Biochemical effects of methomyl on experimental animals

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Introduction
One of the most common and points investigated for health an effect of any chemical is cancer. In 2008, approximately 271,000 cases of kidney cancer were diagnosed around the world, and 116,000 individuals died of kidney cancer Ferlay et al. (2010). Genotoxic effects are considered among the most serious of the possible side effects of pesticides. If a chemical could react to nuclear DNA, it could be caused mutagenic or carcinogenic effects to the exposed organisms. The effects include heritable genetic disease, carcinogens, reproductive dysfunction and birth defects. Glutathione-S-transferases (GSTs) are a large family of Phase II detoxification enzymes that are expressed in many tissues and play critical roles in regulating the conversion of toxic compounds to hydrophilic metabolites. Hayes and Pulford (1995)–Ketterer (1988). Because the differential expression of (GSTs) has been found to markedly influence the anticarcinogenic potential of tissues since it was first suggested as a potential marker for cancer susceptibility in 1986, Seidegard et al., (1986), (GSTs) are currently being investigated as risk biomarkers for various cancers Song (2005)–Sui et al., (2011). Among the (GSTs), the association of the (GSTM1, GSTT1 and GSTP1) genotypes with their individual susceptibilities to cancer has been extensively studied. Individuals who carry homozygous deletions in these genes are thought to be increased risks for malignancies because of their decreased capacity to detoxify potential carcinogens McIlwain et al., (2006), Hayes et al., (2005). The (GSTP1) gene is located on chromosome 11 Hayes et al., (2005), and the single nucleotide polymorphisms (SNPs) in this gene are known to cause genetic damage and increased cancer risk Zimniak et al. (2005). Human genetic polymorphisms in metabolic activation and detoxification pathways appear to be important sources of inter-individual variation in susceptibility to cancer. Individuals who inherit the at-risk alleles of genes for enzymes such as glutathione S-transferases (GST) may fail to be protected against carcinogens in cigarette smoke, diet, industrial processes, and environmental pollution Bell et al., (1993). Two distinct supergene families encode proteins with glutathione S-transferase (GST) activity; firstly, at least 16 genes encode proteins expressed in tissue cytosols and secondly, at least six genes are expressed in membranes Hayes and Strange (2000). The different (GST) enzymes have classically been viewed as part of cell defence against numerous harmful chemicals produced endogenously and in the environment. The general reaction of GST enzymes is the addition of glutathione S-transferase (GST) activity; firstly, at least 16 genes encode proteins expressed in tissue cytosols and secondly, at least six genes are expressed in membranes Hayes and Strange (2000). The influence of the genetic polymorphisms of enzymes, (GSTM1, GSTT1, GSTM1)
using the polymerase chain reaction based genotyping method and micronucleus analysis on farm workers was studied and the obtained results showed significant differences in micronucleus and the polymorphic genes, (GSTM1 and GSTT1), appeared to be associated with evaluated MN frequencies.

Materials and methods

Pesticide
- Common name: Methomyl.
- Commercial name: Lannate
- IUPAC Name: S-methyl-N- (methyl carbamayl) oxyl-thio acetamidate.
- Structure formula: C_5H_{10}N_2O_S
- Used formulathion: Lannate 90% SP.
- This insecticide was applied from Kafr El-Zayat pesticides and chemicals company (Chemical Co.-, Ltd).

Experimental animals
40 adult male albino mice (Mus musculus) were used in this investigation, aged 4-5 weeks and of average weight 20 gram obtained from the Organization of Biological Product and Vaccine Helwan Farm, Cairo, Egypt. The cages were kept in air conditioned room at a temperature of 22°C and a relative humidity of 55% (55-70%) and normal light/dark cycle. Animals were caged in 8 groups (5 animals for each group) they were given oral administration by gastric tube daily for 30 days. They were also monitored daily and abnormal symptoms were recorded.

Experimental protocol: Determination of cytotenic assay: Glutathione-S-transferase T1 (GSTT1) and Glutathione-S-transferase M1 (GSTM1) polymorphisms
Isolation of DNA from liver tissue
At the end of treatment, animals were killed, and liver was removed, weighed and frozen at -40°C. Crude extraction of DNA from the liver tissue was obtained according to Hoffman (2001), before any analysis it is important to determine the concentration and the purity of DNA which has been isolated, this was done by estimating UV absorbance at wave length of 260 and 280 nm.

The polymorphisms of (GSTT1 and GSTM1)
The genotypes of DNA rates liver samples were determined by polymerase chain reaction (PCR) based methods. PCR for the glutathione S-transferase (GSTM1 and GSTT1) was done according to the method describe by Norppa et al., (1995). The β-globin gene primer was included in the PCR reaction to confirm the presence of amplifiable DNA in the samples Bell et al., (1995). PCR was carried out in a total volume of 25µl, containing 10 µg (500 ng) DNA; 10Mm dNTPs; 2.5µl 10x PCR buffer containing MgCl2. The samples were overlaid with 100 µl white light mineral oil, heated to 97 °C for 10 min to denature DNA. The temperature was reduced to 63 °C for 1 min and primers M1 and T1 forward and reverses (50 Picomole) and 2.5 U (5unit/µl) of Taq polymerase were added and heated at 72 °C for 1 min. The reaction was then subjected to 35 cycles of amplification, 94 °C for 30 sec, 59 °C for 30 sec and 72 °C for 45 sec. After 35 cycles, 1µl of PCR product were run on 2% agarose gel in Tris acetate EDTA (TAE) buffer and stained with ethidium bromide. The (GSTT1 and GSTM1) negative genotypes were identified on the bases of the absence of the (GSTT1 and GSTM1) specific DNA fragment.

Fig. (1). Agarose gel electrophoresis of PCR product of the GSTT1 polymorphism (M) DNA marker, (L1) represent DNA patterns of untreated group (control), (L2) represent DNA patterns of treated group (1/20 LD_{50}) (High), (L3) represent...
DNA patterns of treated group (1/40 LD$_{50}$) (Medium) and (L4) represent DNA patterns of treated group (1/80 LD$_{50}$) (Low).  

**Results**

The results of present work revealed that there was higher polymorphism of both (GSTT1) and (GSTM1) in all the applied doses of Methomyl (1/20 LD$_{50}$, 1/40 LD$_{50}$ and 1/80 LD$_{50}$) after 30 days. Polymorphism of both (GSTM1) and (GSTT1) showed positive genotype in all doses. The (GSTM1) polymorphism showed positive genotype in the high and medium doses (1/20 LD$_{50}$ and 1/40 LD$_{50}$) but not in the low dose (1/80 LD$_{50}$). From these results we concluded that tested pesticides exert mutagenic effects in male albino mice Figs. (1 and 2).

**Discussion**

There are several studies of the relationship of GSTs polymorphisms and cancers. Among the members of the GST super family, GSTM1, GSTT1 and GSTP1 genotypes are considered to be the most related to the development of many cancers because their roles in lung cancer, acute leukemia and breast cancer have been identified in previous studies Song (2005), Sergentanis and Economopoulos (2010), Song et al., (2006). In addition the animals treated with Methomyl which has GSTT1 and GSTM1 positive genotype showed higher frequency of MN, although the proper function of these genes was detoxification of the carcinogen and any genotoxins exposure through decreasing the cytogenetic damage, which was measured by the micronucleus induction. These results may be reasonable by the loss ability of GST gene to
reduce the DNA damage due to long continuous exposure to pesticides as environmental pollution. GST enzyme activity is known to be involved in pesticides detoxification Hodgson et al., (1991), whereas glutathione conjugation has also been described in the metabolic activation of certain halogenated alkanes Hallier et al., (1993). Hodgson et al., (1991) Stated GST- mediated glutathione conjugation is known to play a role in detoxification of several groups of pesticides. Lucero et al., (2000) studied the genotypes of GSTM1 and GSTT1 in worker exposed to pesticides, they found that Mn frequency in exposed subject was similar to that of control group and was not affected by the genotype of GSTT1 and GSTM1. Hammam and Abdel-Matthaleb (2007) Found no relation between increase MN and the genotype of GSTT1 and GSTM1. Mammalian glutathione S-transferase (GST) biotransformation of the widely used organophosphorus, methyl parathion (MeP) was investigated in cytosolic fraction isolated from rat and mouse. They found no correlation between hGSTM1, hGSTT1 genotypes and MeP. Changes in erythrocyte delta-aminolevulinic acid dehydratase (ALA-D) after exposure to different pesticides and the effect of the combind polymorphism of enzymes involved in the detoxication of pesticides on the level of the target erythrocyte enzyme were studied as biomarkers of individual susceptibility Hernandez et al., (2005). They found that ALA-D appears to be an important biological indicator of pesticide exposure and GSTT1 is relevant determinants of susceptibility to chronic pesticide. The influence of the genetic polymorphisms of enzymes, GSTM1, GSTT1 using the polymerase chain reaction based genotyping method and micronucleus analysis on farm workers was studied and the obtained results showed significant differences in micronucleus and the polymorphic genes, GSTM1 and GSTT1, appeared to be associated with evaluated MN frequencies. Although GSTs play critical roles in the development of tumors, in fact, it has been found that GSTs activity is lower in renal tumor tissue specimens than in healthy renal tissue distant to the tumor, and it’s also lower than in normal subjects Ahmad et al., (2012).

These data suggest that genetic background or environmental differences may contribute to the discrepancy in the results. With respect to the exposure to pesticides, the occupationally exposed subjects with GSTM1 or GSTT1 active genotypes had a significantly increased risk for cancer compared with those of occupationally exposed subjects with GSTM1 or GSTT1 null genotypes and unexposed subjects. In general, glutathione compounds are excreted easily. However, in specific tissues, these compounds are more reactive than in normal tissues. These phenomena are especially evident in the kidneys Ćorić et al., (2010), Bladeren (2000). These more reactive intermediates damage the kidney tissues directly, and active GST enzymes are required for the formation of such intermediates. Conversely, the GSTs mutant genotype forms inactive enzymes and is responsible for the detoxification of carcinogens Ćorić et al., (2010), Groves et al., (1991) – Pekmezović (2010). This finding suggests that the subjects who are occupationally exposed to pesticides and have an active GSTM1 or GSTT1 variant have a significantly increased risk of cancer.

References


