Evaluation of Antioxidant Activity of Zingiber Officinale (Ginger) on Formalin-Induced Testicular Toxicity in Rats

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***Abstract*—This study was carried out to investigate the possible antioxidant activity of *Zingiber officinale* (ginger) ethanolic extract on formalin-induced testicular toxicity in rats. Twenty male Wistar rats were randomly divided into four groups: (1): control; (2): rats exposed with 10% formalin; (3): rats exposed with 10% formalin and treated with ethanolic ginger extract; (4): rats treated with ethanolic ginger extract. Exposure of 10 % formalin was performed through inhalation while ethanolic ginger extract was administered orally. Determination of malondialdehyde (MDA) and the activities of superoxide dismutase (SOD) and catalase (CAT) were assessed upon harvested testicles. As a result, 10% formalin exposure significantly increased the concentration of MDA as compared to control. Meanwhile, all groups showed significant increase in SOD level as compared to control. There is no significant difference of CAT activities in all experimental groups as compared to control. However, rats exposed with formalin and treated with ethanolic ginger extract significantly increased the CAT activity as compared to the group of formalin exposure only. In conclusion, 10% formalin triggered oxidative stress in testicles with the evidence of the significant increase of MDA concentration. Moreover, ginger exhibit antioxidant properties which proven by the increase of SOD and CAT activities.**

***Index Terms*—Zingiber officinale, formalin, testicles, oxidative stress, antioxidant**

I. Introduction

Zingiber officinale (ginger) belongs to family Zingiberaceae [1]. It is an important ingredient traditionally used in Chinese, Ayurvedic and Tibb-Unani herbal medicines to treat several diseases such as asthma, stroke, and diabetes [2]. Over the centuries, the usage of *Zingiber officinale* has been progressed and increased in pharmaceutical demands. Recent study supported that *Zingiber officinale* has the protective nutraceutical effect against oxidative stress and also reproductive toxicity [3]. Among main active phytochemicals in *Zingiber officinale* such as gingerols, gingerdiol, shogaols, zingerone, and zingibrene are claimed to have antioxidant activity [4]. Some study showed that *Zingiber officinale* treatment provided antioxidant effects by raising tissue concentrations of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) [5]. These antioxidants are important protection against oxidative stress due to their ability to detoxify free radicals, such as reactive oxygen species (ROS).

The imbalance of ROS production and detoxification lead to oxidative stress in tissue. Oxidative stress is directly proportionate to lipid peroxidation, DNA damage, protein damage and induction of apoptosis which will result in cell death [6]. Formaldehyde (FA) is identified as one of the causative agent of oxidative stress.

Formaldehyde (CH_2O) is a colourless, flammable, reactive gas and readily polymerized at room temperature with a pungent odour [7]. It is commercially available as a solution called formalin and according to Occupational Safety and Health Administration (OSHA) it is formed from various proportions of formaldehyde, water, and alcohol [8]. Formaldehyde has been routinely utilized in medical work setting such as hospitals and laboratories. Formaldehyde is an excellent tissue fixative and commonly used for the preservation of tissues [9]. Therefore, exposure to FA occurs significantly among pathologist, hospital housekeeping staff, and laboratory workers [10]. Some epidemiological studies of industrial workers, embalmers and pathology anatomists have indicated association of FA exposure with elevated cancer risks at various sites, including the brain, nasal cavities, lung [11], pancreas [12], lymphohematopoietic system [13], [14] and prostate [15]. Other than cancer effects, recent study shows that long-term exposure of formaldehyde can cause reproductive damage on male rats by producing oxidative stress [16].

This paper describes the role of *Zingiber officinale* as protective nutraceutical agent towards testicular toxicity effect of formalin exposure by measuring the lipid peroxidation activity, malondialdehyde (MDA) and the levels of two antioxidant enzymes, superoxide dismutase (SOD) and catalase (CAT) in rat testicles.

II. Methodology

A. Preparation of Plant Extract

One kilogram of fresh *Zingiber officinale* rhizomes were procured from the local market in Kajang, Selangor and

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were cleaned with tap water. The cleaned rhizomes were peeled and cut into slices and let dried under the sunlight for few days until constant weight was achieved. Approximately 250g dried rhizomes were grounded and extracted with ethanol through double boiling at 60˚C for 12 hours. The ethanol was removed by using rotary evaporator at 40 ℃. The extraction end-product is a pure *Zingiber officinale* appears in dark orange colour. The extract was kept at 4 ˚C until further uses. The extract must first be solubilized with corn oil and 15 % of dimethyl sulfoxide (DMSO) prior to oral administration of the rats at 100mg/kg body weight dosage.

B. Experimental Animals

Twenty healthy male Wistar rats (weighing 150g - 200g), were housed in 38x23x10 cm transparent polycarbonate wire-topped cages (2 rats per cage). They were acclimatized at 12 hours light and 12 hours dark cycle and fed with standard diet and tap water for one week prior experiment procedure commence. Rats were randomly divided into four groups: (1): control (received treatment vehicle, corn oil and 15 % of dimethyl sulfoxide); (2): rats exposed with 10% formalin (4 hrs/day, 5 days/wk, 8 wks); (3): rats exposed with 10% formalin (4 hrs/day, 5 days/wk, 8 wks) and treated with 100 mg/kg body weight of ethanolic *Zingiber officinale* extract (14 days); (4): rats treated with 100 mg/kg body weight of ethanolic *Zingiber officinale* extract (14 days). The duration of 8 weeks of formalin exposure was performed through whole-body inhalation while ethanolic *Zingiber officinale* extract was given orally.

C. The Exposure Procedure

The formalin exposure took place in a transparent polycarbonate inhalation chamber with the dimensions 50x35x30 cm to generate a constant airstream from a daily fixed amount of commercial aqueous solution of formalin. The formalin (37% formaldehyde solution) was given by means of a pipette into a flat dish which was located on the top center of the chamber. The weight and physical observation of rats was recorded in daily basis.

D. Biochemical Assays

After 24 hours post experiment, the rats were sacrificed and the testicles were harvested, weighted, divided equally and immediately frozen in liquid nitrogen to stop the biochemical reaction in organ. The biochemical assays were done on these tissue samples to measure the activity of malondialdehyde (MDA) [17], superoxide dismutase (SOD) [18], catalase (CAT) [19] and protein estimation [20].

E. Statistical Analysis

All statistical analysis was carried out by using Statistical Package for the Social Sciences (SPSS) statistical software version 13.0. For biochemical analysis, two-way ANOVA was used to compare means among four groups. Post- Hoc Dunnett was used to make the comparison between means. All the data were expressed in mean ± standard error of the mean (SEM) and p value less than 0.05 considered as significant.

III. Result

A. Assay For Malondialdehyde (MDA)

Fig. 1 shows the MDA concentration in all experimental groups which are control, formalin exposure only, formalin exposure treated with *Zingiber officinale* and *Zingiber officinale* treatment only. There is significant increase of the MDA concentration in the group of formalin exposure only (1.84618 ± 0.3355378 nmol/mg protein) as compared to control group (0.29252 ± 0.12644 nmol/mg protein). The figure also reveals decreasing pattern of MDA concentration in the group of formalin exposure treated with *Zingiber officinale* as compared to the group of formalin exposure only, however it is not significant.

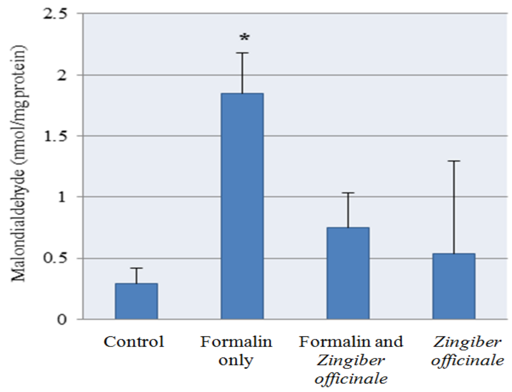

Figure 1. Malondialdehyde (MDA) concentration in all experimental groups. There is significant different ($p<0.05$) between formalin exposure only group as compared to control group.

B. Superoxide Dismutase Activity

Fig. 2 shows the superoxide dismutase (SOD) activity in all experimental groups. Interestingly, the activities of superoxide dismutase in the groups of formalin exposure only (13.515680 ± 1.7683013 U/mg protein), formalin exposure treated with *Zingiber officinale* (11.301940 ± 1.1804805 U/mg protein) and *Zingiber officinale* treatment only (14.110240 ±1.4295342 U/mg protein were significantly increased ($p<0.05$) as compared to control group (3.854800 ± 0.7502381 U/mg protein). However, no significant changes were observed when comparing each group to another.

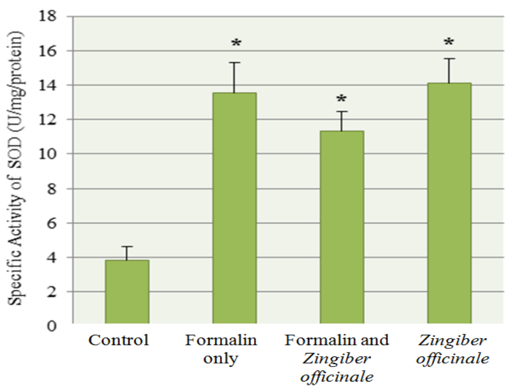

Figure 2. Superoxide dismutase (SOD) activity in all experimental groups. There is significant different ($p<0.05$) in all experimental groups as compared to control group.

C. Catalase Activity

Fig. 3 shows the catalase (CAT) activity in all experimental groups. Even though there was no significant different ($p>0.05$) was observed in all groups as compared to control group however, there is significant increase ($p<0.05$) for the catalase activity in the group of formalin exposure treated with *Zingiber officinale* (249.1139 ± 14.87664 µmol) as compared to the formalin exposure only group (164.9830 ± 18.34530 µmol).

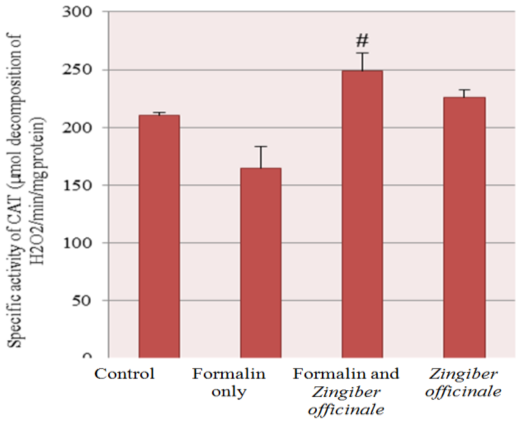

Figure 3. Catalase (CAT) activity in all experimental groups. There is significant different ($p<0.05$) in the group of formalin exposure treated with *Zingiber officinale* as compared to formalin exposure only group.

IV. Discussion

The present study revealed the testicular toxicity effect of formalin exposure along with the role of *Zingiber officinale* as protective nutraceutical agent to this effect. The testicular toxicity effect of formalin and formaldehyde vapours in some mammals including human has been investigated previously, however to our knowledge there are limited reports on the protective role of natural product such as *Zingiber officinale* (ginger) towards testicular toxicity. The procedure of 10% formalin exposure in inducing oxidative stress was adopted from a study by Golalipour *et al.*, (2007) [21]. The rats was placed in the cadaver's room for 18 weeks and resulted in significant increase of MDA concentration as compared to the control group. Surprisingly, the same observation was detected in the present study although the duration of exposure in this study was much shorter (8 weeks).

The result in this study has shown that the exposure to 10% formalin for 4 hours/day, 5 days/week for a consecutive 8 weeks significantly increase the level of MDA in testicles of rats. According to study by Zhou *et al.* in year 2006 [22] reported that the lipid peroxidation product, MDA increased significantly in the testicles of rats exposed to formaldehyde vapour ($10mg/m^3$ for 2 weeks) compared to the control group. An animal study by Ozen *et al.* (2008) [23] also revealed the increase concentration of MDA which portray damaging effect of formaldehyde on testicles. The obtained findings in this study are compatible with the results of the above studies. Recent study suggested that formaldehyde may cross the blood barrier thus trigger oxidative stress by increasing reactive oxygen species (ROS) within the testicles [24]. These substantial ROS might eventually trigger histological changes in seminiferous tubules [23], sperm motility and sperm count [25] which ultimately will induce infertility or sterility in male. Although MDA concentration level in the group of formalin exposure treated with *Zingiber officinale* is insignificantly decreased as compared to the group of formalin exposure only, it may proposed that *Zingiber officinale* has protective effect against testicular toxicity by formaldehyde. This invalidity may be due to small magnitude of sample and by increasing sample magnitude a significant result might be achieved.

As the oxidative stress and ROS are constantly produced and trigger testicular tissue damage, therefore these tissues must be protected by endogenous antioxidants such as SOD, CAT and others. However, in the present of massive extent of oxidative stress and ROS, additional exogenous antioxidants are needed to neutralize the tissue stress effect. These exogenous antioxidants can be found in herb plants, fruits, vitamins and others. In the present study, the role of *Zingiber officinale* as protective nutraceutical agent was analyzed.

Surprisingly, all experimental groups except for control resulted in significant increase of SOD activity in rat's testicles. This may be explained by the testicular physiological state itself. Spermatogenesis requires high rates of mitochondrial oxygen consumption however, due to poor vascularization of the testicles resulted in extremely low oxygen tension. Theoretically, spermatogenesis and Leydig cell steroidogenesis would be highly susceptible to oxidative stress due to the abundance of highly unsaturated fatty acids and ROS generating systems such as mitochondria and various enzymes (xanthine- and NADPH-oxidases). On the contrary, in order to protects itself from the risk, the testicles have developed a sophisticated antioxidant systems comprising both enzymatic (superoxide dismutase, glutathione peroxidase and glutathione-S-transferase) and non-enzymatic (vitamin C and E, zinc, melantonin and cytochrome C) components [26].

Therefore, it is not puzzling to observe high activity of SOD in these three experimental groups because the testicular tissue itself contain not only cytosolic (Cu/Zn) and mitochondrial (Fe/Mn) forms of SOD but also feature special form of extracellular SOD (ex-SOD) which is produced by both sertoli and germ cells [26]. In addition, we suggested that there is definite contribution of SOD from the *Zingiber officinale* due to significant elevation of SOD activity in group of *Zingiber officinale* treatment only as compared to control group. This is proved that the ethanolic extract of *Zingiber officinale* possess an antioxidant property which supported by study done by Morakinyo *et al.* (2010) [27].

In the presence of SOD, superoxide anion (O_2-) is rapidly converted into hydrogen peroxide (H_2O_2) in order to prevent the former from participating in the formation of highly pernicious hydroxyl radicals. The elimination of H_2O_2 is either effected by catalase (CAT) or glutathione peroxidase (GPx), with the latter predominating in the case of the testicles [26]. Although CAT is of limited importance in the testicles, it is still a vital component to detoxify hydrogen peroxide. As observed in this study, there is no significant difference in catalase activity detected in all experimental groups as compared to control group. This is may be due to role of converting hydrogen peroxide has been taken up by GPx as this enzyme are abundant in testes and also catalyzing hydrogen peroxide to oxygen and water [26]. However, the group of formalin exposure treated with *Zingiber officinale* has significantly higher catalase activity as compared to the group of formalin only, proved that *Zingiber officinale* has the ability to elevate CAT activity in formalin-stressed testicles.

V. Conclusion

This study shows that 10 % formalin exposure for 4 hours/days, 5 days/week for consecutive 8 weeks was sufficient to significantly induce oxidative stress in rat reproductive system particularly, testicles. Although the existed testicular antioxidant systems are efficient to overcome oxidative stress, this study suggested that *Zingiber officinale* has protective nutraceutical capacity to help in overcome the oxidative stress induced by the 10% formalin.

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