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EFFECT OF ACETONE AND ALCOHOL EXTRACTS OF SEEDS OF *CORIANDRUM SATIVUM* ON GLUTATHIONE CONTENT OF CCL_4 INDUCED SHEEP LIVER IN VITRO

D. S. Kumbhar*, P. A. Shinde, A. A. Kanase

*Department of Zoology, Dada Patil Mahavidyalaya, Karjat. Dist-Ahmednagar

ABSTRACT

Present work has been designed to check hepatoprotective potency of acetone and alcohol extracts of seeds of *Coriandrum sativum* in *in vitro* system by using CCl_4 induced toxicity in sheep liver cells. Glutathione is a major endogenous antioxidant participates in neutralization of free radicals which prohibited aging and pathogenesis. Different concentrations of seeds of *Coriandrum sativum* extracted in acetone and alcohol were prepared and used for treatment on sheep liver tissue for 03,06,09 and 12 hours to record optical density with the help of colorimeter. Present research work is directly related with the medical field as the experimental results shows that increasing concentration of extracts with CCl_4 is inversely proportional to cell death for some concentrations. Dose dependent variations in glutathione content were also noted at variable time intervals.

Key words: Antioxidant, *Coriandrum sativum*, Free radicals, Glutathione

***Corresponding author: Dr. D.S. Kumbhar** Ph. D.

Department of Zoology, Dada Patil Mahavidyalaya, Karjat. Dist-Ahmednagar

E. mail Address: ds_zoo@yahoo.co.in

1. INTRODUCTION

Present work is designed to test the heapatoprotective potency of acetone and alcohol extracts of *Coriandrum sativum* in *in vitro* system using CCl_4 induced toxicity in sheep liver. Use of liver slices *in vitro* is a popular method to test toxicity, drug effect and cellular metabolism in different

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condition [McCay *et al* 1984, Parrish *et al* 1995]. CCl_4 induces centrolobular necrosis in liver with specific damage to endoplasmic reticulum [Salter, 1978] [Dianzani *et al* 1966] had revived the direct effect of CCl4 on sub cellular particles and also on cells of tissue pieces *in vitro* [Salter, 1978]. A glutathione is simple tripeptide it is a component of an amino acid transport system synthesized in cytosolic compartment and is utilized for a detoxification of free radicals and gives protection. Glutathione is the major endogenous antioxidant produced by the cell. Glutathione participates directly in the neutralization of free radicals

2. MATERIAL AND METHODS

1) *Coriandrum sativum* extract preparation

Dry fruit/seed powder of *Coriandrum sativum* was purchased from the market. Weighted dry powder of known amount of *C.sativum* was extracted in acetone and alcohol (proportion : 1 gm *Coriandrum sativum* powder per ml of acetone/alcohol). Acetone and alcohol were evaporated at room temperature in sterile conditions.The prepared extracts were used for further experiments. These extracts were dissolved in culture medium with varying concentrations. Final doses used were- 1mg, 3mg, 6mg, 9mg, 10mg,30mg, 60mg, 90mg/gm weight of liver tissue for the simultaneous treatment.The culture medium used was M199 (HIMEDIA) with Hank's salt. L-glutamate and $NaHCO_3$was added at the time of preparation to adjust the pH exactly between 7.2-7.4.The acclimatization period was 03 hours.Until next medium change in first 3 hrs acid phosphate leakage was seen and it stopped at 3 hrs and protein content of liver and protein released in medium was initiated at 3 hrs. Thus the period of 3 hrs.was used as equilibrium period for acclimatizing the tissue pieces.

2) The tissues were prepared as follows for experimental schedule

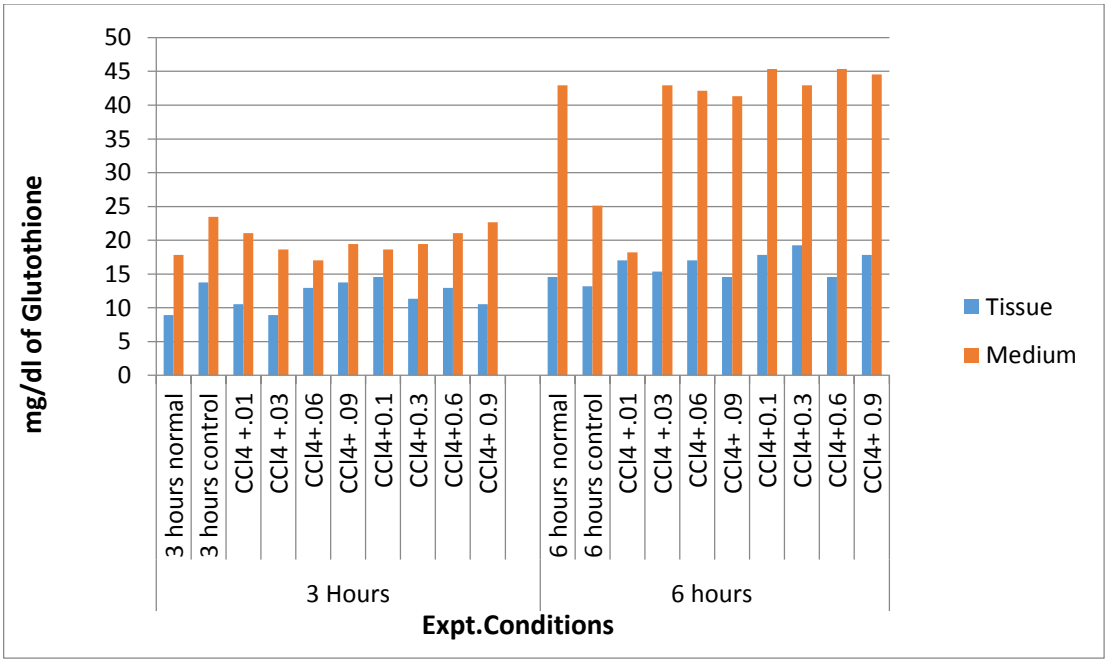
Sheep liver was removed immediately at slaughter house and was immersed in tissue culture medium at in ice bath. The pieces of liver were prepared suitably for above described experimental schedule independently. From the T-flasks the liver pieces were transferred to the experimental tubes.The experiments were conducted as described in experimental protocol after 3 hrs,6 hrs, 9 hrs and 12 hrs. The pieces of liver were used for homogenization and suitable dilutions were made to estimate glutathione

Experimental Protocol (Incubation Temp. 37.5^0 C)

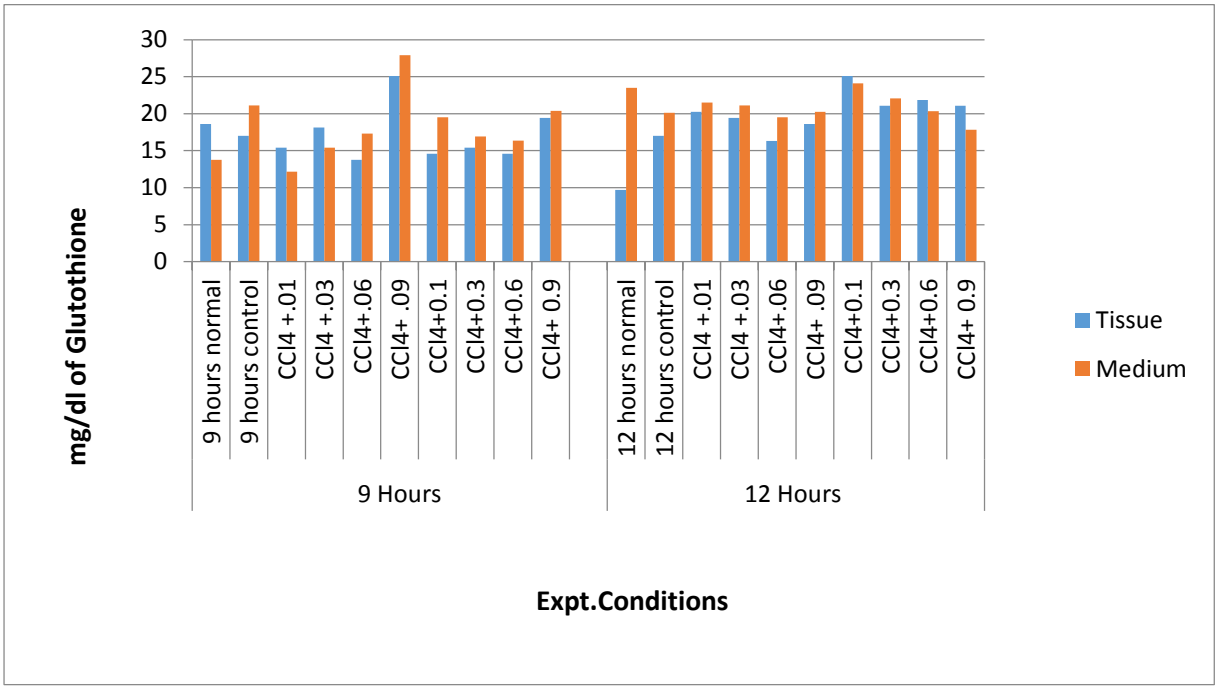
Sr.No	Experimental Conditions (For 03,06,09 &12 Hrs.)	CCl4	*Coriandrum sativum* Acetone /Alcohol extract
1	Liver Control (Only medium)	-	-
2	CCl4 Control	√	-
3	0.01 mg. dose	√	0.01 mg/ml
4	0.03 mg. dose	√	0.03 mg/ml
5	0.06 mg. dose	√	0.06 mg/ml
6	0.09 mg. dose	√	0.09 mg/ml
7	0.1 mg. dose	√	0.1 mg/ml
8	0.3 mg. dose	√	0.3 mg/ml
9	0.6 mg. dose	√	0.6 mg/ml
10	0.9 mg. dose	√	0.9 mg/ml

3. RESULTS AND DISCUSSION

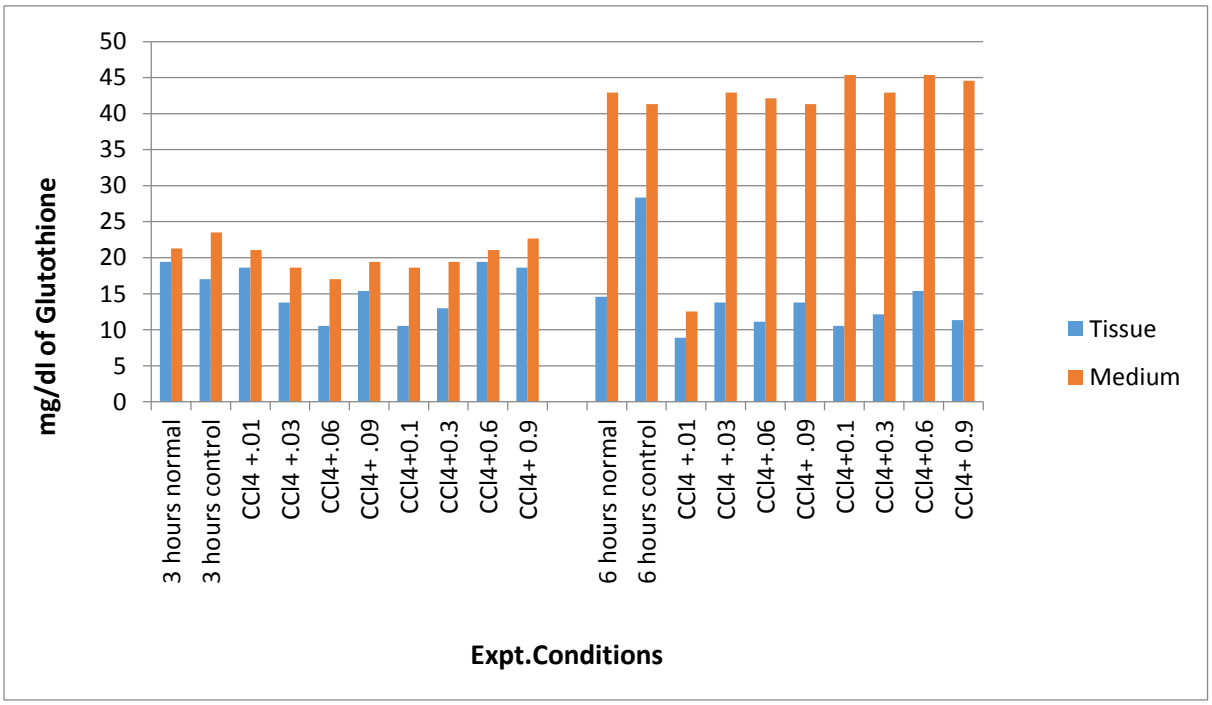
Graph 1: Effect of Alcohol extract of *C.sativum* on glutathione content in Sheep liver *in vitro* (At 3 and 6 hrs of incubation)



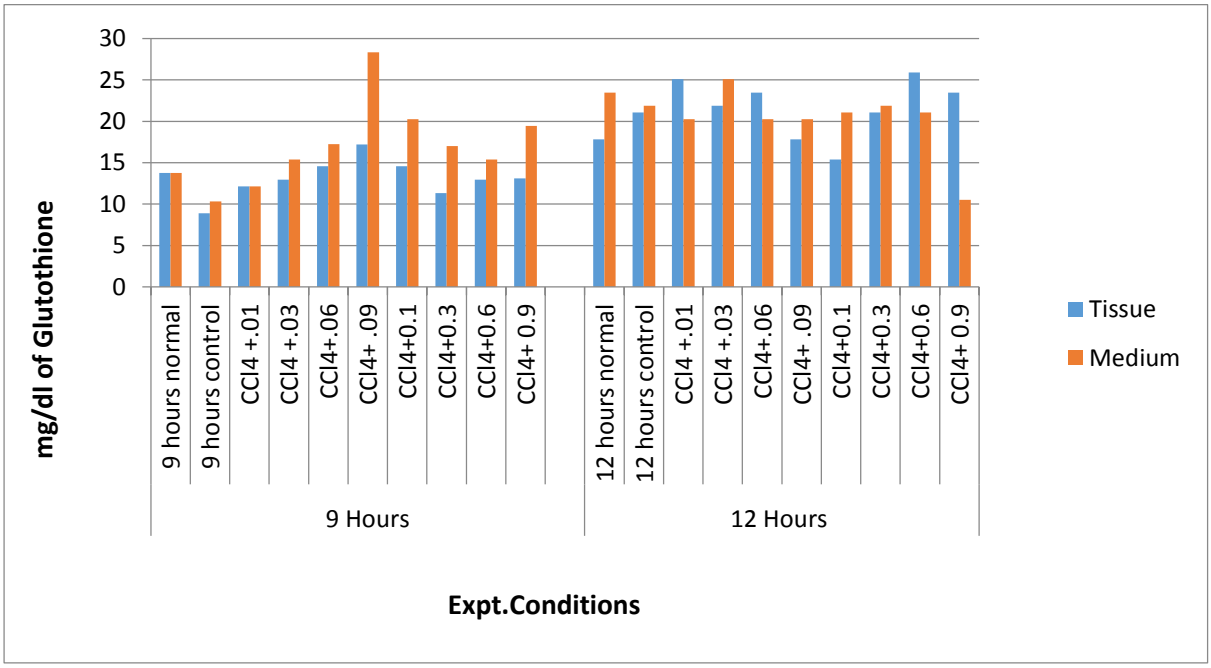
Graph 2: Effect of Alcohol extract of *C.sativum* on glutathione content in Sheep liver *in vitro* (At 9 and 12 hrs of incubation)



Graph 3: Effect of Acetone extract of *C.sativum* on glutathione content in Sheep liver *in vitro* (At 3 and 6 hrs of incubation)



Graph 4: Effect of Acetone extract of *C.sativum* on glutathione content in Sheep liver *in vitro* (At 9 and 12 hrs of incubation)



Alcohol Extract –

At 3 hours, the variations are complementary to the corresponding malonaldehyde content which was thought to be fluctuated in 25% - 62.5% range as observed by Bhogum (2007) in similar conditions of experiment indicating glutathione seems to play free radical scavenger as it is known for the function of free radical scavenger (Caderlas et.al 1996). At 6 hours, corresponding alterations in malonaldehyde content showed oscillating nature in liver and in medium. Though in some experimental conditions liver malonaldehyde content was low , the glutathione content was steady. Bhogum (2007) may be due to sustained injury the glutathione content is continuously utilized and it synthesized in liver (Meister,1991). At 9 hours, corresponding lipid peroxidation alterations in some experimental schedule showed very low malonaldehyde content except 0.06 and 0.09 doses respectively (Bhogum,2007) .The malonaldehyde content in the medium depleted and varied in the steady range. The low levels of lipid peroxidation may have been suppressed by the steady levels of glutathione. The steady levels of glutathione may be from scavenging continuously generating free radicals and continued synthesis of glutathione as adaptive metabolite reactions may be occurring in liver since it is utilized in liver and partially secreted in liver and hence steady glutathione content seems to be present in medium.

At 12 hours, with high concentration dose range of extract the steady glutathione is observed is may be due to lowering the values of lipid peroxidation.

Thus following observations were noted.

1) At 3 hrs,Glutathione effects were low.
2) Alcohol extracts used as low doses showed steady content of glutathione in medium is the indicator of its counter reactions for free radical scavenging.This was more evident at 6 hrs and 9 hrs.
3) Treatment of 12 hrs seems to control free radicals as glutathione content was steady in liver and medium.

Acetone Extract-

With acetone extract, the range of glutathione content alteration in liver was changed significantly but with low range dose it showed increase 3 hrs and 12 hrs, but comparatively low levels alterations were observed with 6 hrs and 9 hrs. The glutathione content in the medium was significantly increased in the range of glutathione throughout the intervals studies but not drastically. When it is considered with reference to malonaldehyde content in the same schedule of experiments (Bhogum,2007), The free radicals scavenger process seems to occure with high concentration doses at 3 hrs.While at 9 hrs, both low and high concentration dose ranges showed depleted free radicals but this was more evident at 9 hrs and 12 hrs. The high concentrations showed two states where free radicals were narrow, and in all these alterations glutathione content in liver and medium was steady at 12 hrs. Thus at 12 hrs at least all high concentration doses show glutathione content that can be managed by these extract doses.

Thus, these doses can be further used for the *in vivo* study to predict the probability of its use as hepatoprotective or antioxidant.

REFERENCES

1. Albano E, Tomasi, A. Persson , J.O, Terelius, Y. Gorici Gatti ,L, Ingelman – Sundberg ,M, & Dianrani M.U. (1991) Role of ethanol-inducible cytochrome P-450 (P-450 IIEI) in catalyzing the free radical activation of aliphatic alcohols *Biochem pharmacol. June 15, 41(12*) 1995-902.
2 Albers B. Bray, D ;Lewis J Raff M. Roberts ,K & Watson J. D, (1996) Molecular biology of cell (3rd ed.) , pub by *Garlard publishing inc New york.*
3. Buege, J.A. & Aust, S.D.(1978) in “method in enzymology" Vol.2 part C.Ed. By: F.Fleischer & L.Packer P-302 *pub.by: Acadamic press*, New York

4. Cadenas E., Packer l. (1996) Handbook of Antioxidants *marcel oekker Inc*. New York .Basal. Hong Kong.
5. Dianzani, M.U. and Poli G (1984) CCl4 induced block of hepatic lipoprotein secretion studies of pathogenesis using isolated rat hepatocytes *front Gastro invest Res,* 8, (liver cirrhosis) 1-13.
6. Farbar E and Fisher, MM (1980) Toxic injury of the Liver part A&B published B.T. *kekker,* New York.
7. Grunert, R.R., & Philips, R.R. (1951) Determination in glutathione Arch. Biochem.30, P-217.
8. McCay PB, Lai E.K, Poyer J.L, DuBose O.M & Janzen E.G (1984) Oxygen & carbon centered free radical formation during carbon tetra chloride metabolism observation of lipid radicals *in vivo & in vitro.*
9. Mehendale, H.M. (1985) Hepatic toxicity, in, Modern toxicology vol.1 Ed by Gupta B.V. & Salunke D.K, Pub by, MD Metropolian book co.pvt 1 ed. N.S Marge, New Delhi 110002, India.P-95.
10. Meister A and Anderson M.E (1984) new aspects glutathione biochemistry and transport--selective alteration of glutathione metabolism. *Nutr Rew*. 42: 397-410 (Medline).
11. Meister A and Anderson ME (1983) Glutathione. *Annu Tev Biochem* 52: 711-760 (Midline).
12. Meister A and Tate SS (1976) Glutathione. Related Y- glutamy1 compounds: Biosynthesis and utilization. *Annu Rev Biochem* 45: 559-604 (Medline).
13. Mc Cay P B, Lai E K Poyer J.L, DuBose, O.M, and Janzen E.G (1984) Oxygen and Carbon centered free radical formation during CCl_4 metabolism observations of lipid radicals in vivo and in vitro J.biochem 259,2135
14. Oshino N, Oshino R. and Chance, B, (1973). The characteristics of the "peroxidatic" reaction of catalyses in ethanol oxidation Biochem J Mar, 131 (3) 555-63.
15. Parrish, A.R., Gandolfi, A.J. & Brendel, K. (1995) Mini review. Precision-cut tissue slices: Application in pharmacology & toxicology. Life sciences 57:1887-1901.
16. Rauckman E.J.,Padilla G.M (1987) The isolated hepatocyte use in toxicology & xenobiotic biotransformation, Acadamic press. *INC, Harcour Brace Jovanovich. Publisher.* North corolina
17. Sakaida, I ;Thomas, A.P. Farber, J.L. (1991) Increase in cytosolic calcium ion concentration can ion dissociated from the killing of cultured hepatocytes by tart butyl hydro peroxide. *Bio chem. Jan* 15,266 (2) 712-22

18. Smith M.T. Thor H Hortizell, P and Orrenius, S. (1982) the measurements of lipid peroxidation in isolated hepatocytes. *Biochem phar, macol* 1982 Jan 1, 31 (1): 19-26.

19. Tribble, D.L. AW, T.Y, Jones D.P. (1987). The Patho physiological significance of lipid peroxidation in oxidative cell injury *Hepatology* Mar-Apr, 7(2): 377-86.