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Comparative study of antioxidants: curcumin and capsaicin on stress induced rats and its effects on liver

Sandhya T Avadhany[1], Saliha C.K[1*]
1.Department of physiology St. John's Medical College, Bangalore

ABSTRACT

Oxidative stress is considered to impair health. The cell damage caused by the free radicals, generated by oxidative stress is believed to be a key reason for the pathogenesis of several diseases. Present study has designed to find out the acute and chronic effect of stress on the liver and to compare the antioxidant and hepatoprotective effect of curcumin and capsaicin. Forty two male wistar rats were examined for ten days. Results of the study revealed that both acute and chronic stress have altered the endogenous antioxidants like glutathione and peroxidase. it also increases the lipid peroxidation. It is found that the level of hepatic glutathione is decreased by 33.63% and 51.51% in acute and chronic groups respectively. The blood glutathione level and the peroxidase enzyme also followed the same trend. MDA, the end product of lipid peroxidation is increased by 18.08% and 32.64% in the same groups. Both the curcumin and capsaicin showed the similar antioxidant and hepatoprotective effect and it regained the endogenous antioxidant level in to normal range. Our data also revealed that there is no significant difference between the antioxidant and hepatoprotective effect of curcumin and capsaicin.

Keyword: MDA: Malonyl dialdehyde, GSH: reduced glutathione, NAFLD: Non-alcoholic fatty liver diseases, NF-kB : nuclear factor kappa B, TNFα : Tumour Necrosis Factor alpha

*Corresponding Author Email: salihac.k111@gmail.com
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INTRODUCTION

Introduction: There are several studies proving the effect of stress on liver and it is well known that the organ which is severely affected by stress is liver[1]. Before explaining the effect of stress on body, it is worthwhile to define what stress is. Hans Selye is the pioneer in stress research. He introduced and popularized the word stress to the research world. According to Hans Selye "stress is a nonspecific response of the body to any demand upon it"[2]. He defined stress as a threat to the homeostasis of an organism. An attempt to maintain the homeostasis is called adaptive or stress response[3]. The enzymatic and non- enzymatic antioxidants present in the cell protects themselves against the dangerous reactive oxygen species generated during stress. Important enzymatic antioxidants are catalase (CAT), Superoxide dismutase (SOD), glutathione peroxidase and the non-enzymatic antioxidants are reduced glutathione (GSH), vitamin A, E and C [4]. There is a balance between the Reactive Oxygen Species and the antioxidant level in the body. However chronic activation of stress impairs the balance between the ROS (Reactive Oxygen Species) and antioxidant level and shifts towards the ROS leading to a condition called oxidative stress [5]. Increased level of free radicals can react with proteins [6], DNA [7], and cell membrane causing cell death [8]. Main ROS such as superoxide, hydroxyl radical, nitric oxide radical and hydrogen peroxide [9] are produced during normal metabolic process like respiration, phagocytosis, electron transport chain reaction, activation of oxidase enzymes [10,11] and metabolism of substance like arachidonic acid [12]. Physiological level of ROS has beneficial role in body, it acts as mediator for apoptosis mainly pre-cancerous cells, phagocytosis, gene expression and cell proliferation [13]. Excess production of ROS due to stress adversely affect the body. Oxidative stress and associated free radical are mainly recognized as a factor in pathogenesis of different diseases like diabetic complications [14], IBS [15], muscular dystrophy, hepatitis [16], immune system decline [17], aging [18,19] and neurological diseases [20]. It is reported that free radicals are the main factors for the pathogenesis of at least 50 diseases [21].

In our study we focused to see the effect of stress on hepatocyte and find out the hepato protective and antioxidant effect of curcumin and capsaicin. It is well documented in previous studies about the effect of stress on hepatocytes. Researchers reported that majority of chronic liver diseases are associated with oxidative stress [22]. It induces the apoptosis of the hepatocyte, leading to liver injury. Increased levels of free radical increases the level of pro inflammatory cytokine level by the activation of its transcription factor NF-kB (Nuclear Factor- kappa B) [23]. This pro inflammatory cytokines enhances the caspases which leads to apoptosis of hepatocytes. NF-kB also cause the

activation of TNFα (Tumour Necrosis Factor alpha), which eventually lead to cell destruction [24]. Another physiological pathway connecting oxidative stress and liver diseases is, high level of ROS and oxidized NAD(P)H increase membrane permeability transition (MPT) by increasing intracellular Calcium [25] or mitochondrial serine protease [26]. The prime factor for the pathogenesis of NAFLD (Non Alcoholic Fatty Liver Disease) is oxidative stress and associated mitochondrial dysfunction [27,28] Hepatic disorder constitute one of the main causes of world-wide mortality. There was a 25% increase in liver disease deaths between 2001 and 2009. This is in contrast to other major causes of death, which has been declared that liver disease kills people at a much younger age. 90% of people with liver disease dying in hospital are under 70 years old [29]. This shows the challenge in managing the end of life care for patient group. Several studies has been conducted for obtaining a suitable solution for hepatic disease but we are far from effective treatment. "We are what we ate" [30], so there is increasing interest to find out the antioxidant effect of commonly used food. In this study we try to observe and compare the effect of antioxidant rich food supplement like curcumin and capsaicin in stress related liver disease. Curcumin and capsaicin have been proved that they can reduce the level of pro inflammatory mediators released from macrophages.

Curcumin is an active constituent in turmeric, an Indian spice and it has a long history of use in ayurvedic medicine. It shows wide range of pharmacological anti-inflammatory [31], antioxidant and anti-cancer properties [32-34] Chemically it is di-feruloylmethane ($C_{21}H_{20}O_6$), contain more than one functional group like phenyl rings, β-di-keto group and carbon–carbon double bonds, all are capable for free radical scavenging. Some studies claimed that among all these functional group phenolic group is essential for the detoxification reaction [35,36] Curcumin shows antioxidant properties by two method, first one is the direct scavenging of ROS [37,38] and later by modulating cellular signals [39,40] Studies have shown that curcumin inhibits the activation of NF-kB, which is associated with the proliferation of tumor cell [41]. It also suppress the other inflammatory markers like COX, LOX and iNOS [42-44] Recent study on curcumin proved that curcumin is a unique chemotherapeutic compound, unlike other chemotherapeutic agent, it acts only on affected cell and does not cause damage to the neighboring normal cell [45,46] Beneficiary of curcumin against liver fibrosis [47], Alzheimer's disease [48,49], diabetes [50,51] skin diseases [52] and auto immune diseases [53,54] have also been reported. Another spice which we have selected in our study is capsaicin. Like curcumin it also contain phenolic group. Chemically it is 8-methyl-N-vanillyl-6nonenamide ($C_{18}H_{27}NO_3$), it is a pungent and active component in capsicum. Both the spices have structural

similarities[55]. Like curcumin, capsaicin also has the antioxidant[56], antimutagenic, anti-inflammatory [57,58] and anticarcinogenic activities [59]. It's reported that capsaicin suppress the growth of hepatic carcinoma cell [60] and leukemic cell [61]. Recent studies showing that capsaicin have a role in managing prostate cancer [62,63] Capsaicin prevent the activation of NF-kB by suppressing the degradation of IκBα factor. In our study we compare the antioxidant and hepato protective properties of these commonly used spices [64].

MATERIALS AND METHOD

Animals

Forty two male albino rats 6-8 weeks old weighing 180-200 gms were used for the experiment. The rats were divided into seven groups of six rats. Animals were caged individually and maintained in an ideal laboratory conditions room temperature at 22-24^{0}C and 12h light and dark cycle. The rats were fed with standard pellet and water ad libitum. All studies were conducted in accordance with the CPCSEA (Control for Protection to Cruelty to Experimental Animals), India guidelines. The study protocol was approved by IAEC.

Experimental design

The animals were divided in to seven groups as follows

Group I – normal control

Group II – exposed to acute water immersion stress

Group III – exposed to chronic water immersion stress

Group IV – acute water immersion + curcumin administration

Group V – chronic water immersion + curcumin administration

Group VI – acute water immersion + capsaicin administration

Group VII – chronic water immersion + capsaicin administration

Rats assigned to control group were kept in a standard laboratory conditions without being exposed to any type of stress. Rats assigned to stress group were exposed to forced swimming stress for one hour in a glass water tank. Forced swimming stress is considered as the ideal method for giving emotional stress to the experimental animals [65] The temperature of water in the tank was maintained at 28^{o}C. Acute groups were exposed to water immersion stress for one day, whereas seven days of stress of 1hr a day were given for chronic group. Curcumin and capsaicin were purchased from Arjuna chemicals and given orally to respective group with a concentration of 30mg/kg (average 6mg/rat) for 10 days [66,55] Rats were sacrificed at the end of treatment. Blood collected in heparin tubes from retro orbital venous sinus for blood GSH estimation. Liver tissues

were taken out, one part was frozen in liquid nitrogen and kept in -80^{0}C until being used for biochemical assays and the other part was used for histopathology study. In the present study GSH level in both blood and liver were estimated by using Ellman's reagent [67], MDA by using TBAR method[68] and peroxidase estimation by pyrogallol method [69]. Tissue samples are homogenized in 0.1M ice cold phosphate buffer solution and centrifuged at 10000 rpm for 10 minutes at 4oC and collected the supernatant

Estimation of malonyldialdehyde (MDA)

Liver homogenate was mixed with the TBA-TCA- HCl solution and incubated in boiling water bath for 10 minutes. Cooled in room temperature and centrifuged again at 1000 rpm for 10 minutes. Optical density of the supernatant was measured against the blank at 532. Concentration of MDA was calculated by using extinction co efficient (ε = 1.56 x 105M$_{-1}$ Cm $^{-1}$) and the result were expressed as nanomol MDA/gram of tissue.

Estimation of reduced glutathione (GSH)

Heparinized blood samples were used for the estimation of blood glutathione level. 750 micro liter of precipitating reagent and 450 micro liter distilled water were added to 50 micro liter of sample. Centrifuged at 10000 rpm for 45 minutes. Collected the supernatant and added 0.3M phosphate buffer (pH= 8) and DTNB solution (300 micro molar in 0.1 M phosphate buffer). Incubated at dark for 5 minutes and OD reading measured against the blank at 412 nm wave length. Concentration of GSH was calculated by using glutathione standard curve.

Estimation of peroxidase

5% pyrogallol (0.32ml), 30% hydrogen peroxide solution (0.16 ml) and phosphate buffer (pH=6, 0.32ml) added to the sample. Absorbance was noted at every 20 seconds for 3minutes. Enzyme activity was calculated by using the formula (ΔA420/20 second Test - ΔA420/20 Blank) x 3 x df/ 12x 0.1

RESULT AND DISCUSSION

Effect of stress

The effect of acute and chronic water immersion stress on blood and hepatic GSH level is depicted in table 1. Hepatic GSH level was decreased by 33.63% in acute stress and 51.51% in chronic stress. Blood glutathione level was also decreased by 15.8% and 28.5 % in acute and chronic stress respectively. Activity of peroxidase enzyme also showed the similar results. It is decreased by 44.38% in acute stress and 54.55% in chronic stress. The results also revealed that there is a drastic increase in MDA level in acute and chronic stressed group. In acute group there is an increase of

18.08% and in chronic water immersion group 32.64%. Histopathological results showed sinusoidal dilation, congested blood vessel, kupffer cell hyperplasia and portal inflammation in stress induced rats.

Effect of pretreatment of curcumin and capsaicin.

Administration of curcumin and capsaicin restored the GSH and MDA values in stress induced rats. All antioxidant markers are altered in stressed group compared to the control, however all the markers showed a marked recovery in response to curcumin and capsaicin administration to stress induced rats compared to untreated stressed rats.

Table 1: effect of water immersion stress and hepatoprotective effect of curcumin and capsaicin on rats.

	Group 1 (control)	**Group 2 (Acute stress)**	**Group 3 (chronic stress)**	**Group 4 (acute+curcumin)**	**Group 5 (chronic+curcumin**	**Group 6 (acute+capsaicin**	**Group 7 (chronic+capsaicin**
Blood glutathione level (mg/ml of blood	0.344±0.03	0.289±0.04*	0.246±0.03**	0.368±0.01	0.351±0.04	0.358±0.03	0.334±0.02
Hepatic glutathione (mg/gm tissue)	1.02±0.1	0.68±0.2*	0.49±0.18**	0.93±0.23	0.78±0.15	0.87±0.18	0.78±0.14
Hepatic MDA level (nmole/gm of tissue)	11.05±0.9	13.04±1.51*	14.44±1.86**	11.17±1.6	11.84±1.16	11.1±1.3	11.8±1.03
Hepatic peroxidase (U/gm of tissue)	7.79±1.9	4.33±0.9**	3.54±0.4**	10.58±1.69	7.87±2.9	10.79±2.5	9.29±1.89

** = strong significance ($p<0.01$) ; * significant ($p<0.05$)

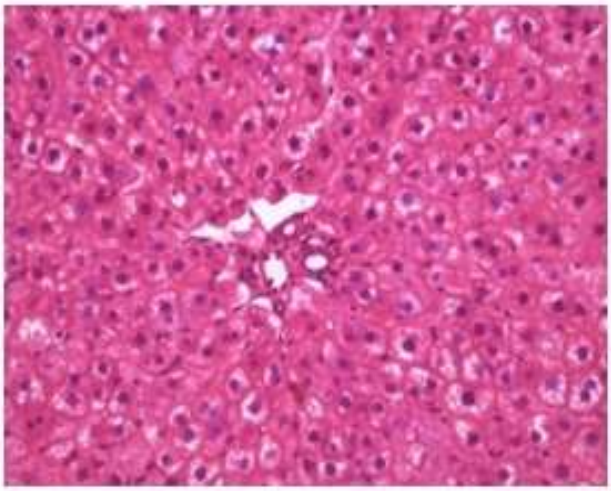

Figure 1: Control rat liver

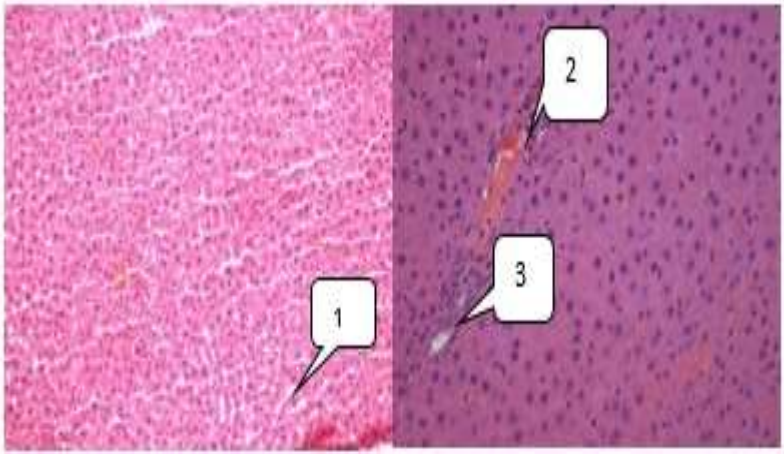

Figure 2: Acute stress induced rat liver

Kupffer cell hyperplasia , 2. Capillary congestion, 3.portal inflammation

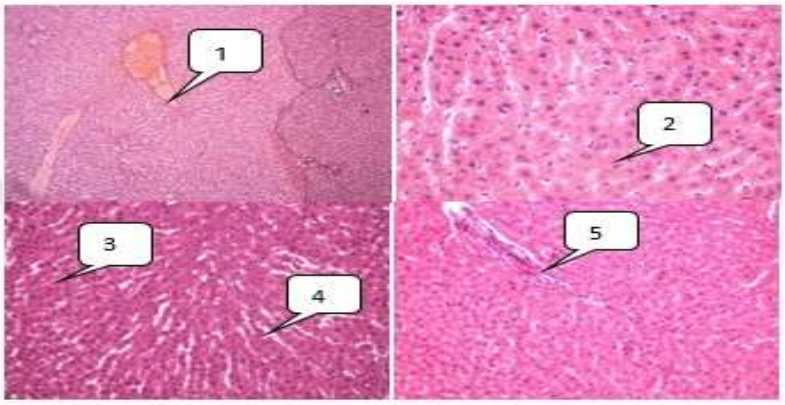

Figure 3: Chronic stress induced rat liver

1. Congested blood vessels 2. Kupffer cell hyperplasia 3 Steatosis. 4. Sinusoidal dilatation 5. Portal inflammation

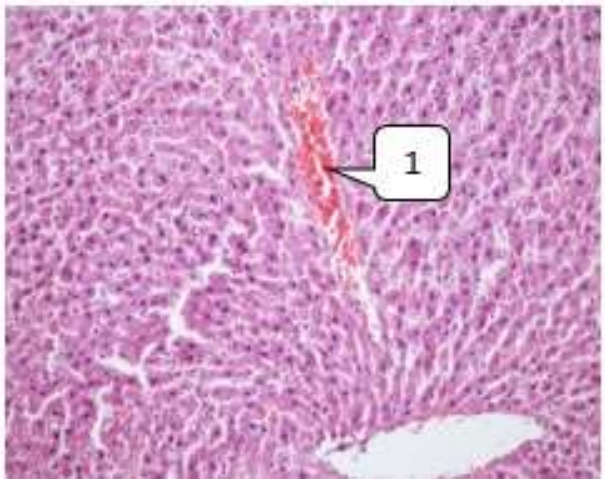

Figure 4: Acute stress + curcumin

1. Vascular congestion

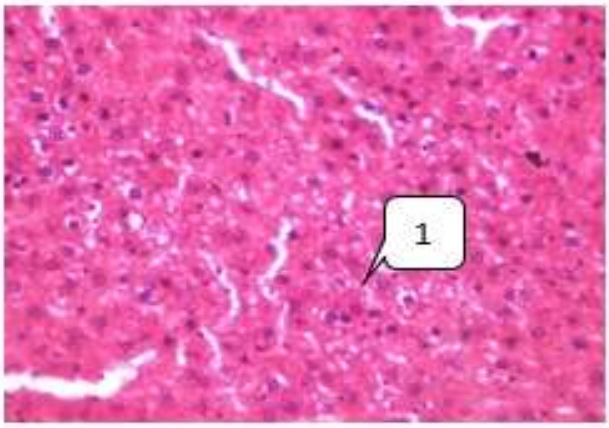

Figure 5: Chronic stress + curcumin

1. mild steatosis

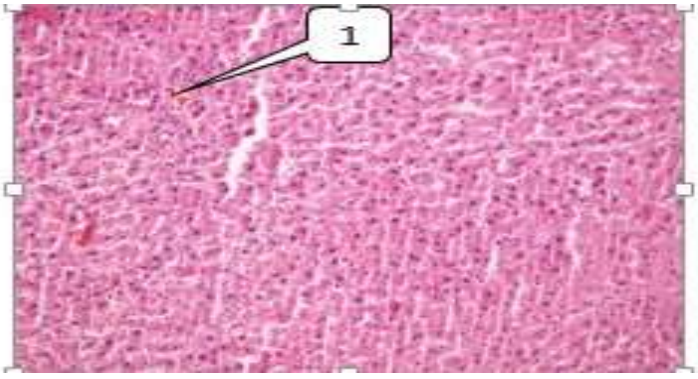

Figure 6: Acute stress + capsaicin

1. Capillary congestion and mild portal infiltrate

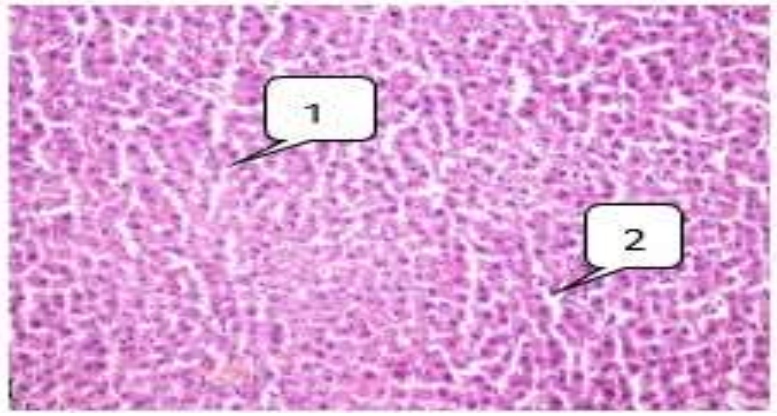

Figure 7: Chronic stress + Capsaicin

1. Mild steatosis 2. Sinusoidal dilation

This study investigated the potent hepato protective and antioxidant properties of curcumin and capsaicin in acute and chronic stress, induced by water immersion stress. In the study, hepatotoxicity induced by water immersion stress was assessed by biochemical and histopathological parameters. Selected markers showed significant difference in stress induced groups and it restored to its normal value in curcumin and capsaicin treated groups. Several studies have demonstrated that ROS generated from oxidative stress has a major role in the pathogenesis of several disease mainly liver diseases. In our study MDA level shows significant increase both in acute and chronic stressed groups. MDA is the end product of lipid per oxidation and considered as an ideal marker for cellular injury [70]. Previous studies also reported that administration of both acute and chronic stress cause significant elevation of MDA in different tissues [71-72] However some of the previous reports are inconsistent with this [73-74]. It is universally accepted that increased level of corticosterone is a sign of stress. Evidence indicates that there is a positive correlation between corticosterone level and MDA and it suggested that increased corticosterone secretion during stress accelerates the lipid per oxidation through ROS[75].

MDA level in all groups are presented in table. MDA level in curcumin and capsaicin treated groups were lower than stress induced group. These observations show that curcumin and capsaicin inhibit the lipid peroxidation and protect the cell from stress induced damages. Previous studies also proved the hepato protective effect of curcumin and capsaicin in iron induced hepatotoxicity [76.] Another study reported that curcumin prevent the generation of highly active hydroxyl radical, which initiate the lipid per oxidation by inhibiting the oxidation of Fe2+ with hydrogen peroxide [77-78] Primary mechanism for the lipid per oxidation is the activation of pro inflammatory mediators by the activation of NFkB. It is reported in recent papers that both curcumin and capsaicin reduce the activation of NFkB [79-80]

Our study also demonstrated that concentration of reduced glutathione (GSH) in blood as well as liver was progressively decreased in acute and chronic group. Glutathione is an important non enzymatic antioxidant. Concentration of GSH is high in liver compared to other tissues, about 10% of total GSH is present in hepatocyte [81]. Chemically glutathione is α L-glutamyl-L-cysteineglycin and more than 90% of cellular non protein thiole groups are present in it. It has been reported that depletion of GSH enhances the apoptosis of cell [82]. Depletion of GSH level has been reported in liver cirrhosis [83] and chronic hepatitis [84]. It is also proved that blockage of GSH/GST detoxification system in tumor cell lines enhances the chemo sensitivity [85]. A significant increase in GSH levels was observed in capsaicin and curcumin-treated rats. Reports from other studies demonstrated that curcumin increase the GSH level in hepatocytes [86] and kidney [87]. An in-vitro study reported that curcumin enhances the formation of GSH [46]. Peroxidase, another important endogenous antioxidant enzyme has similar effect. Recent study shows that curcumin treatment increased the antioxidant status of mouse exposed to different doses of gama radiation [88] and aflatoxin induced oxidative stress[89]. Study of Chakraborti et al reported that, glutathione depletion may lead to an increased lipid peroxidation, possibly due to the lowering of the cellular defense system against endogenous toxic intermediates [90.]

Hitopathological findings also supported the biochemical observations. Histopathological parameters comprised of sinusoidal dilation, congested blood vessel, hepatic steatosis, kupffer cell hyperplacia and portal inflammation in stressed rats. It has been reported that kupffer cells are more sensitive to oxidative stress[91]. The proinflammatory cytokines released during stress act on the kupffer cell and initiates the liver inflammation. At the same time the present result revealed that the pre- treatment of curcumin and capsaicin administration was able to reduce the hepatotoxicity induced by stress. Previous studies have been reported with similar antioxidant effect of curcumin and capsaicin.

CONCLUSION

In summary, exposure to stress induces production of free radicals and lead to the oxidative damage. Our results also suggest that oral administration of curcumin and capsaicin elevated the activity of glutathione, peroxidase concentrations and lowered the lipid peroxidation in rat liver exposed to water immersion stress. Our findings indicate that use of curcumin and capsaicin in our food may be one of benefit to reduce the effect of stress and generation of highly active free radicals. Both the curcumin and capsaicin have similar beneficial effect as an antioxidant and hepatoprotective agent.

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