Review Article

Toxic effects of formaldehyde on the nervous system

Hussein Abdu[1], Yamrot Kinfu[2] and Asrat Agalu[1*]

[1]Wollo University, college of medicine and health sciences, Dessie, Ethiopia.
[2]Addis Ababa University, College of health sciences, school of medicine; department of anatomy, Addis Ababa, Ethiopia.

Formaldehyde is a flammable, colourless gas with pungent odour and readily polymerized at ambient temperature. It is one of the major pollutants in indoor air. It is found in the environment as a result of natural processes and from man-made sources and it is widely used in industries and medical settings. Several symptoms might be manifested related to formaldehyde –exposure among which lethargy, decrease in motor activity, and loss of appetite is common. Formaldehyde resulted in minor headache to irreversible neurotoxicity and brain cancer. The neurotoxic effects produced by formaldehyde exposure are dependent on the concentration of formaldehyde and duration of exposure which is more pronounced in concentrated and increased duration of exposure. Acute exposure to low concentrations it leads to stimulation while in higher concentrations it acts as central nervous system depression. The formaldehyde exposure in adult and postnatal period results in an increase oxidant substances though it causes a decrease in the antioxidant enzymatic activity in the rat frontal cortex and hippocampus. It was indicated that formaldehyde induces several characteristics of neurotoxicity. Anatomists, histologists, pathologists, medical students, embalmers and members of industries utilizing formaldehyde is more exposed to formaldehyde gas. Therefore, it is recommended to use personal protective equipment, maintaining high room air exhaust rates and concentrations of formaldehyde in work places should be monitored by using standard measuring machines.

Key words: Toxicity, neurotoxic, formaldehyde, nervous system, concentration.

INTRODUCTION

Physical and Chemical Features of Formaldehyde

Formaldehyde (FA) (formula: HCHO; chemical name: methanal) is a member of the aldehyde family and is one of the simplest organic molecules (Kanter, 2010; Songur et al., 2003; Yamato et al., 2005). It is a flammable, colourless gas with pungent odour and readily polymerized at ambient temperature, and is one of the major pollutants in indoor air (WHO, Regional Office for Europe, 2000). The theoretical solubility of formaldehyde in water is 95% (w/w) at 120°C. However, at room temperature, pure aqueous solutions contain formaldehyde in the form of methylene glycol and its oligomers. Aqueous solutions containing more than 30% (w/w) FA become cloudy at room temperature due to the formation of larger poly-glycols (**table 1**) (Ullmann, 1985).

Formaldehyde is rarely found in its original state because it has a short half-life in air (approximately 30-50 minutes) (WHO, 1999) and decomposes in light to form a toxic substance. In liquid form (formalin) it is stable over time (US ATSDR, 1997; WHO, 1999). It is highly soluble in water, as well as in most organic solvents, and is a highly reactive molecule that can be irritating to tissues through direct contact. Furthermore, it causes cytotoxicity through the formation of strong deoxyribonucleic acid (DNA) –protein cross-links, as well as cross-links with other molecules, For example, amino acids (Gurel et al., 2005; Zhou et al., 2006).

Formaldehyde is easily absorbed through the respiratory and gastrointestinal tracts, and it is metabolized to formic acid (format) in the nasal mucosa, liver, and erythrocytes of living organisms, and is then excreted in the urine and feces, or is converted into carbondioxide and exhaled. There are at least seven enzymes that catalyse the oxidation of FA in animal tissues, namely aldehyde dehydro-

*Corresponding: author: Email-asratagl@yahoo.com

genase, xanthine oxidase, catalase, peroxidase, aldehyde oxidase, glyceraldehyde-3-phosphate dehydrogenase, and a specific Nicotine amide Adenine-dinuclotide-dependent FA dehydrogenase (Gabriel et al., 2005; Solomons and Cochrane, 1984).

There are long-standing concerns about the adverse health effects of FA exposure, including carcinogenicity, for professionals exposed to formalin-based fixatives, such as pathologists, anatomy students, nurses, and embalmers, and for workers exposed to FA in manufacturing. Recently, public awareness of this issue has been raised in the wake of Hurricane Katrina, as high levels of FA have been found in the temporary housing trailers provided by the Federal Emergency Management Agency. At the same time, a large number of workers are exposed to FA in their workplaces (OSHA, 1995).

During an anatomy class, the evaporation of FA from the cadavers, embalming fluid, could negatively affect medical students and instructors' health. The Ministry of interior of Thailand declared in 1977 that FA concentration during eight hours working periods could not be over 3 parts per million (ppm) while the threshold limit value of the American Conference of Governmental Industrial Hygienists is 0.3 ppm (OSHA, 1995).

Sources of Formaldehyde

Formaldehyde is ubiquitously present in the environment as a result of natural processes and from man-made sources (Ullmann, 1985; WHO, Regional Office for Europe, 2000). But, it does not accumulate in the environment, because it is broken down within a few hours by sunlight or by bacteria present in soil and water. People, animals, and plants actually produce low levels of FA during normal metabolic processes and it is metabolized and excreted quickly. Thus, it does not accumulate in the body. According to the International Program on Chemical Safety, the natural formation of FA from other molecules may contribute up to 70-90 percent of the total atmospheric FA (FETEG, 2002).

Formaldehyde is found in nature, domestic air, cosmetics, cigarette smoke, and in the polluted atmosphere of cities from the incomplete combustion of organics (i.e. Methane or other gases, wood, coal, oil, tobacco and gasoline), photochemical smog, and off-gassing of products containing FA (FETEG, 2002; Franklin, 2000; Kanter, 2010; Kim, 2001; Smith, 1992; Sanger et al., 2008; Ullmann, 1985; Usanmaz et al., 2002). Indoor sources of FA include upholstery, permanent press fabrics, carpets, pesticide formulations, and cardboard and paper products whereas outdoor sources include emissions from fuel combustion (motor vehicles), industrial fuel combustion (power generators), oil refining processes, and other uses (copper plating, incinerators, etc.) (WHO Regional Office for Europe, 2000). Thus, everyone living in society may be exposed to FA.

Uses of Formaldehyde

Formaldehyde is produced and used worldwide on a large scale, predominately in industries for the production of resins, manufacturing of building materials, paper, carpets, textiles, paint, particle board, and plywood and as a component of numerous household products (US ATSDR, 1997; WHO, 1999; US EPA and TEACH, 2007; Public Review Draft, 2007; WHO International Agency for Research on Cancer (IARC), 2006). FA is also used in the manufacture of melamine, Polyacetal, and phenolic resins. It is also used as a preservative, a hardening and reducing agent, a corrosion inhibitor and a sterilizing agent. It is also used in agriculture, concrete and plaster additives, cosmetics, disinfectants, fumigants, photography, and wood preservation. In addition, it is widely used in medical settings and employees in these areas may be highly exposed to it. In particular, anatomists and medical students having dissection sessions are the most common subjects that can be exposed to Formaldehyde gas (Sarnak, 1999; WHO IARC, 2006).

Harmful Effects of Formaldehyde

Formaldehyde was declared as a Priority Existing Chemical on 5 March 2002 in response to occupational and public health concerns (NICNAS, 2006; OECD SIDS, 2000). Recently, many cases of poisoning, allergy, asthma, pulmonary damage, cancer and death were reported as a result of FA exposure from contaminated foods, drinking water, and polluted indoor air (Tang et al., 2009).

genated to CO_2 and exhaled. The presence of glutathione and FA dehydrogenase in epithelial cells of the respiratory tract varies with location and influences the amount of FA reaching the blood. While glutathione-bound FA are rapidly metabolized, free FA in cells can form DNA-protein cross-links (Public Review Draft, 2007). The toxicity of FA is of concern to all who work closely with it (Kilburn et al., 1987; Public Review Draft, 2007).

Acute Effects of Formaldehyde

There appears to be a consensus on the range of acute toxic and irritant effects of FA on man. The acute effects of FA exposure appear to be largely as a result of its irritant properties. However, some individuals experience symptoms that are a result of previous sensitization following acute high FA exposure, or long term low level exposures. The organ systems usually affected include the eyes, upper and lower respiratory tract, skin and CNS. Irritation of the eyes results in a burning sensation, lacrimation, and conjunctivitis (Solomons and Cochrane, 1984).

Ingestion of liquids containing 10-40% FA cause severe irritation and inflammation of the mouth, throat, and stomach. Severe stomach pains will follow ingestion with possible loss of consciousness and death. Ingestion of dilute FA solutions (0.03- 0.04%) may cause discomfort in the stomach and pharynx (Ozen *et al.*, 2003; Yale University office of environmental health and safety, 2005).

Inhalation of FA is highly irritating to the upper respiratory tract and eyes. Concentrations of 0.5to 2.0 ppm may irritate the eyes, nose, and throat of some individuals. Concentrations of 3 to 5ppm may also cause tearing of the eyes and are intolerable to some persons. Concentrations of 10 to 20 ppm cause difficulty in breathing, burning of the nose and throat, cough, and heavy tearing of the eyes and 25 to 30 ppm causes severe respiratory tract injury leading to pulmonary edema and pneumonia. A concentration of 100 ppm is immediately dangerous to life and health (Yale University office of environmental health and safety, 2005).

Inhalation of high concentrations (> 120 mg/m3) of FA caused hyper salivation, acute dyspnea, vomiting, muscular spasms, convulsions and finally deaths. Histopathology examination showed respiratory tract irritation, bronchioalveolar constriction and lung edema (OECD SIDS, 2002). Effects found microscopically in rats following exposure to FA (10 ppm) for 4 hours included ciliar lesions, cellular swelling and excess secretion of mucus by goblet cells (OECD SIDS, 2002).

Formalin is a severe skin irritant and a sensitizer. Contact with formalin causes white discoloration, drying, cracking, and scaling. Prolonged and repeated contact can cause numbness and a hardening or tanning of the skin. Previously exposed persons may react to future exposure with an allergic eczematous dermatitis or hives (Solomons & Cochrane, 1984; Yale University office of environmental health and safety, 2005). Formaldehyde solutions splashed in the eye can cause injuries ranging from transient discomfort to severe, permanent corneal clouding and loss of vision. The severity of the effect depends on the concentration of FA in the solution and whether or not the eyes are flushed with water immediately after the accident (Yale University office of environmental health and safety, 2005).

Chronic Effects of Formaldehyde.

It is accepted that FA is toxic and slightly carcinogenic over certain concentrations, and the harmful effects of FA increase under room temperature conditions, because the molecule easily evaporates (Feron et al., 1991; Franklin *et al.*, 2000; Gurel *et al.*, 2005; Ozen et al., 2002; Songur et al., 2003; 2010). Repeated and prolonged exposure increases the risk. Various animal experiments have conclusively shown FA to be a carcinogen in rats. In

humans, FA exposure has been associated with cancers of the lung, nasopharynx and oropharynx, and nasal passages (Yale University office of environmental health and safety, 2005). Also prolonged or repeated exposure to FA may result in respiratory impairment. Rats exposed to FA at 2ppm developed benign nasal tumors and changes of the cell structure in the nose as well as inflamed mucous membrane of the nose. Structural changes in the epithelial cells in the human nose have also been observed (Yale University office of environmental health and safety, 2005). Some persons have developed asthma or bronchitis following exposure to FA, most often as the result of an accidental spill involving a single exposure to a high concentration of FA (Yale University office of environmental health and safety, 2005).

According to IARC*,* FA is classified as a weak genotoxic, probable carcinogenic agent for humans (group 2A) (Takeuchi, 2004). Exposure to FA is a predisposing factor for the occurrence of cancer in the nasal cavity, paranasal sinuses, and leukaemia. According to WHO, FA does not have a high carcinogenic potential in humans (WHO IARC, 2006; Takeuchi, 2004). FA is thought to have weak carcinogenic effects through the formation of protein cross-links and the promotion of cell proliferation in the human respiratory tract (WHO IARC, 2006; Takeuchi, 2004).

According to the studies of Ozen et al. (2002; 2003); Songur et al. (2003) and Zararsiz et al. (2006), FA inhalation was observed to cause a reversible decrease in food and water consumption, and in body weight (body weight gain) in rats. In addition, Martin (1990) and Saillenfait et al. (1989) also reported that inhalation of FA (10–40 ppm) during the gestation period caused a reduction in maternal food consumption, a decrease in body weight gain, and lower birth weight of pups. These toxic effects may occur by central inhibition or most likely from inhibition of nucleic acid and protein synthesis (Ozen et al., 2003; Songur et al., 2010). FA is accepted as toxic over certain doses and the chances of harmful effects are increased at room temperatures because of its volatility (Kanter, 2010).

Formaldehyde Neurotoxicity

Neurotoxicity is any effects on the structure or function of the central and/or peripheral nervous system related to exposure of a chemical substance. Recently, neurotoxicity has become an important endpoint in hazard identification and assessing the risks of chemicals. The nervous system is of particular interest because mature neurons are generally incapable of regeneration (Takeuchi, 2004).

The disorders of the nervous system due to chronic

Table 1. Physical and chemical features of formaldehyde.

Chemical name	Methanol
Chemical Family:	Aldehyde
Molecular formula	HCHO
Structural formula	H
	|
	C = O
	|
	H
Molecular weight	30.03 k_{Da}
Description	Colourless liquid, pungent odor
Boiling point:	214 deg. F (101 ^{o}C)
Specific Gravity	1.08 (H20=1 at 20 ^{o}C)
PH:	2.8-4.0
Solubility in Water	Miscible Solvent; Soluble in alcohol, acetone
Vapor Density	1.04 (Air= 1 at 20 ^{o}c)
Odor Threshold:	0.8-1 ppm
Other names (Synonyms)	Formalin; Formic aldehyde, Methaldehyde Methyl aldehyde, Methylene oxide, Formalith, Formol; Morbicid, Oxomethane, Oxymethylene, Paraform, Methylene Glycol, Formalin (Methanol-free), Methanal; Methylene Oxide, Tetraoxymethalene,

Cited from: (Public Review Draft, 2007; OECD and SIDS, 2002; Yale University office of environmental health and safety, 2005).

exposure to industrial chemicals are usually very hard to recover, and sometimes tend to become gradually more serious even after removal from the exposure. In addition, the normal cascade of brain development during fetal and newborn life may be exquisitely sensitive to disruption by chemicals, resulting in long lasting and profound nervous system dysfunction (Takeuchi**, 2004).**

Formaldehyde can cause nervous system damage by its known ability to react with and form cross linking with proteins, DNA and unsaturated fatty acids (Thrasher *et al.*, 1990).

These mechanisms could cause damage to virtually any cell in the body, since all cells contain these (proteins, DNA and unsaturated fatty acids) substances. FA can react with the nerve proteins (neuroamines) and nerve transmitters (For example catecholamine) which could impair normal nervous system function and cause endocrine disruption (Thrasher et al., 1990).

Acute effects of FA gas mostly occur on the organs which have direct contact with the external environment. The most common acute toxicities occur in the form of headaches, malaise, insomnia, anorexia and dizziness (Kanter, 2010).

Chronic exposure to FA has been associated with immunological hypersensitivity as measured by elevated circulating IgG and IgE auto antibodies to human serum albumin. (Public Review Draft, 2007).

In addition, a decrease in the proportion of T-cells was observed, indicating altered immunity. As reported by Thrasher et al., *(*1990), long-term exposure to formaldehyde was associated with auto antibodies, immune activation, and FA -albumin adducts in patients occupationally exposed, or residents of mobile homes or of homes containing particleboard sub-flooring. The authors suggested that the hypersensitivity induced by FA may account for a mechanism for asthma and other health complaints associated with FA exposure (Public Review Draft, 2007; Songur et al., 2010; Thrasher et al., 1990).

Long-term exposure to FA (such as14–30 years) may cause irreversible neurotoxicity and is related to brain cancer (astrocytoma) (Kanter, 2010).

Furthermore, inhaled FA has been shown to cause behavioural and memory disorders in rats and has been classified as 'probable neurotoxic' (Bedino, 2004; Kanter, 2010). This review paper attempts to review the works of different authors which have been done to determine FA neurotoxicity on different parts of the nervous system.

Review of published articles on formaldehyde neurotoxicity

RESULTS AND DISCUSSION

The Effects of Formaldehyde on Biochemical Parameters

The response for any toxic substance first starts at the chemical level and any changes that occur in the structures are observed as the damage continues.

Table 2. MDA, GSH-Px and SOD values in the hippocampus of the three groups of rats (n=7 for each group) (Ozdem *et al.,* 2007).

Group	MDA(nmol/g)	GSH-Px(U/g)	SOD(U/g)
I(control)	20.3±0.6	54.2±2.1	192.1±5.1
II(FA)	31.4±0.9	74.3±3.3	262.4±5.6
III(FA+CAPE)	24.0±0.7	57.2±2.2	221.6±3.9
P(IvsII)	0.001	0.001	0.001

Values are expressed as means ± SD.

Ozdem et al. (2007) investigated the neurotoxicity of FA on hippocampus and the protective effects of caffeic acid phenethyl ester (CAPE) against these toxic effects. For this purpose, 21 male Wistar rats (weighing 200-250 g) were used. The rats were kept in plexiglas cages (4 rats/cage) where they received standard chow (supplied from Elazig plant, Elazig, Tutkey) and water ad libitum in an air- conditioned room with automatically regulated temperature (21±1°c) and lighting (07.00-19.00h). The animals were divided in to three groups. The rats in group I (n=7) were used as the controls. The rats in group II (n=7) were injected with FA (10mg/kg, ip) daily. The rats in group III (n=7) received CAPE (10mg/kg, ip) daily while exposed to FA. At the end of the eight days experimental periods, all rats were sacrificed by decapitation. The brains of rats were removed and hippocampus tissues were obtained from all the brain specimens. Some of the hippocampus tissue specimens were washed twice with cold saline solution, placed into glass bottles, labelled, and stored frozen (-30 °c) for eventual determination of SOD, GSH-Px and MDA production. The other hippocampus tissue specimens were used for light microscopic and immune histochemical evaluations (Ozdem *et al.*, 2007).
important parameter for the determination of oxidative damage, was significantly higher in the FA administered group than in the control group (p<0.001). The rats that were exposed to FA together with CAPE administration had decreased SOD; GSH-Px and MDA levels compared to FA administered rats (**table 2**) (Ozdem et al., 2007).

Another study was conducted by Zararsiz et al. *(*2007) to investigate the protective effects of melatonin against FA- induced neurotoxicity in pre frontal cortex of rats for this study, also 21 adult male Wistar rats (weighing 310-320 g) were used and maintained under controlled temperature (21±1°c) and controlled light conditions (light, 07.00-19.00 h). Food (standard pellet diet) and tap water were supplied ad libitum. The animals were divided in to three groups. The rats in group I (n=7) were used as the controls. The rats in group II (n=7) were injected with FA (10mg/kg, ip) every other day. The rats in group III (n=7) received melatonin daily ip (25 mg/kg) while exposed to FA. At the end of 14- day experimental

period, all rats were killed by decapitation. The brains of rats were removed and the prefrontal cortex tissues were obtained from all the brain specimens. Some of the prefrontal cortex tissue specimens were washed twice with cold saline solution, placed into glass bottles, labelled, and stored frozen (-30 °c) for eventual determination of SOD, GSH-Px and MDA production. The other prefrontal cortex tissue specimens were used for immunehistochemical evaluation (Zararsiz et al., 2007).

Zararsiz et al. *(*2007) reported the biochemical findings from the rats exposed to FA. The values of SOD and GSH-Px (oxidative/antioxidant enzymes) were significantly decreased compared to those in the control group (p<0.001) that is contradicted with the study done by (Ozdem et al., 2007). This difference may be due to the differences in duration of exposure. The MDA level which is considered to be an important parameter of oxidative damage determination and a marker for lipid per-oxidation of the tissue, was significantly higher in FA administered group than in the control group (p<0.001) that is in line with the study conducted by (Ozdem et al., 2007). The rats that were exposed to FA together with melatonin administration had increased SOD and GSH-Px enzyme levels and decreased MDA levels as compared to FA administered group. Bax-stained cells were not observed in the control group while FA administered rats had dense immune positive cells. The group that was exposed to FA along with melatonin administration had many fewer immune positive cells **(table 3)** (Zararsiz et al., 2007).

In the studies conducted by Gurel et al., (2005) and Sarsilmaz *et al*., (2003) on FA neurotoxicity, FA was observed to affect cerebral oxidant/antioxidant systems and cause oxidative damage. The reactive oxygen species (ROS*)*, including singlet oxygen, hydrogen peroxide, superoxide anion, and hydroxyl radical, are essential for normal biological processes and are produced physiologically. The excessive production and accumulation of ROS can become hazardous to cells and tissues (Songur et al., 2010).

These ROS are important mediators of cellular injury and play a role in oxidative stress, and can be present in condition where toxicity is occurred. Oxidative stress that

Table 3. MDA, GSH-Px and SOD values in prefrontal cortex of the three groups (7 per group) Zararsiz et al., 2007).

Parameters	I(control)	II(FA)	III(FA+MEL)
MDA(nmol/g)	11.3±0.7	17.8±0.8*	11.6±0.5[+]
GSH-Px(U/g)	241.4±17.1	54.6±2.5*	168.3±11.7*[+]
SOD(U/g)	246.1±32.0	136.8±25.6*	253.6±21.3[+]

Values are expressed as means ± SD; MEL, melatonin. *p<0.001;**p<0.01vs. control group;[+]p<0.001 vs. FA group.

are initiated by ROS can be regulated by cellular defence mechanisms of the body, including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (Songur et al., 2010). The brain has a high content of unsaturated fatty acids and requires very high amounts of oxygen. Since the rates of oxidative metabolic activities in the brain are relatively high and antioxidant enzymes activities are low, the neurons in the CNS are more vulnerable to toxicity or ischemia (Irmak et al., 2003; Songur et al., 2010).

In another study reported by Gurel et al. (2005) and Zararsiz et al. (2006; 2007), exposure to FA during the adult period (10 mg/kg, 10 days, intra peritoneal (ip)), resulted in an increase in oxidant substances (malondialdehyde (MDA) and protein carbonyl (PC)) , but causes a decrease in the antioxidant enzymatic activity such as SOD and CAT, in the rat frontal cortex and hippocampus (Gurel et al., 2005; Songur et al., 2010; Zararsiz et al., 2006; 2007).

In another study exposure to FA during the early postnatal period was also found to cause an increase in the levels of MDA and resulted in a decrease in the activity of glutathione peroxidase (GSH-Px) and SOD at postnatal day (PND)30, in the rat cerebellum (Songur et al., 2010). Since FA is metabolized by FDH enzyme and this enzyme is dependent on glutathione, a decrease in GSH-Px activities resulted in oxidative damage of cells and tissues.

The Effect of Formaldehyde on Neuronal Morphology

Songur et al., (2003) were conducted a research to investigate the effects of FA gas inhalation during the early postnatal period on the heat shock proteins 70 kDa (Hsp70) synthesis and morphological changes in the hippocampus in developmental process of rats. These investigators prepared vitreous quadrangular chambers (20×50×100 cm). Two holes were opened into each chamber for inflow and outflow of air. The FA gas was generated by thermal depolymerisation of parformaldehyde at 70-90 °c. It was pumped from production area to chambers on desired levels and times.

Healthy and mature male and female albino wistar rats used were mated. The groups were arranged with pups born at the same time, and put in the chambers after birth. FA gas was given into vitreous chambers at concentrations 0 (control), 6 ppm and 12 ppm for 30 days (6 hours/day; 5 days/week), respectively. Its concentrations were regularly monitored with the FA monitor After the treatment, rats were divided in to nine groups for decapitation at 30th, 60th and 90th days. Pups were kept in plastic cages and transferred in to the chambers only during the treatment. During the study, the temperature of the chambers and room air were maintained at 25±2°c, the humidity at 40-45%, and light was 12 h day/ 12 night cycle. Food and water were provided and libitum during the FA treatment and rest of the time (Songur et al., 2003).

In the study reported by Songur et al. (2003) on the FA neurotoxicity, inhalation of FA at concentrations of 6 ppm and 12 ppm, during the early postnatal period (PND30), resulted in an increase in pyknotic neuron counts in the rat hippocampus pyramidal cell layer. These increases in pyknotic neuron counts in the tested rats were continued throughout the PND60 period. However, there were no significant changes in the PND90 group of rats (Songur et al., 2003, 2010). Because of FA inhalation, there was an increase in immune staining of heat shock protein 70 (Hsp70) in the rat hippocampus, particularly at the exposure concentration of 12 ppm, and for the PND30 group rats. But in the PND60 and PND90 rat groups, Hsp70 immune staining decreased (Songur et al., 2003; 2010).

A stereological study done by Sarsilmaz et al., (2007) to determine whether exposure of neonatal rats to FA had either early or delayed effects on the numbers of pyramidal cells in the hippocampus. Neonatal Wistar rats were exposed to 0 ppm (control group), 6 ppm and 12 ppm (high concentration group) of FA concentrations throughout the 30 day period following the birth by placing them for 6 h/day in a glass chamber containing FA vapour. Then, some of the animals from each FA treated group were anesthetized and decapitated at the day 30, and the remaining ones were killed at the day 90. The brains were removed immediately and fixed in 10%

Table 4. The numbers (number/μm^2) of apoptotic neurons in the frontal cortex tissues of A (control), B (FA treated) and C (FA treated with NS) groups (Kanter, 2010).

Groups	Frontal cortex tissue
A	11.7±1.2
B	87.3±9.3a
C	49.8±4.5b

Values are expressed as means ±SD, n=10 for each group; [a]$p<0.0001$ compared to A group; [b]$p <0.001$ compared to A group; [b]$p<0.01$ compared to B group.

neutral buffered FA solution. The optical fractionator counting method was used to estimate the total number of pyramidal cells in the hippocampus. There were concentration- related- volume changes of hippocampus at PND 30 and 90; low concentration of FA significantly increased, whereas high concentration decreased the volume of hippocampus in comparison of the control at PND 30.

In both hippocampus and cerebral hemisphere, an age related volume decrease in both control and low/high concentration groups were found. On the other hand, there were significant age-related reductions in the total number of pyramidal cells at 90 days of age irrespective of the groups examined. Rats treated with high concentration FA were seen to have significantly fewer pyramidal cell neurons than either the animals treated with low concentration FA or control groups (p< 0.01). These observations indicate that pyramidal cells in the hippocampus may be vulnerable to FA exposure during the early period of life. This means it was observed that FA inhalation at 12 ppm concentration during the early PND caused a reversible decrease in the volume of cerebral hemispheres and in the hippocampal pyramidal cell layer. The decrease was evident in both PND30 and PND90 groups of rats, and thus, the damage appeared to be permanent (Sarsilmaz *et al.,* 2007). This' result was consistent with the study done by Songur *et al.,* (2003).

Aslant *et al.,* (2006) also reported that after inhalation exposure to both 6 and 12 ppm FA for the PND30 group, the volume of the dentate gyrus (DG) were observed to significantly increase in the rat brain. This increase in DG volume was also observed at the 6ppm FA inhalation level for the PND90rat group. In addition, exposure to 12ppm FA inhalation for the PND90 group caused a decreased in the total number of granular cells of the DG, in comparison to the control group and the 6ppm FA inhaled rat groups (Songur et al., 2010). From the results of the above studies reported by Sarsilmaz et al. (2007) and Songur et al. (2003), inhalation of FA during the early postnatal period resulted in an increase in apoptosis, a decrease in neuronal development, and damage to the hippocampal formation.

Another study was conducted by Kanter (2010) on protective effects of *Nigella sativa* on FA induced neuronal injury in frontal cortex. Thirty healthy male wistar albino rats, weighing 250-300 g and averaging 12 weeks old were utilized in the study. The animals were housed in individual cages (360×200×190mm) 1 month before the start of the experiments. Food and tap water were available at libitum. In the widow less animal quarter automatic temperature (22±2°c) and lighting controls (light on at 07 AM and off at 09 PM: 14 h light/ 10 h dark cycle) was performed. Humidity ranged from 50% to 55%. The rats were randomly allotted in to one of three experimental groups: control, FA treated and FA treated with NS; each group contains 10 animals. FA treated and FA treated with NS groups received ip injection of 10 mg/kg FA for 10 days at the same time in the in the morning. The rats in the NS treated group was given NS (in a dose of 400mg/kg body weight) once a day orally for 10 days starting just after FA injection. Control and FA untreated rats were injected with the same volume of isotonic NaCl as the FA treated animals that received NS. After the treatment, the animals were sacrificed and frontal cortex tissues were removed for histological investigation. Frontal cortex tissue were harvested from the sacrificed animals, and the tissues were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 5 mm thickness and then, stained with haematoxylin and eosin. Histological specimens were examined in light microscopy (Kanter, 2010).

In the study of Kanter, (2010), the morphology of neurons in the frontal cortex tissues of the control group was normal while in the FA treated group the most consistent findings occurring in the histological tissue sections stained with heamatoxyline-eosine were those indicating severe degenerative changes, shrunken cytoplasm and extensively dark pyknotic nuclei in neurons of the frontal cortex tissues.

In the NS-treated rats' frontal cortex tissues, the intensity of neuronal damages was less than in the only FA treated group. On the other hand, the number of apoptotic neurons was increased significantly in FA treated rats compared to control (p< 0.0001) and FA treated
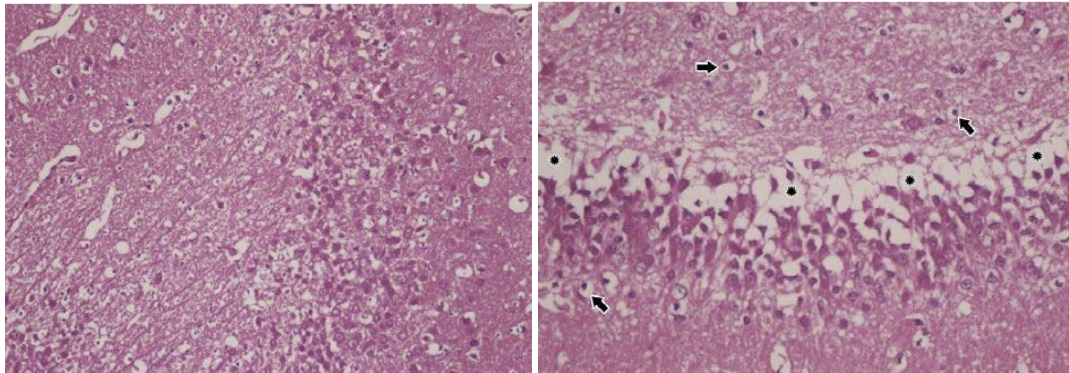
Figure 1. (a) Hippocampus section from control group showing a normal histological appearance **(b)** Pyknotic cells (**arrows)** and vacular degeneration areas (**asterisks)** were observed in hippocampus of FA administered rats (Ozdem *et al.,* 2007).

with NS (p<0.01) rats' frontal cortex tissues (**table 4**) (Kanter, 2010).

Ozdem *et al.*, (2007) also reported that in the light microscopic evaluation of the hippocampus tissue sections of the control group that were stained with HE had a normal appearance and the nuclei of the neurons had normal sizes and regular membranes (Figure 1a) but, the histological appearance of the hippocampus tissue sections obtained from FA administered rats revealed apoptotic cells with fragmented nucleus. Furthermore, shrinking pyknotic cells were at the point apoptotic bodies and vacular degeneration areas (Figure 1b). FA administration caused severe degenerative changes, shrunken cytoplasm and extensively dark pyknotic nuclei in neurons of the hippocampus tissues. The observation of Ozdem *et al.*, (2007) is in agreement with the study of Kanter, (2010). And Songur et al. (2003) which suggests that exposure to FA caused severe degenerative changes in neurons of frontal cortex and hippocampal tissues.

In another study by Gurel et al. (2005) it was also revealed that exposure to FA (10 mg/kg, 10 days, ip) increased pyknosis and decreased neuronal number in the adult rat frontal cortex and hippocampus that is in agreement with studies of Kanter, (2010), Ozdem et al., (2007) and Zararsiz et al., (2007). Furthermore, FA administration under similar conditions increased apoptosis in the rat prefrontal cortex and caused an increase in the immune-reactivity of a pro-apoptotic protein (Zararsiz et al., 2006; 2007). In their studies, Zararsiz et al. (2006; 2007) also reported the Bax protein induces cytochrome C release from the mitochondrial membrane to the cytoplasm, which initiates the apoptotic process (Sarsilmaz et al., 2007; Songur et al., 2010).

Sari et al. (2004) reported that chronic exposure to low levels of FA in rats caused an increase in the number of corticotrophin releasing hormone (CRH) neurons in the hypothalamus and adrenocorticotropic hormone (ACTH) cells in the pituitary gland, with an increase in ACTH-

mRNA expression in a dose-dependent manner. From these results, FA inhalation was suggested to increase activity of the hypothalamic-pituitary-adrenal (HPA) axis so as to mitigate FA neurotoxicity (Sari *et al.*, 2004).

A series of studies have addressed the effects of long-term repeated exposures to FA on altered functioning of the HPA axis (Sorg *et al.*, 2001a) and on neurobehavioral changes in rats (Sorg *et al.*, 2001b). Sorg *et al.,* (2001a) study FA's effects on the HPA. These investigators measured corticosterone levels in the blood of male Sprague-Dawley rats 20 or 60 min following acute exposures to FA (0.7 or 2.4 ppm). All groups showed increased corticosterone levels above naive basal levels at 20 min followed by a return to baseline by 60 min, with no differences between treatment groups. A second experiment assessed the effects of repeated FA exposure (1 h/day, 5 days/week for 2 or 4 weeks) on basal corticosterone levels and those after a final challenge. Basal corticosterone levels were increased above naive values after 2 week exposure to air or 0.7 ppm FA. By 4 weeks, corticosterone levels in the air group returned to naive values, but remained elevated in the 0.7 ppm formaldehyde group. There were no differences in basal corticosterone levels among either 2.4 ppm exposed groups. After a final air or FA challenge, the 2 and 4 week air and 0.7 ppm FA groups had elevated corticosterone levels similar to their acute response, while in the 2 and 4 week 2.4 ppm FA groups, corticosterone levels were higher than their acute response levels, indicating enhanced reactivity of the HPA axis to subsequent FA. It thus appears that repeated low-level formaldehyde exposure alters HPA axis functioning and the release of stress hormones (Public Review Draft, 2007; Sorg *et al.*, 2001a).

The Effects of Formaldehyde on Behaviour

Several symptoms have been observed during studies of FA-exposure; such as lethargy, decrease in motor activity,

and loss of appetite (Ozen et al., 2003; Songur et al., 2003; Zararsiz et al., 2006; 2007).

The CNS is one of the most important systems affected by the effects of FA. Anatomists, histologists, pathologists, cadaver embalming technicians, dissection students, and nurses working at dialysis units are occupationally subject to FA exposure. There have been reports of malaise, headache, indigestion, balance and sleep disorders, and mental and memory disorders due to FA exposure (Kilburn et al., 1987; Songur et al., 2010). Moreover, the reports of severe fatigue and feeling of thirst, irritability, lethargy, and behavioural and sensory-emotional disorders in the people working in the industrial areas where FA is regularly used are also suggestive of neurotoxicity of FA (Kilburn et al., 1987; Songur et al., 2010).

Kilburn et al. (1987) and Songur et al. (2003) also reported that FA exposure resulted in extensive neurobehavioral impairments such as malaise, headache, indigestion, sleep disorders, and memorial disorders.

Sorg and Hochstatter, (1990) were studied on behavioural sensitization after repeated FA exposure in rats. Three behaviours were assessed in rats repeatedly exposed to FA inhalation. In the first series of experiments, rats were given high dose FA exposure (11 ppm)1 hr/day for 7 days or low dose FA exposure (1 ppm either 1hr/day for 7 days or 1hr/day for 5 days/week).

Within a few days after discontinuing daily FA, cocaine-induced locomotor activity was evaluated after high dose FA or 20 days of low dose FA inhalation. That shows exposure to high level FA or repeated low level FA produces cross-sensitization to cocaine's locomotors activating effects Sorg and Hochstatter, (1990). Sorg and Hochstatter, (1990) also reported that inhalation of FA at 11 ppm for 7 days or 1 ppm for 20 days was observed to cause an increase- induced locomotor activity and a conditioned fear response to odour, suggesting that FA may cause chemical encephalopathy.

CONCLUSION

Everyone living in the society is exposed to formaldehyde. It has many detrimental effects on various tissues of the body including skin, eye, gonads, gastrointestinal system and respiratory tract including CNS. It is reported that malaise, headache, indigestion, balance and sleep disorders, and mental and memory disorders are common due to FA exposure to. Many psychological diseases in adult humans are dependent on factors that occurred prenatally or during the early postnatal period. The neurotoxic effects produced by FA exposure are dependent on the concentration of FA and duration of exposure which is more pronounced   in concentrated and increased duration of exposure. The FA exposure in adult and postnatal period results in an increase oxidant substances though it causes a decrease in the antioxidant enzymatic activity in the rat frontal cortex and hippocampus. It was indicated that FA induces several characteristics of neurotoxicity. Acute exposure to low concentrations of FA leads to stimulation of the CNS, while exposure in to concentrated FA leads to CNS depression.

Since complete prevention from FA exposure is impossible it is recommended that exposure to chemicals during Anatomical Dissection should be minimized through regular air monitoring to evaluate working conditions, maintaining high room air exhaust rates, proper operation of the ventilated anatomy tables, using personal protective equipment, and by following the Safety Guidelines. Generally monitoring Concentration using standard measuring machines, avoiding household products that are made up of chipboard, melamine, synthetic fibers, and plastics, it is preferred and should be offered to use formaldehyde-free wood products, marble, and natural fibers, ventilating houses, keeping temperature and humidity at low levels by the use of air conditioners. It is recommended for individuals who are exposed to FA in their working place to take *Nigella sativa,* vitamins E, melatonin, fish omega-3 fatty acids and caffeic acid phenethyl ester (CAPE).

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