Ameliorating effect of *Tetracarpidium Conophorum* on Oxidative Stress Markers and Liver Markers of Welders Exposed to Oxy-acetylene Fumes in Anambra State

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Abstract

The welding fume generated during the welding processes could have severe adverse effect to Welders. The knowledge and awareness of these adverse effects and the actions toward this deleterious effect are important factors in the prevention of these hazards among the welders. This study assessed the extent of oxidative stress and liver injuries caused by welding fumes and estimate the possible ameliorating effect of African walnut (*Tetracarpidium Conophorum*). The study was carried out in commercial city of Ihiala and uli, in Ihiala Local Government Area of Anambra State of Nigeria. It was a cross sectional and interventional study design. A total of forty welders who consented were identified from a list of registered workshops. They were interviewed using semi-structured interviewer-administered questionnaires. Baseline samples were collected for oxidative stress and liver markers. They were later fed with two pieces of African walnut (3 times per day for period of one month). Eight milliliters of blood were collected for analyses. Data obtained was analyzed using SPSS. Results showed that the MDA levels welders were significantly higher when compared with the non-exposed group ($P < 0.05$). The mean levels of SOD and GPx activities significantly lower in exposed group than non-exposed. The mean levels of Selenium and Zinc were also significantly lower in exposed than non-exposed ($P < 0.05$). The liver markers AST and ALT were significantly higher in exposed group of welders when compared with non-exposed group. Intervention results showed that MDA levels was significantly reduced when compared with the baseline study of the exposed group ($P < 0.05$). The mean levels of SOD and GPx activities were significantly higher after being fed with *T. Conophorum* (13.06 ±2.462 and 15.43±1.842) and 0.661±0.087 and 0.781±0.0806. The mean levels of Selenium and Zinc were also significantly higher after the intervention ($P < 0.05$). The liver markers AST and ALT were significantly reduced after intervention when compared with the baseline study. However, the mean levels of AFP, ALP, Albumin, Protein, total bilirubin and conjugated bilirubin did not differ significantly between the baseline and interventional studies ($p > 0.05$). Welding processes may induce oxidative stress which may lead to toxicities of various organs especially liver and kidney. African walnut (*T. conophorum*) may be beneficial in reducing the rate of oxidative stress thereby protecting the vital organs of the body.

Keywords: Welders; African walnut (*T. conophorum*); oxidative stress markers; liver markers.

INTRODUCTION

According to the International Standard Classification of Occupations (ISCO), welders perform their daily role by working on metal parts using heat from flame or other sources of heat (ILO, 1968). Welding exposes welders to a variety of work-related hazards, which may be deleterious to their health. There are many kinds of welding processes in Nigeria which include the use of oxy acetylene flame and electric welding using electricity. The health of workers in the welding environment is a major concern in the under developed and developing countries. The high speed particles and occasional explosions of the oxyacetylene gas vessels may also lead to severe burns and other physical injuries (Shaikh& Bhojani, 1991). The excessive lighting (glare) and exposure to ultra violet radiation may lead to `arc eye' or `flash burn' injuries to the cornea, photo keratosis and double vision and consequent retinal damage (Norn & Franck, 1991). Welders are also exposed to noxious metal fumes containing many toxic metals, including manganese (Mn), cadmium (Cd), and some non- toxic trace elements chromium (Cr) and cobalt which may probably become toxic when exposed to higher quantities. (OSHA, 1981). Some conditions suspected to be associated with welding exposures, including metal fume fever, pulmonary fibrosis, bronchitis, and asthma, have been attributed to oxidative stress and inflammatory processes in the lung (Coggen, *et al*, 1994). Generation of ROS (superoxide, hydroxyl radicals, and hydrogen peroxide) and nitrogen species (nitric oxide and peroxynitrite) is main goal of these processes. The imbalance between generation of ROS and anti -oxidant mechanisms; superoxide dismutase (SOD) and glutathione peroxidase (GPx) and other antioxidant enzymes may lead to oxidative stress. Apart from the direct damage caused by the toxic metals generated by metal fumes, production of reactive oxygen species is a particularly destructive aspect of oxidative stress and more aggressive radical species can cause extensive cellular damage resulting to tissues and organ damage.

There have been reports of carcinogenic and mutagenic effects due to chronic exposure to welding fumes in animals that may be extrapolated to man (Pasamen *et al.*, 1986). Other organs, which may be affected by welding fumes, include liver, kidneys (Vyskocil *et al.*, 1992; Nuyts, *et a.l,* 1995), and the reproductive organs leading to reduction in sperm count and fecundity (Mortensen, 1988; Bonde, 1990). Therefore, there is need for natural protective means of cubbing this deleterious effect of these injuries caused by welding fumes.
The use of medicinal plants to cure many ailments has been a tradition in different parts of the world. *Tetracarpidium conophorum* (African walnut) is a member of the Euphorbiaceae family. The seed is edible and widely cultivated. The Yoruba speaking people of Nigeria call the walnut *awusa* or *asala, while the Igbo's call it ukpa* and the Hausa's call it *gawudi bairi* (Chijoke, Anosike & Ani 2015; Kanu, *et al.*, 2015). In Sierra Leone, it is called *musyabassa* and in western Cameroon, it is known as *kaso* or *ngak*, among other local names (Burkill, 1985). Walnut has been known to contain vital nutrients such as protein, vitamins and minerals including essential fatty acids (Kim & Lee, 2002)

The aim of this study was to use a human population known to be chronically exposed to welding fume to investigate:
1. If there is any liver toxicities among welders by assessing the liver markers.
2. Whether or not occupational exposure to welding fume altered the homeostasis of essential trace elements, such as Selenium and zinc in biological fluids.
3. The status of the oxidative stress among career welders following chronic exposure by using oxidative biomarkers.
4. Whether introduction of African walnuts (*Tetracarpidium conophorum*) could reverse the toxicities caused by welding fumes and particles.

MATERIALS AND METHODS

MATERIALS

The study was carried out in Ihiala, a town in Anambra State of Nigeria. It was of a cross sectional prospective and interventional study design. The total population of welders in the local government area (LGA) was included in the study. They were identified from a list of registered workshops obtained from their association headquarters in the LGA. After verbal consent, detailed personal, and medical questionnaire was completed by the welders through personal interview. To validate the use of the questionnaire, twenty welders from separate local government in Anambra State, Nigeria were selected and pre-tested while walking them through. Each question was fully explained to the respondents to ensure adequate understanding of the issues being sought after.

Sample Size

The study included all the fifty welders who consented and occupationally exposed to oxyacetylene fumes for more than five years (workers group), and fifty workers never occupationally exposed to oxyacetylene fumes (control group). All the included subjects in the two groups were from urban areas. .

Plant seed Collection and Identification

Mature nuts of *Tetracarpidium conophorum* (African walnut) were purchased from a local market in Ihiala, Anambra State. The walnut was identified and authenticated by the Department of Plant Science Chukwuemeka Odumegwu Ojukwu University, Uli.

Samples Collection and Intervention

Eight milliliters (8 ml) of blood samples were collected from all subjects who were included in the study prior to commencement of intervention and was stored at -20 ˚C for analysis of oxidative stress markers (GPx, SOD and MDA) within one week of collection, while the remaining aliquot was stored at -20 ˚C for analysis of liver markers (ALT, AST, ALP, Albumin, total protein, total and conjugated bilirubin).

Thereafter, the subjects were fed with *Tetracarpidium conophorum* (African walnut) for a period of one month (three walnuts given three times daily after each meal) under strict supervision.

Eight milliliters (8 ml) of blood samples were collected from all subjects who complied with rules after one month study for analysis of oxidative stress markers (GPx, SOD and MDA) and liver markers.

METHODS

Quantitative determination of Alpha Feto-Protein (AFP) was carried out by sandwich enzyme immunoassay technique using ELISA Kit.

Total Protein was measured by the Biuret Method, Albumin by Dye-binding Method.

SOD was assayed by colorimetric method of Misra and Fredovich (1972).

The activity of glutathione peroxidase was determined the method of Rotruck *et al.* (1973).

MDA level was determined by the colorimetric method of Gutteridge and Wilkins (1982).

AST AND ALT were determined using the spectrophotometric method of Bergermeyer *et al.* (1978). ALP was assayed using the spectrophotometric method of Schlebusch *et al.* (1974). Total Bilirubin was assayed using the method of Jendrassik and Grof (1938).

Trace metal contents was determined using atomic absorption spectrophotometer.

All analyses were done at Onamec, Nnewi and Springboard Laboratories LTD Awka, Anambra State, Nigeria.

Statistical Analysis

The collected data and the laboratory results were computed. Statistical analysis was done using SPSS version 21. The quantitative results were expressed as means ± standard deviation (SD). Independent t-test was used to compare two independent variables. P value <0.05 were considered significant.

RESULTS AND DISCUSSION

Welding is a common small-scale industry in Nigeria and as with other occupations, is associated with its work place hazards that can affect the health of those engaged in it. Present research finding shows that there is increase in plasma levels of AST and ALT which serve as reliable indices of assessment of damage to the parenchymatous cells of liver (Table 1). This was in concordance with that observed by Okolie *et al*. (2005) and Newhouse *et al*. (1998). These observed significant increases in the activities of these two liver enzymes are pointers to crude acetylene-induced lesions in liver tissues. This is also supported by the pronounced depression in SOD and GPx activities in liver (Table 2), which suggests that the deleterious effects of crude acetylene may occur through a mechanism involving oxidative stress. The level of MDA which is an index of lipid peroxidation is significantly higher in welders when compared with the control un-exposed group (Table 2). SOD and GPx are among mercenary of biological antioxidant systems that protect membranes from oxidative damage by reactive oxygen species. It is likely that the observed elevation of tissue SOD may be an initial adaptive response to cope with the toxic effects of crude acetylene. Since ALP and AFP were increased, though not significantly, it is possible that this increase may become significant at higher doses of crude acetylene exposure and prolonged inhalation periods. However, the concentrations of albumin, total protein and total bilirubin were not significant. This implies that the toxic gases may not affect the synthesis function of the liver. The oxidative stress results confirm what has been reported in previously published literature, in that welding fume exposure may result in systemic oxidative stress and alterations in trace metals metabolisms, both in animals (Li *et al*., 2004; Melissa *et al.,* 2014) and in humans (du Plessis *et al*., 2010; Graczyk *et al*., 2016). Du Plessis *et al*. (2010) also found increased oxidative stress in circulating mononuclear cells in male welders occupationally exposed to welding fumes. These findings could be attributed to the ROS generated during the welding processes.

From our interventional studies, African walnut (*T. conophorum*) was able to significantly reduce the raised liver enzymes (ALT and AST), and MDA levels (Table 3 & 4). It however, increases SOD and GPx activities associated with the exposure of oxy-acetylene fumes when compared with the base line study. A similar study was carried out by Akomolafe *et al*. (2015) who stipulated that the antioxidant properties could be attributed to its radical scavenging ability. This study therefore confirms the ameliorating effect of *T. Conophorum* on Oxidative Stress Markers and Liver Markers of Welders Exposed to Oxy-acetylene Fumes

Table 1: Liver Markers of Exposed Group and Control

Liver Markers	Exposed (welders)	Non-exposed	P Value
AFP(µmol/min)	3.83 ± 3.11	3.14 ± 1.478	0.66
ALT(µmol/min)	30.84 ± 5.37	18.42 ± 2.97	0.01*
AST(µmol/min)	26.07±4.49	15.24±3.90	0.00*
ALP(µmol/min)	49.32±10.62	46.049±5.02	0.34
ALB(µmol/L)	42.12 ± 4.16	41.28 ± 4.14	0.50
PROT(µmol/L	**66.42±4.16**	**64.32±4.01**	**0.26**
Total. Bil. (µmol/L)	10.25±1.64	9.23 ±0.91	0.63
Conj. bil.(µmol/L)	2.66 ±0.38	2.99±1.0 5	0.86

* Significant at 0.05 level

Table 2: Oxidative Stress Markers and Trace Elements among exposed group and control group.

Oxidative Stress markers/Trace elements	Exposed (welders)	Non- exposed	P Value

GPx(µmol/min)	0.61 ± 0.08	0.78 ± 0.08	0.01*
SOD(µmol/min)	13.06±2.46	15.43 ± 1.84	0.01 *
MDA(µmol/L)	2.32 ±0.63	1.88 ± 0.45	0.03*
Se(µmol/L)	1.96 ±0.37	2.17 ± 0.54	0.01*
Zn(µmol/L)	3.17±0.89	4.73 ± 0.86	0.00*

Table 3: Liver Markers of Exposed Group and after intervention

Liver Markers	Exposed (Base line)	After Intervention	P Value
AFP(µmol/min)	3.83 ± 3.11	3.04 ± 2.67	0.75
ALT(µmol/min)	30.84 ± 5.37	22.35 ± 1.97	0.01*
AST(µmol/min)	26.07 ± 4.49	16.32 ± 2.90	0.00*
ALP(µmol/min)	49.32 ± 10.62	44.49 ± 6.92	0.74
ALB(µmol/L)	42.12 ± 4.16	43.35 ± 4.14	0.20
PROT(µmol/L	**66.42 ± 4.16**	**67.49 ± 3.08**	**0.16**
Total. Bil. (µmol/L)	10.25 ± 1.64	9.93 ± 0.81	0.13
Conj. bil.(µmol/L)	2.66 ± 0.38	2.92 ± 1.90	0.86

* Significant at 0.05 level

Table 4: A Comparison of Oxidative Stress Markers and Trace Elements of baseline and after intervention

Oxidative Stress markers/Trace elements	Base line	Intervention	P Value
GPx(µmol/min)	0.61 ± 0.08	0.72 ± 0.079	0.01*
SOD(µmol/min)	13.06 ± 2.46	13.43 ± 1.74	0.10
MDA(µmol/L)	2.32 ±0.63	1.46 ± 0.310	0.03*
Se(µmol/L)	1.96 ± 0.372	1.71±0.540	0.71
Zn(µmol/L)	3.17 ±0.892	3.739±0.92	0.56

* Significant at 0.05 level of significance

CONCLUSION

The African walnut (*T. conophorum*) should be explored for the production of walnut flour and cake. If incorporated in diet will enhance protection against occupational based toxicities because of its great potential.

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COMPETING INTERESTS

The authors declare that they have no financial or personal relationships which may have inappropriately influenced them in writing this article.

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