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RESEARCH ARTICLE

AN EXPERIMENTAL STUDY ON ALBINO RATS TO COMPARE THE HEPATOPROTECTIVE EFFECT OF ORALLY ADMINISTERED DL-METHIONINE AND N-ACETYLCYSTEINE ON THE LIVER INJURY CAUSED BY POSITIVE CONTROL DRUG DICLOFENAC SODIUM

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Abstract

Introduction: Liver, main organ for biotransformation of drugs, during which most of the drugs which are lipophilic in nature are made hydrophilic by the biochemical processes in the liver cells resulting into water-soluble products which get excreted in urine or bile. Adverse drug reactions (ADRs) pertaining to liver, can be of mainly two types, predictable or dose dependent and idiosyncratic. To counter the oxidative stress and cellular damage, several antioxidant enzymes have been developed including superoxide dismutase (SOD), Glutathione peroxidase and catalase, that detoxify the reactive oxygen species and the cells contained endogenous antioxidants that scavenge the free radicals and reduces the cellular damage of which glutathione plays a major role. Synthesis of glutathione is regulated at the substrate level by cysteine, which is synthesized from homocysteine via the trans-sulfuration pathway N-Acetylcysteine used as a specific drug in the treatment of Paracetamol overdose, serves as a prodrug to L-Cysteine, which is a precursor to the antioxidant Glutathione. L-methionine, a precursor of L-cysteine, which is considered to have antioxidant activity, is found to be a precursor to glutathione as well. Methionine is particularly important in opposing the toxicity of free radicals generated by various toxins. Hence, supplementation of the same has been proposed to have a greater role in reducing the toxic effect on liver. The aim was to study the comparative hepatoprotective effect of orally administered DL-Methionine and N-Acetylcysteine on the liver injury induced by positive control Diclofenac Sodium.

Materials and Methods: The healthy albino rats were grouped for the experimental study, in various groups (n=6). After overnight fasting, rats belonging to Group I Vehicle treated Group (n=6) were administered Distilled Water 10 ml/kg orally; Group II to V were administered single oral dose concomitantly with Diclofenac sodium (96 mg/kg & 240 mg/kg p.o.) and DL-Methionine (700 mg/kg p.o. & 1400 mg/kg p.o.) respectively. Group VI and VII were administered single oral dose concomitantly with Diclofenac sodium (96 mg/kg & 240 mg/kg p.o.) and N-Acetylcysteine 450 mg/kg p.o., respectively. Serum samples from each rats were collected after 24-hours of post-treatment, to study the comparative effects of DL-Methionine and N-

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Acetylcysteine on the liver injury induced by positive control Diclofenac Sodium through Haemato-biochemical changes.

Results: The vehicle-treated group showed no significant alteration in the level of liver function tests and liver morphology. On concomitant administration of DL-Methionine 700 mg/kg with Diclofenac sodium 96 mg/kg and 240 mg/kg it was observed that there occurred significant reduction ($p < 0.001$) in the serum SGOT and SGPT levels as compared to control and positive control group. The hepatoprotective effect of DL-Methionine 700 mg/kg and N-Acetylcysteine 450 mg/kg on concomitant administration with positive control drug Diclofenac sodium 96 mg/kg were compared for their effect on serum SGPT levels and was observed that there was no statistically significant decrease in the levels of SGPT when compared between the two groups of hepatoprotective agents. The hepatoprotective effect of DL-Methionine 700 mg/kg and N-Acetylcysteine 450 mg/kg on concomitant administration with positive control drug Diclofenac sodium 240 mg/kg, were compared for their effect on serum SGPT levels. It was observed that there was a significant reduction ($p <0.05$) in SGPT levels, with DL-Methionine 700 mg/kg and N-Acetylcysteine 450 mg/kg. However, in both the doses of DL-Methionine, there were no statistically significant changes in the Total Serum Bilirubin, serum Alkaline Phosphatase & serum Gamma-Glutamyl Transpeptidase (GGTP) levels.

Conclusion: We have compared for the effectiveness of DL-Methionine and N-Acetylcysteine for their hepatoprotective effect against Diclofenac-induced hepatotoxicity, where we have observed, both DL-Methionine and N-Acetylcysteine reduces the serum transaminases levels, that were elevated due to the hepatotoxic effect of diclofenac sodium. However, there was no much of a difference of hepatoprotective effect of both DL-Methionine and N-Acetylcysteine. In summary, our present study reinforced the concept that hepatotoxicity induced by drug can be protected by administering NAC even in cases of non-paracetamol induced liver injury.

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Introduction:-

Liver, main organ for process of biotransformation of drugs, during which most of the drugs which are lipophilic in nature are made hydrophilic by the biochemical processes in the liver cells resulting into water-soluble products which get excreted in urine or bile. Adverse drug reactions (ADRs) pertaining to liver, can be of mainly two types, predictable or dose dependent and idiosyncratic. Premarketing recruits a relatively low number of patients, frequently insufficient to fully judge the true incidence of hepatotoxicity [1,2]. The number of drugs associated with adverse reactions in the form of liver injury is extensive [3]. It has been observed that patient who suffers with hepatotoxicity due to one Non-Steroidal Anti-Inflammatory Drugs, often is susceptible to the same type of reaction on rechallange or on administering the drug belonging to the same group (sister-drug) e.g. Diclofenac and Tiaprofenic acid [4]. To counter the oxidative stress and cellular damage, several antioxidant enzymes have been developed including superoxide dismutase (SOD), Glutathione peroxidase and catalase, that detoxify the reactive oxygen species and the cells contained endogenous antioxidants that scavenge the free radicals and reduces the cellular damage of which glutathione plays a major role [5-7].

Synthesis of glutathione is regulated at the substrate level by cysteine, which is synthesized from homocysteine via the trans-sulfuration pathway. Many agents including N-Acetylcysteine (NAC), Silymarin, Antioxidants, S-Adenosine Methionine, Ursodeoxycholic acid, or a combination of these have been used in the treatment of drug induced liver injury and other forms of liver toxicity [5-7]. There is therefore the growing need to research into liver diseases and hepatoprotective remedies.

N-Acetylcysteine was introduced in the 1970s and still is the only clinical antidote against paracetamol-induced liver injury. Later, it got established as an effective and safe treatment for Paracetamol toxicity, which was also analyzed for its effectiveness in the treatment of non-paracetamol liver failure for which N-acetylcysteine was found to be hepatoprotective [9, 10]. L-methionine, a precursor of L-cysteine, which is considered to have antioxidant activity, is found to be a precursor to glutathione as well. Methionine is particularly important in opposing the toxicity of free radicals generated by various toxins [6].

The aim was to study the comparative hepatoprotective effect of orally administered DL-Methionine and N-Acetylcysteine on the liver injury induced by positive control Diclofenac Sodium.

Liver is involved in almost all biochemical pathways to growth, immunity, nutrition and reproduction, also known to degrade/metabolize the drugs, enzymes, hormones, cytokines and various other chemicals. **Hepatotoxicants** are exogenous compounds of clinical relevance and may include overdoses of certain medicinal drugs, industrial chemicals, natural chemicals (microcystins), herbal remedies and dietary supplements [11,12]. Non Steroidal Anti Inflammatory Drugs (NSAIDs) such as Diclofenac, used as both prescriptive and Over The Counter (OTC) medications, causing a risk of 1-8 cases per 100000 patients per year use of NSAIDs causing liver injury [13, 14]. Diclofenac is Non-Steroidal Anti-Inflammatory Drug (NSAID), indicated in the relief of all grades of pain and inflammation associated with a wide range of conditions, including arthritic conditions, acute musculo-skeletal disorders and other painful conditions resulting from trauma [15]. Diclofenac and its metabolites undergo glucuronic acid and sulphate conjugation mediated by UDP-Glucuronosyl Transferase (UGT) 2B7 [16]. The study of Haemato-biochemical and Histopathological changes help in detecting the injury to the hepatocytes. Synthesis of glutathione, the most abundant mammalian antioxidant, is regulated at the substrate level by cysteine, which is synthesized from homocysteine via the trans-sulfuration pathway. Glycine-n-methyltransferase is most abundant in the liver [16, 17]. Hence, supplementation of the same has been proposed to have a greater role in reducing the toxicity in liver diseases [5].

Drug-induced liver injury (DILI) is a potential complication of many drugs. This is not surprising given the fact that the liver plays a central role in drug metabolism [13-16].

Materials & Methods:-

Ethical approval:

The study was conducted after the research protocol (no. SVU/PH/IAEC/25-04-2009/01) was accepted and approved by the Institutional Animal Ethics Committee (IAEC), (CPCSEA Reg. No.: 947/PO/Re/S/06/CPCSEA, Dated 30-06-2006) of S. B. K. S. Medical Institute and Research Centre, Sumandeep Vidyapeeth Deemed to be University, Piparia, Vadodara; which is registered under Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment, Forest & Climate change, Govt. of India, New Delhi.

Experimental Study Design and Methodology [21, 24-28]:

Experimental study was conducted in Young healthy albino rats of either sex weighing 150 – 400 gm body weight which were quarantined before starting the study, and were housed in poly-propylene rat-cages, rice husk used as bedding, acclimatized and were given free access to food and purified drinking water, ad libitum. They were housed under a controlled ambient temperature (24° ± 2°C), relative humidity (55% ± 5%), and in a 12-hour light-/dark rhythm; throughout the experiment. Study was conducted for duration of one year, between May 2015 and August 2015.

The drugs used were Diclofenac sodium (DFN), DL-Methionine (MET) and N-Acetylcysteine (NAC), and the chemicals included 10% Formalin, Xylene, Hemotoxylin and Eosin stains. To evaluate the levels of liver enzymes[24-28], the diagnostic kit reagents (Erba Diagnostics, Manheim) of serum Glutamic-Pyruvic Transaminase (SGPT), Serum Glutamic-Oxaloacetic Aminotransferases (SGOT), Serum Alkaline Phosphatase, Serum bilirubin – Direct and Indirect Bilirubin, Total Bilirubin; Serum Gamma-Glutamyl Transpeptidase (GGTP) were used. Gross appearance of liver and determination of liver morphology changes, after each drug administration, was studied after liver removed after dissection.

Other equipments used such as Digital weighing balance, intragastric cannula, Collecting glass tubes, light ether anaesthesia, glass capillary tube, cotton, distil water, normal saline, syringe, glass instruments, glass petridish,

labeled specimen collection jars, dissection box and various misc. equipments used for biochemical estimation of liver enzymes and histopathological studies.

Experimental Study Groups:
The albino rats were grouped for the experimental study, in various groups as shown in **[Table number 1].**

Table no. 1:- Experimental Study Design.

Sr. No.	Groups	n	Treatment	Duration of treatment
	Group I: Control	6	Distilled Water (Vehicle treated) 10 ml/kg body weight [18]	24 hours overnight fasting
	Group II: Positive control drug	6	Positive control drug Diclofenac sodium 96 mg/kg p.o. [19] + DL-Methionine 700 mg/kg p.o. [20, 21,22]	24 hours overnight fasting
	Group III: Positive control drug	6	Positive control drug Diclofenac sodium 240 mg/kg[19] + DL-Methionine 700 mg/kg p.o. [20, 21,22]	24 hours overnight fasting
	Group IV: Positive control drug	6	Positive control drug Diclofenac sodium 96 mg/kg p.o. [19] + DL-Methionine 1400 mg/kg p.o. [20, 21,22]	24 hours overnight fasting
	Group V: Positive control drug	6	Positive control drug Diclofenac sodium 240 mg/kg [19] + DL-Methionine 1400 mg/kg p.o. [20, 21,22]	24 hours overnight fasting
	Group VI: Positive control drug	6	Positive control drug Diclofenac sodium 96 mg/kg[19] + N-Acetylcysteine 450 mg/kg p.o.[23]	24 hours overnight fasting
	Group VII: Positive control drug	6	Positive control drug Diclofenac Sodium 240 mg/kg[19] + N-Acetylcysteine 450 mg/kg p.o.[23]	24 hours overnight fasting

Photographs no. 1 – 8:- The plan of work and methodology:

Photo 1. Weighing albino rat

Photo 2. Weighing of drugs

Photo 3a & Photo 3b. Preparation of drug solutions for oral drug administration

Photo 4.Isolated rat liver samples

Photo 5. Wax-block preparation of liver

Photo 6. Tissue processing

Photo 7. Wax block slicing for slide preparation

Photo 8:- Prepared histopathology slides.

Results & Discussion:-

Statistical analysis:

All the observed data were collected and entered in the Microsoft excel sheet. Values to be compared were analyzed statistically. All results were expressed as Mean ± SEM. All calculations were performed using statistical software SPSS version 21.0 computer-based. Results were compared and analyzed by using repeated measures Analysis of Variance (ANOVA) and post hoc and values were considered to be significant when P values were less than or equal to 0.05 (**$p \leq 0.05$**).

Study of hepatoprotective effects of DL-Methionine by concomitant administration of positive control group:

After overnight fasting, as mentioned in **Table no. 1,** the albino rats (n=6 in each group) belonging to group II, III, IV, V were administered DL-Methionine[20, 21,22,29] and positive control drug Diclofenac sodium[19] concomitantly and in group VI & VII N-Acetylcysteine[23] with positive control drug Diclofenac sodium concomitantly; dose and route as depicted in **Table no. 1**. Group I was administered distilled water [18].

The volume administered was maintained at 10 ml/kg [18] in all the experimental animals. Following this, 24 hours later the blood samples were collected by glass capillary method from retro orbital plexus of eye[21] and the serum was separated after centrifugation method at 3000 rpm and was preserved at − 20° C temperature till further analysis. The serum samples were then analyzed for the estimation of the liver enzymes.

As mentioned in **Table no. 2** shows the comparison of serum SGPT levels after concomitant administration of hepatoprotective agents and positive control groups.

Table no. 2:- Comparison of serum SGPT levels after concomitant administration of hepatoprotective agents and positive control groups:

Sr. No.	Group (n=6)	Mean ± SEM
1.	Control DW 10 ml/kg	32.83 ± 2.91
2.	DL-Methionine 700 mg/kg	50.33 ± 7.06
	N-Acetylcysteine 450 mg/kg	39.00 ± 2.70
1.	Diclofenac sodium 96 mg/kg + DL-Methionine 700 mg/kg	57.17 ± 5.19
	Diclofenac sodium 96 mg/kg + N-Acetylcysteine 450 mg/kg	44.67 ± 5.17
2.	Diclofenac sodium 240 mg/kg + DL-Methionine 700 mg/kg	69.17 ± 3.57*
	Diclofenac sodium 240 mg/kg + N-Acetylcysteine 450 mg/kg	51.50 ± 6.08*
3.	Diclofenac sodium 96 mg/kg	147.67 ± 13.72
	Diclofenac sodium 96 mg/kg + DL Methionine 1400 mg/kg	43.00 ± 4.25***
4.	Diclofenac sodium 240 mg/kg	236.50 ± 24.01
	Diclofenac sodium 240 mg/kg + DL Methionine 1400 mg/kg	69.50 ± 6.76**
5.	Diclofenac sodium 96 mg/kg	147.67 ± 13.72
	Diclofenac sodium 96 mg/kg + N-Acetylcysteine 450 mg/kg	44.67 ± 5.17***
6.	Diclofenac sodium 240 mg/kg	236.50 ± 24.01
	Diclofenac sodium 240 mg/kg + N-Acetylcysteine 450 mg/kg	51.50 ± 6.08***

Note:
*** p value < 0.05 = significant,**
****p <0.001 = highly significant and**
*****p value < 0.0001 = very highly significant, values are presented as Mean ± SEM**

For serum SGPT, as shown in **Table no. 2**, we have done comparison between hepatoprotective agent DL-Methionine at dose of 700 mg/kg shows values of Mean± SEM is 50.33 ± 7.06 with hepatoprotective agent N-acetylcysteine 450 mg/kg shows Mean± SEM is 39.00 ± 2.70 which is not significant.
In the comparison between the group of rats treated with Diclofenac sodium 240 mg/kg+ DL-Methionine 700 mg/kg shows values of Mean± SEM is 69.17 ± 3.57; with the group of Diclofenac sodium 240 mg/kg + N-acetylcysteine 450 mg/kg shows values of Mean± SEM is 51.50 ± 6.08; which is significant with **P value 0.036**.

In the comparison between the group of rats treated with Diclofenac sodium 96 mg/kg shows values of Mean± SEM 147.67 ± 13.72; with the group of Diclofenac sodium 96 mg/kg + DL-Methionine 700 mg/kg shows values of Mean± SEM is 57.17 ± 5.19; which is significant with **P value 0.001**.

In the comparison between the group of rats treated with Diclofenac sodium 96 mg/kg shows values of Mean± SEM is 147.67 ± 13.72; with the group of Diclofenac sodium 96 mg/kg + N-acetylcysteine 450 mg/kg shows values of Mean± SEM is 44.67 ± 5.17; which is significant with **P value 0.0001**.
In the comparison between the group of rats treated with Diclofenac sodium 96 mg/kg shows values of Mean± SEM is 147.67 ± 13.72; with the group of Diclofenac sodium 96 mg/kg + DL-Methionine 1400 mg/kg shows values of Mean± SEM is 43.00 ± 4.25; which is significant with **P value 0.0001**.

In the comparison between the group of rats treated with Diclofenac sodium 240 mg/kg shows values of Mean± SEM is 236.50 ± 24.01; with the group of Diclofenac sodium 240 mg/kg + DL-Methionine 700 mg/kg shows values of Mean± SEM is 69.17 ± 3.57; which is significant with **P value 0.001**.

In the comparison between the group of rats treated with Diclofenac sodium 240 mg/kg shows values of Mean± SEM is 236.50 ± 24.01; with the group of Diclofenac sodium 240 mg/kg + N-acetylcysteine 450 mg/kg shows values of Mean± SEM is 51.50 ± 6.08; which is significant with **P value 0.0001**.

In the comparison between the group of rats treated with Diclofenac sodium 240 mg/kg shows values of Mean± SEM is 236.50 ± 24.01; with the group of Diclofenac sodium 240 mg/kg + DL-Methionine 1400 mg/kg shows values of Mean± SEM is 69.50 ± 6.76; which is significant with **P value 0.001**.

Table no. 3:- Comparison of serum SGOT levels after concomitant administration of hepatoprotective agents and positive control groups.

Sr. No	Group (n=6)	Mean± SEM
1.	Control DW 10 ml/kg	126.00 ± 15.07
2.	DL-Methionine 700 mg/kg	184.50 ± 6.58**
	N-Acetylcysteine 450 mg/kg	114.67 ± 16.92
3.	Diclofenac sodium 96 mg/kg + DL-Methionine 700 mg/kg	295.00 ± 22.87***
	Diclofenac sodium 96 mg/kg + N-Acetylcysteine 450 mg/kg	107.17 ± 21.47
4.	Diclofenac sodium 240 mg/kg + DL-Methionine 700 mg/kg	395.83 ± 20.95***
	Diclofenac sodium 240 mg/kg + N-Acetylcysteine 450 mg/kg	240.67 ± 9.92
5.	Diclofenac sodium 96 mg/kg	1220.83± 130.50
	Diclofenac sodium 96 mg/kg + DL-Methionine 1400mg/kg	225.17 ± 9.27**
6.	Diclofenac sodium 240 mg/kg	1490.00 ± 168.88
	Diclofenac sodium 240 mg/kg + DL Methionine 1400 mg/kg	301.83 ± 22.76**
7.	Diclofenac sodium 96 mg/kg	1220.83± 130.50
	Diclofenac sodium 96 mg/kg + N-Acetylcysteine 450 mg/kg	107.17 ±21.47***
8.	Diclofenac sodium 240 mg/kg	1490.00 ± 168.88
	Diclofenac sodium 240 mg/kg + N-Acetylcysteine 450 mg/kg	240.67 ± 9.92**

Note:
*** p value < 0.05 = significant,**
****p <0.001 = highly significant and**
*****p value < 0.0001 = very highly significant, values are presented as Mean ± SEM**

Table no. 4:- Comparison of Total Serum Bilirubin levels after concomitant administration of hepatoprotective agents and positive control groups.

Sr. No	Group (n = 6)	Mean± SEM
1.	Control DW 10 ml/kg	0.70 ± 0.08
2.	DL-Methionine 700 mg/kg	0.83 ± 0.03
	N-Acetylcysteine 450 mg/kg	0.80 ± 0.05
3.	Diclofenac sodium 96 mg/kg + DL-Methionine 700 mg/kg	0.95 ± 0.08
	Diclofenac sodium 96 mg/kg + N-Acetylcysteine 450 mg/kg	0.80 ± 0.05
4.	Diclofenac sodium 240 mg/kg + DL-Methionine 700 mg/kg	1.01 ± 0.09
	Diclofenac sodium 240 mg/kg + N-Acetylcysteine 450 mg/kg	0.93 ± 0.04
5.	Diclofenac sodium 96 mg/kg	1.07 ± 0.12
	Diclofenac sodium 96 mg/kg +	0.88 ± 0.09

	DL Methionine 1400 mg/kg	
6.	Diclofenac sodium 240 mg/kg	1.25 ± 0.11
	Diclofenac sodium 240 mg/kg + DL-Methionine 1400 mg/kg	1.12 ± 0.20
7.	Diclofenac sodium 96 mg/kg	1.07 ± 0.12
	Diclofenac sodium 96 mg/kg + N-Acetylcysteine 450 mg/kg	0.80 ± 0.05
8.	Diclofenac sodium 240 mg/kg	1.25 ± 0.11
	Diclofenac sodium 240 mg/kg + N-Acetylcysteine 450 mg/kg	0.93 ± 0.04

Note:
*** p value < 0.05 = significant,**
****p <0.001 = highly significant and**
*****p value < 0.0001 = very highly significant, values are presented as Mean ± SEM**

Table no. 5:- Comparison of serum Alkaline Phosphatase levels after concomitant administration of hepatoprotective agents with positive control drug.

Sr. No	Group (n = 6)	Mean± SEM
1.	Control DW 10 ml/kg	106.17 ± 23.15
2.	DL-Methionine 700 mg/kg	92.00 ± 16.19
	N-Acetylcysteine 450 mg/kg	194.00 ± 38.72
3.	Diclofenac sodium 96 mg/kg + DL-Methionine 700 mg/kg	151.17 ± 8.42**
	Diclofenac sodium 96 mg/kg + N-Acetylcysteine 450 mg/kg	223.83 ± 18.43
4.	Diclofenac sodium 240 mg/kg + DL-Methionine 700 mg/kg	136.83 ± 27.79
	Diclofenac sodium 240 mg/kg + N-Acetylcysteine 450 mg/kg	161.50 ± 49.97
5.	Diclofenac sodium 96 mg/kg	153.83 ± 32.01
	Diclofenac sodium 96 mg/kg + DL-Methionine 1400 mg/kg	133.83 ± 16.07
6.	Diclofenac sodium 240 mg/kg	229.00 ± 32.06
	Diclofenac sodium 240 mg/kg + DL-Methionine 1400 mg/kg	134.50 ± 31.48
7.	Diclofenac sodium 96 mg/kg	153.83 ± 32.01
	Diclofenac sodium 96 mg/kg + N-Acetylcysteine 450 mg/kg	223.83 ± 18.43
8.	Diclofenac 240 mg/kg	229.00 ± 32.06
	Diclofenac 240 mg/kg+ N-Acetylcysteine 450 mg/kg	161.50 ± 49.97

Note:
*** p value < 0.05 = significant,**
****p <0.001 = highly significant and**
*****p value < 0.0001 = very highly significant, values are presented as Mean ± SEM**

Table no. 6:- Comparison of serum GGT levels after concomitant administration of hepatoprotective agents with positive control drug.

Sr. No	Group (n = 6)	Mean± SEM
1.	Control DW 10 ml/kg	2.33 ± 0.56
2.	DL-Methionine 700 mg/kg	6.25 ± 1.72*
	N-Acetylcysteine 450 mg/kg	1.47 ± 0.23
3.	Diclofenac sodium 96 mg/kg + DL-Methionine 700 mg/kg	2.42 ± 0.30

	Diclofenac sodium 96 mg/kg + N-Acetylcysteine 450 mg/kg	1.77 ± 0.50
4.	Diclofenac sodium 240 mg/kg + DL-Methionine 700 mg/kg	1.38 ± 1.62*
	Diclofenac sodium 240 mg/kg + N-Acetylcysteine 450 mg/kg	2.20 ± 0.29
5.	Diclofenac sodium 96 mg/kg	3.03 ± 1.40
	Diclofenac sodium 96 mg/kg + DL Methionine 1400 mg/kg	2.76 ± 0.88
6.	Diclofenac sodium 240 mg/kg	1.60 ± 0.28
	Diclofenac sodium 240 mg/kg + DL-Methionine 1400 mg/kg	2.98 ± 0.44*
7.	Diclofenac sodium 96 mg/kg	3.03 ± 1.40
	Diclofenac sodium 96 mg/kg + N-Acetylcysteine 450 mg/kg	1.77 ± 0.50
8.	Diclofenac sodium 240 mg/kg	1.60 ± 0.28
	Diclofenac sodium 240 mg/kg + N-Acetylcysteine 450 mg/kg	2.20 ± 0.29

Note:
*** p value < 0.05 = significant,**
****p <0.001 = highly significant and**
*****p value < 0.0001 = very highly significant, values are presented as Mean ± SEM**

Limitation(S):
The study was conducted in a group of six albino rats, due to reduction in the number of animals per group, based on the principles of Replacement, Reduction and Refinement. The study is done on experimental small lab animals hence; further research can be carried out in different species of animals with other routes of administration also.

Conclusion:-

The present study is a comparison of the effectiveness between DL-Methionine and N-Acetylcysteine for their hepatoprotective effect against Diclofenac-induced hepatotoxicity, where it was observed, both DL-Methionine and N-Acetylcysteine reduces the serum transaminases levels, that were elevated due to the hepatotoxic effect of diclofenac sodium and these observations were further proved by the histopathological findings as well. However, there was no much of a difference of hepatoprotective effect of both DL-Methionine (700 and 1400 mg/kg) and N-Acetylcysteine (450 mg/kg).

Thus, it is concluded that although no much of a statistically significant difference is found between DL-Methionine and N-Acetylcysteine on its hepatoprotective activity, both have found to be hepatoprotective, in the doses used against the hepatotoxicity caused by diclofenac sodium (96 and 240 mg/kg). The present study is novel as it is the first of its kind to investigate the possible effects of the orally administered DL-Methionine as hepatoprotective agent against diclofenac-induced hepatotoxicity at various dose. Also, NAC is used as hepatoprotective agent for the first time against non-paracetamol-induced liver injury induced in rats.

In summary, our present study reinforced the concept that hepatotoxicity induced by drug can be protected by administering NAC even in cases of non-paracetamol induced liver injury.

References:-

[1] Aithal PG, Day CP. The natural history of histologically proved drug induced liver disease. Gut 1999; 44:731–5.
[2] Avila MA, Berasain C, Prieto J, Mato JM, Garcia-Trevijano ER, Corrales FJ. Influence of impaired liver methionine metabolism on the development of vascular disease and inflammation. Current Medicinal Chemistry-Cardiovascular & Hematological Agents. 2005 Jul 1;3(3):267-81.
[3] Biour M, Jaillon PJ. Drug-induced hepatic diseases. Pathol Biol (Paris) 1999; 47:928-937.
[4] Bareille MP, Montastruc JL, Lapeyre-Mestre M. Liver damage and nonsteroidal anti-inflammatory drugs: case non-case study on the French pharmacovigilance database. [in French] Therpaie 2001; 56:51–5.

[5] Mato, J. M. and Lu, S. C. Role of S-adenosyl-L-methionine in liver health and injury. Hepatology. 2007; 45: 1306–1312. doi:10.1002/hep.21650
[6] Accessed online: https://pubchem.ncbi.nlm.nih.gov DL-Methionine
[7] Green JL, Heard KJ, Reynolds KM, Albert D. Oral and intravenous acetylcysteine for treatment of acetaminophen toxicity: a systematic review and meta-analysis. West J Emerg Med 2013; 14: 218- 226.
[8] Lee T, Lee YS, Yoon SY, Kim S, Bae YJ, Kwon HS, Cho YS, Moon HB, Kim TB.. Characteristics of liver injury in drug-induced systemic hypersensitivity reactions. J Am Acad Dermatol 2013; 69: 407-415.
[9] Lee WM, Hynan LS, Rossaro L, Fontana RJ, Stravitz RT, Larson AM, et al. Intravenous N-acetylcysteine improves transplant-free survival in early stage nonacetaminophen acute liver failure. Gastroenterology. 2009;137:856–64. 864.e1.
[10] Singh A., Bhat T. K., Sharma O. P. Clinical biochemistry of hepatotoxicity. Journal of Clinical Toxicology. 2011;(S4, article 001) doi: 10.4172/2161-0495.S4-001.
[11] Willett KL, Roth RA, Walker L (2004) Workshop overview: hepatotoxicity assessment for botanical dietary supplements. Toxicol Sci 79: 4-9.
[12] Papay JI, Clines D, Rafi R, Yuen N, Britt SD, et al. (2009) Drug-induced liver injury following positive drug rechallenge. Regul Toxicol Pharmacol 54: 84-90.
[13] Lazarou J, Pomeranz BH, Corey PN. JAMA. 1998 Apr 15;279(15):1200-5. Incidence of adverse drug reactions in hospitalized patients: a meta-analysis of prospective studies.
[14] Bekkering GE, Bala MM, Reid K, Kellen E, Harker J, Riemsma R, Huygen FJ, et al. Epidemiology of chronic pain and its treatment in The Netherlands. Neth J Med. 2011;69(3):141-153.
[15] K. D. Tripathi. Essentials of Medical Pharmacology. Chapter 14. Nonsteroidal Antiinflammatory Drugs And Antipyretics-Analgesics. 6th edition, page 184-193.
[16] King C, Tang W, Ngui J, Tephly T, Braun M: Characterization of rat and human UDP-glucuronosyltransferases responsible for the in vitro glucuronidation of diclofenac. Toxicol. Sci. 2001; 61:49-53.
[17] Masubuchi, N., Makino, C., and Murayama, N. Prediction of in vivo potential for metabolic activation of drugs into chemically reactive intermediate: correlation of in vitro and in vivo generation of reactive intermediates and in vitro glutathione conjugate formation in rats and humans. Chem. Res. Toxicol. 2007; 20, 455–464.
[18] Ferguson HC. Dilution of dose and acute oral toxicity. Toxicol Appl Pharmacol. 1962; Nov 4: 759-762. DOI: 10.1016/0041-008x(62)90106-0.
[19] Assessed online: https://livertox.nlm.nih.gov Diclofenac.
[20] Dass, Ervilla. Effects of Oral N-Acetylcysteine on the Haemato- Biochemical and Histopathological Changes on the Liver of Albino Rats. Journal of Clinical & Diagnostic Research . Jan 2021, Vol. 15 Issue 1, p10-13. 4p.
[21] Ervilla E. Dass, Shah K. K. Paracetamol and conventional antimalarial drugs induced hepatotoxicity and its protection by methionine in rats. Indian Journal of Experimental Biology, 38(11): 1138-1142.
[22] Dass, Ervilla, et al. Diclofenac-Induced Liver Toxicity in Albino Rats: Dose-Dependent Study. Indian Journal of Research, Volume-7, Issue-1, January-2018.
[23] Veena Nayak, Gincy T.B, Prakash M, Chitralekha Joshi, Soumya S. Rao, Somayaji S N, Nelluri Venu Madhav, Bairy KL. Hepatoprotective activity of Aloe vera Gel against Paracetamol Induced Hepatotoxicity in albino rats. Asian J Pharm Biol Res. 2011; 1(2). 94-98.
[24] Humason, G. L. 1979. Animal Tissue Techniques, 4th Ed. W.H. Freeman, San Francisco.
[25] Thomas L. Alanine aminotransferase (ALT), Aspartate aminotransferase (AST). In: Thomas L, editor. Clinical Laboratory Diagnostics. 1st ed. Frankfurt: TH-Books Verlagsgesell schaft; 1998. p
[26] Gerhard Schumann , Rainer Klauke , Francesca Canalias , Steffen Bossert-Reuther , Paul F.H. Franck , F.-Javier Gella , Poul J. Jørgensen , Dongchon Kang , Jean-Marc Lessinger , Mauro Panteghini and Ferruccio Ceriotti. IFCC primary reference procedures for the measurement of catalytic activity concentrations of enzymes at 37 8C. Part 9: Reference procedure for the measurement of catalytic concentration of alkaline phosphatase. Clin Chem Lab Med 2011; 49(9):1439–1446.
[27] Pearlman, P.C. and Lee, R.T. Detection and measurement of total bilirubin in serum, with use of surfactants as solubilizing agents. Clin. Chem. 1974; 20: 447-53.
[28] Persijn J. P., Vander Silk W. A new method for the determination of gamma-glutamyltransferase in serum. J. Clin. Chem. Clin. Biochem. 1976; 14, 421 – 427.
[29] Dass E, Goswami S, Mehta M. Effects of DL-Methionine at various doses per se on liver in albino rats: An experimental study. Asian J Pharm Pharmacol. 2020;6(3):204-09.