in sero-positive rheumatoid arthritis and correlated the lactic acid concentration to synovial fluid pH, and also the increase in lactate/pyruvate ratio to increase in the free cytoplasmic NADH/NAD+ ratio. Diclofenac has been a prime suspect in causing cell injury due to its ability to covalently bind to macromolecules in situations where intracellular levels of NADH, NADPH, GSH, and other reducing agents are very low. Goolveld et al. (1991) reported alterations in the non-protein bound low molecular mass metabolites such as acetate, citrate, lactate and glutamine represent markers of reactive oxygen radical activity during inflammation of the joints. Elevated plasma lactate concentrations may be caused by cytokine-mediated alterations in specific organ systems responsible for lactate homeostasis. The elevated lactate/pyruvate ratio correlates to the increased NADH/NAD ratio. Elevated lactic acid level in adjuvant treated (FCA) and with diclofenac sodium treated group reveals heperlactemia situation. This implicates upon lactate permeability responses to migration and recruitment of leukocytes to sites of inflammation. Concomitantly polymorphs derived lymphokines can also be attributed to hyperlactemia. Elevated lactate levels in diclofenac sodium treatment further suggest macromolecules depletion besides diclofenac can bind with cellular macromolecules, such mechanism corroborate the idea that alterations in metabolites may be related to characteristics of inflammation.

Lipids and free fatty acids

Lipids comprise one of the most important classes of complex molecules present in animal cells and tissues. The cells, tissues and organs are determined by the processes of lipids metabolism, which include lipids transport, consumption and intracellular utilization, de nova synthesis, degradation and excretion. The process of lipid metabolism requires the involvement of numerous proteins with different functions. These proteins together with their genes are the components of the lipid metabolism system. The interest for the lipid metabolic system is due to its important role in the vital activity of the organism and to the fact that the distortion in its functioning are among the causes of different human diseases.

Lipid metabolites produced by phospholipase action and subsequent intracellular modifications are extensively involved in cellular signaling process and produce wide range of physiological and pathological effects including inflammatory response, cell differentiation and proliferation. The inflammatory and destructive changes are brought by the mediators of local hormones, which are in turn produced by a variety of cell types and lipid mediators (Naughton, 1993). That the altered fatty acid profile is likely to be the cause of inflammation (Haugen, 1994) and its attribution in initiating the lipid mediators of inflammation, such as platelet activating factors synthesis, cyclo-oxygenase and lipo-oxygenase enzymes suggest the significance of lipids and free fatty acids in FCA induced inflammation (Sperling, 1991).

Clinical studies have revealed that pro- inflammatory cytokines like Interferon-alpha, IL-1 and IL-6 released from the inflammatory tissue, which in turn stimulates the hypothalamic–pituitary–adrenal axis and cause the release of Corticotrophic Releasing Hormone (CRH), epinephrine (EN) and nor-epinephrine (NEN) which could cause lipolysis. Diclofenac at therapeutic concentrations are potent inhibitors of PGE2 and IL-6 production (Henrotin et al., 1989). Good correlation between in vitro PGE2 inhibition and in vivo activities for diclofenac and its metabolites indicating that inhibition of prostaglandin synthesis is a major mechanism responsible for their pharmacological actions (Boettcher et al., 2001).

Decreased total lipid in adjuvant treatment and increased in diclofenac sodium treatment noticed in the present study. Free fatty acid level increased in liver and kidney and testis of FCA treatment, but FCA+FIA treatment showed decrease.

Diclofenac sodium treatment showed decrease in the liver, kidney and testis. This speculates the diclofenac pharmaco-toxicological behaviour and further strengthening the significance of lipids and free fatty acids role in inflammation beside their role in synthesis in lipid mediators.

Uric acid

Purine nucleotide degradation generally was attributed for the increase of uric acid production (Asplin, 1996). Uric acid production was correlated besides to deficiency of renal function, and also to neurological dysfunction. It was also revealed that anaerobic environment represents a predisposing factor for the acceleration of purine nucleotide degradation (Yamanaka et al., 1992). Most likely, the compartmentalization of purine nucleotide catabolism is linked to different nucleotides formation sites, but it is essentially involved in an extremely fine, controlled regulation of nucleotide degradation, as required by the very important role that such compounds play the vital role in the cell. In this way, nucleotide synthesis, degradation, and export of final products can be compared to the behaviour of all other well known antithetical metabolic pathways and reactions, such as glycogen synthesis and breakdown, fatty acids synthesis and b -oxidation, hexokinase and glucose-6-phosphate phosphatases activity, which are either differently compartmentalized or submitted to extremely fine regulation, or else occur at diffe-rent times. In addition pharmacological agents also affect the uric acid concentration either by increasing its production or by decreasing its excretion (Banks et al., 1995).

Elevated uric acid level in both adjuvant groups and decreased against Diclofenac treatment in the present study, implies the nucleoside and nucleotide pathways. Though the uric acids is attributed as one of metabolic factors in arthritogenesis in the specific tissue foci as in the case of osteoarthritis and rheumatoid arthritis. It may be construed that the uric acid formed may be transported to systemic circulation to reach the target tissue foci viz., musculoskeletal region. In this context, uric acid is being transported as protein bound urate or as 3-ribosl-uric acid from one part of the body to another through blood is of interest to mention here (Tojo et al., 1968; Wiltmore and Gillbert, 1972). In this present study the elevated uric acid levels in kidney and testis of diclofenac treated rats implicate upon diclofenac sodium toxicity. Besides uric acid production corroborated to renal deficiency and neurological dysfunction along with protein nucleotide degradation.

Enzymes

Abundance of lysosomal enzymes in some tissues and the abnormal fragility of the enzymes and their discharge have been implicated in a number of pathological phenolmena involving inflammatory process including rheumatoid arthritis (Dingle, 1961; Rao et al., 1980). Lysosomal mechanisms represent the prime factor in the chronic erosion of rheumatoid arthritis (Stephens, 1995). The phosphatase being of lysosomal origin, its marked increase in the tissue may signify its role in inflammation. The drop in pH associated with anaerobic metabolism and phagocytosis were correlated to the increased release of lysosomal enzymes (Lussier et al., 1984). Moreover the activation of neutrophils is by the infiltration of soluble and insoluble materials by surface receptors leading to the release of oxygen-free radicals which could be attributed to bring about the inflammation (Gale et al., 1984). Moore et al. (1994) have demonstrated the immune complexes in the superficial layer of rheumatoid arthritic cartilage have suggested that, they can provide an anchorage and a trigger of polymorphs activation. Its function in inflammation is further strengthened by the reports that other proteolytic enzymes like cathepsin, collagenase and plasminogen activators etc., and their activity may blocked by the lower pH that prevails in the *tissues in vivo* (Eeckout and Vals, 1975). It could be as a result of damage from reactive oxygen species or proteolytic enzymes or a combination of both.

On the other hand, the enzymes which were found to complex with immunoglobulins, were LDH, ALP. Such complexes were seen in liver, bone, placenta and intestinal isoenzymes. The constitution studies after papain digestion showed that the association site of the enzymes was in Fab' portion immunoglobulins (Mackawa et al., 1986). The encounter of neutrophils with immune complexes and complement components in the bulk phase or on a surface leads to their secretion of lysosomal hydrolases, especially neutral proteases, which provoke tissue injury. Thus, the secretion of lysosomal enzymes and generation of reactive oxygen species is a part of a stimulus-secretion response to immune complexes and complement components. Concomitantly immune complex involvement in the activation of neutrophils and consequent release of hydrolytic enzymes also well understood Gale et al. (1984). The macrophage phagocytosis may result in cell lysis and release of intra-cellular lactate dehydrogenase enzymes (Missieh, 1996). However changes in the activity of LDH enzyme correlated to glycolytic activity and arachidonic acid metabolism suggest that inhibitors of cyclooxy-genase and lipoxygenase pathways exert a direct protective effect against the hypoxia/reoxygenation induced cell injury (Kim et al., 2003).

The changes in the activity of transaminases imply the changes in the concentration of glutamate and aspartate in the extra-cellular environment. Such alterations of their concentrations by the adjuvant may be expected since both glutamate and aspartate are employed in many metabolic pathways (Bradford, 1985), since the liver is the main organ involved in the metabolism and the toxicity of xenobiotics. Isolated rat hepatocytes have been in-creasingly used in recent years as a model to identify pharmacological and toxicological responses of drugs. However, it is generally recognized that isolated hepatocytes retain most of their functions only for a short period. Lactic dehydrogenase (LDH), glutamic-oxalacetic transaminase (GOT), glutamic-pyruvic transaminase (GPT) increase will be taken as serum markers of liver injury seem to for liver cell damage induced by oxygen free radicals and the efficacy of drug (Donnelly et al., 1998). GPT activity is at-

tributed indication of diclofenac-associated hepatotoxicity might be more common than previously recognized (Ciccolunghi et al., 1978). This ranges from mild, transient elevations in serum transa-minases to pronounced heaptocellular and/or cholestatic injury, which rarely lead to fatal fulminant hepatitis. Elevated activity of ACP, LDH and decreased ALP, GPT and GOT to adjuvant treatment was attribute the inflammation induced due to the administration of the adjuvant and further changes such as ALP elevation and elevated activity of GOT in kidney and testis and GPT in the liver region due to diclofenac activity implicate the drug toxicity. Several fold decrease of LDH and GPT in diclofenac sodium treatment can be taken as toxic manifestation of the drug. The degenerative changes in the liver region due to the adjuvant and diclofenac sodium treatment and necrotic changes observed in the testis imply the inflammatory and drug toxic manifestations.

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