EFFECTS OF PESTICIDE EXPOSURE ON HUMAN HEALTH IN PUNJAB

Ushma J. Shah and S. M. S. Chahal

ABSTRACT
Situated in North-West India, Punjab is an agriculture dominating state and pesticides have become essential element of its crop economy. Pesticide residues were detected in the blood samples of the villagers’ breast milk, bovine milk and also in vegetables and fruits grown in the state. The health issues related to chronic pesticide exposure include eye irritation, pulmonary, neurologic and kidney problems, cancer, mutagenesis, faetotoxic and teratogenic effects, immunological changes and effect on fertility. Various national and international studies, including researches in Punjab state, have demonstrated harmful effects of pesticides on human health. High incidences of cancers are reported in Punjab. Numerous studies have indicated that farmers are more vulnerable to leukaemia, non Hodgkin’s lymphoma (NHL), and brain, prostate and skin cancers. In utero and childhood exposure to pesticides shows significant association with increased risk for NHL. Genotoxic effects of pesticides were shown by DNA damage studies using comet assay and deletion in GSTM1 and GSTT1 genes was observed in pesticide exposed individuals of Punjab. A relationship was also observed between mothers exposed to pesticides and recurrent abortions and premature births in the state. Similarly, a study showed that pregnant women exposed to pesticides have an escalated risk of birth defects, including cleft lip and neural tube defects. Gestational and childhood exposures to organophosphorus pesticide act as developmental neurotoxicant targeting the developing brain. An epidemiological study has demonstrated relationship between organochlorine pesticide exposure and Parkinson’s disease; Dieldrin was also reported as a potential etiological agent of Parkinsonism. In addition, occupational exposure to insecticides was found to be associated with frequent infections and immunological abnormalities. Repeated exposure to chlorpyrifos has been reported to cause immunological changes in peripheral lymphocyte phenotypes. A research showed linkage of pesticide exposure and decreased sperm quality. Thus, agriculture workers and villagers inhabiting Punjab are highly exposed to various types of pesticides and there is direct and indirect evidence to demonstrate that the chemicals included in them have numerous adverse health effects.

Keywords: Health, Farm pesticides, Genotoxicity, Punjab

Ushma J. Shah and S. M. S. Chahal, Department of Human Biology, Punjabi University, Patiala, Punjab, India
INTRODUCTION

The pesticides are defined as the chemical substances used to prevent, destroy, repel or diminish any pest including insects, rodents, weeds and microorganisms (Blindauer et al., 1999). The pesticides are toxic and poisonous by nature to kill pests. Because of their toxic properties, they are also harmful to humans and animals. Pesticides are classified according to particular organism on which they act. These include insecticides, herbicides and fungicides, the former being the most dangerous to human health. Synthetic pesticides are very popular among agriculture workers as they are widely available, simple to apply and provide efficient economic returns (Mathur et al., 2005). These agrochemicals have become essential in demanding agriculture work to enhance the crop production and safe storage. In this respect, pesticides are beneficial to human population. Progress and advancement in agriculture technology in India have resulted in remarkable increase in the pesticide usage in the country.

Health risks have been found to be significantly associated with those who are environmentally or occupationally exposed to pesticides (Le Bailly et al., 1998). Individuals may be exposed to these agrochemicals through direct or indirect routes. Direct exposure occurs to individuals who spray pesticides in farms and orchards etc. while indirect exposure occurs through intake of water, air, dust and food contaminated with pesticides (Alavanja et al., 2004). Either simultaneously or successively farmers are exposed to various types of pesticides or a combination thereof. Such exposures include mixture of different chemicals making it difficult to diagnose effect of a particular chemical (Kamel and Hoppin, 2004). Different factors play important role in determining degree of pesticide exposure including method of application, usage of protection measures, maintenance of hygienic conditions, taking care of spillage and knowledge of risk factors (Gomes et al., 1999; Buchanan et al., 2001; Dosemeci et al., 2002; Alavanja et al., 2004). Some studies of occupational pesticide exposure have classified as exposed all members of an occupational group, typically farmers or farm workers and sometimes also considering job duration, and therefore potential for misclassification with such an approach is high. In fact, farm owners who employ others to apply pesticides may have limited personal exposure to pesticides; and among pesticide applicators, exposure time can vary widely (Kamel and Hoppin, 2004).

Adverse effects of pesticides have been reported worldwide, but from India such studies are limited as yet. In spite of the fact that overall consumption of pesticides in India as a whole is lower than in the developed countries of the world, there is still a widespread contamination of water, soil and air with pesticide residues (Mathur et al., 2005). The present paper reviews the available literature regarding effects of pesticide exposure on human health, with special reference to the people of North-West border Indian state of Punjab.

Punjab is an agriculture dominating state and pesticides have become essential element of its crop economy. Pesticide residues were detected in the blood samples (Mathur et al., 2005) and breast milk (Kalra et al., 1994) of the villagers as well as in
the bovine milk (Kalra et al., 1999) and vegetables and fruits grown in the state (Mathur et al., 2005). A study found presence of organochlorine pesticide residues in maternal and cord blood samples of North Indian population (Pathak et al., 2008). In Bhatinda district of Punjab, cotton crop is grown highest in the country and is known for excessive use of pesticides. The Punjab Agricultural University, Ludhiana recommend only 7 sprays of pesticides on cotton within six months, but it is sprayed as many as 32 times in the district (Menon, 2005). Usage of empty containers of pesticides for storing the food items was also observed in the state (Mathur et al., 2005). Evidently these studies demonstrate continuous pesticide exposures on villagers of Punjab and their possible harmful effects on them.

Most of the time pesticide toxicity may go undiagnosed as villagers, especially women (London et al. 2002); have poor access to medical care (Moses et al., 1993). Thus, farm workers who are not investigated for pesticide poisoning may also have high levels of pesticide exposure or related health problems (Kamel and Hoppin, 2004). The need to evaluate past as well as current exposure has limited the utility of biomarkers and because most modern pesticides are not persistent, studies of chronic exposure rely primarily on questionnaire-based methods. Biomarkers are however, useful in some situations. For example, organochlorines have a long half-life and therefore serum levels can be used as a marker of exposure to these pesticides (Kamel and Hoppin, 2004).

**HEALTH EFFECTS OF PESTICIDES**

Various national and international studies, including researches in Punjab, have documented harmful effects of pesticides on human health. The health problems associated with pesticide exposure include immediate irritating effects, and acute as well as chronic effects leading to major diseases. The health issues related to chronic pesticide exposure include eye irritation, pulmonary, neurologic and kidney problems, cancers, mutagenesis, fetotoxic and teratogenic effects, immunological changes and affects on fertility (Antle and Pingali, 1994). In mild organophosphate poisoning, symptoms include headache, dizziness, nausea, vomiting, pupillary constriction, excessive sweating, tearing, and salivation. Severe cases of such poisoning develop muscle weakness and twitches, bronchospasm and changes in heart rate sometimes leading to convulsions and coma. An intermediate syndrome, occurring few days after exposure, is characterized by muscle weakness and can be fatal if respiratory muscles are affected. Two to five weeks after exposure, patients may develop organophosphate induced delayed polyneuropathy, a well characterized syndrome involving sensory abnormalities, muscle cramps, weakness and even paralysis, primarily in the legs (Kamel and Hoppin, 2004).

**Cancerous Effects**

Cancer is a complex multifactorial disease in which both genetic and environmental factors are responsible. Within last couple of decades the occurrence of cancer has increased dramatically in Punjab. Various different long-term health effects of
pesticide exposure have been studied among which cancer is focused the most. In general term, cancer is uncontrolled proliferation of cells. Of different types of cancers, several specific types have been found to be dominating in agriculture workers (Lebailly et al., 1998). Not many studies have been carried out to confirm that pesticides are responsible for various incidences of cancer and other diseases in Punjab, albeit the research worldwide has shown that pesticides do produce such effects. In a survey conducted in two blocks of Bhatinda and Rupnagar districts of Punjab, as many as 7441 deaths due to cancer were recorded from 1993 to 2003. Cancer of female reproductive system i.e. breast, uterus/cervix and ovary was found more common in Talwandi Sabo block of Bhatinda district, whereas cancer of blood and lymphatic system, esophagus and bones were found more frequent in Chamkaur Sahib block of Rupnagar district (Mathur et al., 2005). Evidences regarding pesticide exposure and breast cancer are contradictory; some studies showed no correlation while others showed strong association. In India, high levels of pesticides were found in breast cancer patients (Mathur et al., 2005). Association was also found between organochlorine pesticide exposure and colorectal cancer (Howsam et al., 2004).

Numerous studies have indicated that farmers are more vulnerable to leukaemia, non-Hodgkin's lymphoma (NHL), and brain, prostrate and skin cancers (Dich and Zahm, 1997). Occupational pesticide exposure has been also found to be associated with cancers of hematopoietic cells, lymphatic system and soft tissue sarcoma (Morrison et al., 1992; Weisenburger, 1993; Blair and Zahm, 1995; Ritter, 1997). Phenoxy acid herbicide exposure was also found to be correlated with risk of soft tissue sarcoma and NHL (Zahm et al., 1990, 1992; Smith and Christophers, 1992).

As the lymphocytes and cells of lymph system play major role in immune system, NHL significantly represses functions of immune and blood cells (Mathur et al., 2005). In utero and childhood exposures to various pesticides, including phenoxyacetic acid herbicides, and lindane and organophosphorus pesticides, showed significant correlation with increased risk for NHL (Zahm, 1992; Mathur et al., 2005). These evidences document significant role of pesticide exposure in the occurrence of human cancer.

**Neurotoxic Effects**

Pesticide exposure is associated with increases in prevalence of several symptoms, with little evidence for specificity. The most common neurotoxic symptoms include headache, dizziness, fatigue, insomnia, nausea, chest tightness, and difficulty in breathing, suggesting cognitive (confusion, difficulty concentrating), motor (weakness, tremor) and sensory (numbness, tingling, visual disturbance) dysfunctions. The association studies on pesticide exposure and neurotoxicity, are mainly targeted on long-term or occupational exposure. For this, both current and previous exposures are relevant and need to be considered. For subjects who remain in the same occupation, current exposure may not reflect past exposure because both available products and methods of use are may change over time (Kamel and Hoppin, 2004). Neurotoxicity can be linked with various different types of pesticides,
including organophosphates (OPs) and carbamate and organochlorine insecticides and fungicides, but only the former have been studied widely (Keifer and Mahurin, 1997). Gestational and childhood exposures to OPs act as a developmental neurotoxicant targeting the developing brain (Pope, 1999; Barone et al., 2000).

Neurobehavioral tests are usually supplemented with tests of sensory or motor functions. One frequently used test is vibration sensitivity that evaluates peripheral somatosensory function. There are evidences to suggest that the test is not affected by moderate pesticide exposure (Kamel and Hoppin, 2004). In one study exposure to organophosphates showed decreased vibration sensitivity (Stokes et al., 1995) and in another study, exposures to mixed pesticides showed decreased sensitivity as well as signs of peripheral neuropathy, including decreased peripheral nerve conduction (Cole et al., 1998). Similarly, few studies have considered motor function such as tremor and weak grip strength but little inferences can be made about its relationship to pesticide exposure (Kamel and Hoppin, 2004). Recurrent pesticide exposure is found to be associated with decreased psychomotor function (Gomes et al. 1998; Kamel et al. 2003) or loss in psychomotor function. Deficiency in psychomotor function could be caused by impairment of sensory input, motor output, or associative delays caused by effect of pesticides (Kamel and Hoppin, 2004). Exposure to herbicide N,N'-dimethyl-4,4'-bipyridylium (paraquat) or dithiocarbamate pesticide (maneb) during gestation or childhood can permanently harm the dopaminergic neurons and increase susceptibility to subsequent neurotoxicant exposures (Thiruchelvam et al., 2000). Dithiocarbamate fungicides are also known to have powerful dopaminergic activity that augments neurotoxicity (Thiruchelvam et al., 2002).

Epidemiological studies suggest that exposure to pesticides is a plausible environmental risk factor for Parkinson’s disease (PD) (Mandel et al., 2000). A study by Baldereschi et al. (2003) demonstrated that such exposures increased the risk of PD 1.5-7 fold than the unexposed population. An another epidemiological study has shown relationship between exposure to organochlorine pesticide such as dieldrin (a mitochondrial poison) and Parkinsonism (Fleming et al., 1994); the residual levels of the pesticide were diagnosed in brain tissue samples of one third of PD patients compared with the controls (Sanchez-Ramos et al., 1998). Besides PD, dementia and Alzheimer’s disease are also found to be associated with occupational exposure to pesticides (Kamel and Hoppin, 2004). Several enzymes including glutathione-s-transferases (GSTs), cytochrome P-450 family (CYP), esterases, flavin mono-oxygenase (FMO) and paraoxonases (PONs) are typically engaged in initial metabolism of pesticides i.e. activating or inactivating them (Ecobichon and Joy, 1994).

Different studies have demonstrated that pesticide exposures may cause single nucleotide polymorphisms (SNPs) in genes encoding for these enzymes, leading to increased risk of PD. GST gene family encodes genes that are critical for certain life processes as well as for detoxication and toxification mechanisms via conjugation of reduced glutathione (GSH) with numerous substrates such as pharmaceuticals
and environmental pollutants (Nebert and Vasiliou, 2004). A CYP family gene CYP2*D6 is a toxin metabolizing regulatory gene involved in the detoxification of environmental chemicals and toxicants (Hodgson and Levi, 1996). Strong association between polymorphism of this gene due to pesticide exposure and PD has been reported (Ecóbichon and Joy, 1994).

Genotoxic Effects

A variety of biomarkers are available to assess transient and permanent genotoxic responses, most studies have focused on cytogenetic aspects including sister chromatid exchanges (SCEs) and chromosomal aberrations (Lebailly et al., 1998). Carbonell et al. (1995) observed an increase in chromosomal aberrations in lymphocytes studied during the period of intense spraying. Significant increases in one or several cytogenetic biomarkers were observed in the exposed group compared to the control group. Occupational exposures to pesticides lead to a higher level of DNA damage in lymphocytes of greenhouse floriculturists (Peluso et al., 1996). A study on workers engaged in insecticide production demonstrated increased SCEs in the exposed group (Laurent et al., 1996). Most of such investigations have been carried out on pesticide applicators exposed to broad categories of pesticides.

In addition to the traditional cytogenetic methods, the single cell gel electrophoresis (comet assay) allows evaluation of DNA fragmentation resulting from a variety of DNA damages such as single- and double- strand breaks and alkali-labile sites, including abasic sites (Lebailly et al., 1998). A recent research indicated genotoxic effects of pesticides by DNA damage study using comet assay and deletion in GST*M1 and GST*T1 genes in pesticide exposed individuals of Punjab (Abhishek et al., 2010). Thakur et al. (2010) observed a relationship between mothers exposed to pesticides and recurrent abortions and premature births in the state. Similarly, a study showed that pregnant women exposed to pesticides have an escalated risk of birth defects, including cleft lip and neural tube defects (Garry et al., 1996). These studies suggest a link between pesticide exposure and genotoxic effects.

Immunological Effects

such asmet al. ding abasic sites)The chemicals present in pesticides such as organochlorine, organophosphate and carbamate etc. can modify immune system. Pesticides were reported to influence immune system by deregulating, suppressing or stimulating its function. Most of the pesticides can do all the three modifications but it depends on duration, concentration and dose of the exposure. Apart from these, the level of accumulated particles and nutritional status of the exposed person also play important roles (Rea and Liang, 1991). Occupational exposure to insecticides was found to be associated with frequent infections and immunological abnormalities (Daniel, 2002). Repeated exposure to chlorpyrifos has been reported to cause immunological changes in peripheral lymphocyte phenotypes (Thrasher et al., 2002). Exposures to fungicide pentachlorophenol (McConnachie and Zahalsky,
1991), insecticide chlorpyrifos (Thrasher et al., 1993) and germicide chlordane (Broughton et al., 1990) were found to have association with lymphocyte abnormalities such as increased activation and excessive auto antibodies. Elevated autoimmunity and immune changes were also observed in farm and factory workers exposed to pesticides (Brusilovskii et al., 1973; Kozintseva et al., 1973; Katsenovich and Usmanova, 1973; Nikolaev et al., 1988). Autoimmune complexes were reported to be increased in organochlorine exposed rural workers and organophosphate exposed urban workers (Krivoruchko and Dzhabarov, 1989). Similarly, organochlorines exposed agriculture workers and occupational pest control applicators develop autoimmunity (Nikolaev and Isayeva, 1975). Pesticide factory workers and villagers with symptoms of pesticide poisoning display modifications in B and T cells and increased antibodies (Ruzybakyev and Fedorina, 1983; Abdullayev and Ruzybakyev, 1986; Nikolaev et al., 1988).

Various different pesticides may have capability to alter proteins (Jakoby, 1980). Alteration of proteins resulting in haptons is the simplest change observed in immunological effects of pesticide exposure (Fan et al., 1989). Apart from proteins, direct cytotoxic effects triggered by mercury containing insecticides have also been reported (Takenchi et al., 1962). Most of the organochlorine pesticides have been reported to deregulate complements (Rea, 1977). These pesticide exposures were found to be linked with direct T-cell triggering and occasionally they also repress suppressor T-cells (Rea et al., 1986). The correlation has been observed between high levels of organophosphate and organochlorine pesticides and Behcet's disease (Ishikawa et al., 1973). These pesticides may also alter the receptor sites for hormones which may damage sodium pump (Reeves et al., 1975; Jakoby, 1980). Thus, both laboratory and clinical studies demonstrate that pesticides of all categories may influence the immune system in humans. Nonetheless, improvement in immune parameters occurs when pesticides are removed from the body (Rea and Liang, 1991).

Effect on Male Fertility

Spermatozoa or their precursors may be damaged by pesticides which can result in reversible or irreversible impaired spermatogenesis, depending on the stage of differentiation affected by the chemical. Damage to spermatogonia causes irreversible impaired sperm production because these stem cells are not replenished (Bretveld et al., 2007). A study showed linkage between pesticide exposures and decreased sperm quality (Swan and Kruse, 2003). Eaton et al. (1986) reported that nematocide exposure caused azoospermia with no revival after as long as seven years. The fertility can be temporarily decreased due to the effect of pesticides leading to reduction in cell number, changes in cell structure, motility and viability of spermatozoa. However, these adverse effects are short termed as stem cells produce new cells via spermatogenesis, after the harmful chemical has been eliminated from the body (Giwercman and Bonde, 1998).

The such aset al. ling abasic sites) Sertoli cells can also be affected by pesticides. As these cells are crucial for proliferation and differentiation of spermatogenic cells,
changes or damage in them is very harmful. Moreover, the Sertoli cells can not be regenerated after puberty. Therefore, high level of impairment in these cells is directed towards irreversible damage to spermatogenesis (Monsees et al., 2000). Similarly, the Leydig cells can also be found to be affected by pesticide exposure. The reduced testosterone concentration in the serum and testicular tissue can occur due to impairment of the function of these cells (Walsh et al., 2000; Ronco et al., 2001; Friedmann, 2002).

Some of the pesticides are known to diminish steroidogenesis by inhibiting particular enzymatic steps in the hormone biosynthesis pathways (Kasper and Harrison, 2005). This process leads to decrease in expression of steroidogenic acute regulatory (StAR) protein (Walsh et al., 2000a,b; Walsh and Stocco, 2000), which has important role in steroidogenesis by transferring cholesterol to inner mitochondrial membrane. Due to suppression of this protein expression, testosterone production by the Leydig cells is inhibited causing adverse effect on fertility (Walsh et al., 2000a). The reduced testosterone synthesis may occur through action of pesticides which can disrupt endocrine system. Interference with hormone receptor recognition and binding is a mechanism of endocrine disruption. Some pesticides can interact with the steroid hormone family of nuclear estrogens receptors (ER) and androgen receptors (AR), both being widely distributed in male reproductive tissues (Bretveld et al., 2007).

The central nervous system (CNS) is very important in the integration of hormonal and behavioural activities, and disturbances in these finely tuned mechanisms can severely impair normal adaptive behaviour and reproduction (Bretveld et al., 2007). The neurotoxic effects of pesticides are well known and therefore, it is acceptable that pesticides have ability to disrupt the coordination activity of CNS and brain cell functions (Crisp et al., 1998). This disruption may cause impotence by failure in achieving erection, difficulties in ejaculation and affecting other reproductive functions (Thomas, 1975). Hence, pesticide exposure has significant adverse effect on male fertility.

CONCLUSIONS

Pesticides comprise a large number of distinct substances with dissimilar structures and diverse toxicity which act through different mechanisms. Different studies have reported strong correlation between pesticide exposure and various types of cancers, especially leukaemia and lymphoma. However, no genetic polymorphism has been studied which links exposure to pesticide and susceptibility to different cancers. Pesticides of all categories may influence the immune system resulting in human dysfunction. Pathophysiological pathways explaining the role of pesticide exposure on sperm quality and male fertility have been documented.

Historically, most studies have focused on neurotoxicity of organophosphates, albeit other types of pesticides, including organochlorines, carbamates, fungicides, and fumigants, have also been studied. No study has evaluated the association of herbicides with symptom prevalence or neurobehavioral performance, but these
chemicals have been implicated as risk factors for Parkinson's disease. Studies of neurotoxicity have documented an increase in symptom prevalence and changes in neurobehavioral performance reflecting cognitive and psychomotor dysfunction, but some others found little effect of pesticide exposure on sensory or motor function or direct measures of nerve function. Although it is important to identify classes of pesticides and even specific chemicals associated with neurotoxicity, it is also important to recognize that most workers are exposed to complex mixtures of pesticides, which may contribute synergistically to neurotoxicity.

Occupational exposure to pesticides may significantly modify the DNA damage, but these changes seem to be transient. The increase of DNA damage level in the middle and at the end of the spraying season is probably not due to an accumulation of damages, although it negatively correlates with the number of days without pesticide use. Moreover, pregnant women exposed to pesticides have high risk of having children with disabilities as pesticide is reported to be fetotoxic and teratogenic. such aset al. ding abasic sites) However, there are certain limitations for studying pesticide exposure because it is affected by many factors, including the multiple chemicals involved, uncertainty regarding the degree of exposure related to specific job tasks or other events, and contributions from multiple sources of exposure, including sources unrelated to occupation. Nonetheless, such studies are successful in demonstrating role of pesticides in providing susceptibility to different health effects.

As rural population of Punjab is having both acute and chronic exposures to various pesticides, the contemporary agriculture workers and villagers inhabiting the state are at high risk of developing life threatening disorders and diseases. Empirical data available from the state supports this hypothesis showing high rate of cancer and other disorders in its recent history.

REFERENCES


Effect of Pesticide Exposure on Human Health in Punjab


Kozintseva, P., L.Kuznetsova and D. Zinchenko 1973. [Clinical significance of the detection of antipesticide circulating antibodies]. Gigiena I Toksikologii Novykh Pestitsidov i Klinika


Effect of Pesticide Exposure on Human Health in Punjab


Clinical Profile of Inversion Y in People of Gujarat, West India

Frenny J. Sheth¹, Ushma J. Shah², Manisha J. Desai¹ and Jayesh J. Sheth¹

¹Institute of Human Genetics, “FRIGE House”, Jodhpur Gam Road, Satellite, Ahmedabad 380 015, Gujarat, India
²Department of Human Biology, Punjabi University, Patiala 147002, Punjab, India


ABSTRACT The present cytogenetic study was carried out on a total of 1,408 male subjects inhabiting West Indian state of Gujarat diagnosed for various clinical conditions. Inversion Y (inv(Y)) was found in 1.67% of Down syndrome, 55.56% of multiple congenital anomalies, 0.47% of ambiguous genitalia and 2.22% of recurrent pregnancy loss subjects; the overall incidence of the trait was 2.20%. The present results revealed that inv(Y), considered a normal variant, had heterogeneous distribution in different clinical conditions in people of Gujarat. Detailed molecular studies are desirable to validate this observation.

INTRODUCTION

In humans, the male is referred to as the heterosexual sex, due to the presence of 2 different sex chromosomes, X and Y. The Y chromosome causes testis differentiation and therefore determines maleness (Sinclair et al. 1990). This chromosome passes from father to son, and unlike other chromosomes, most of it escapes meiotic recombination (Jobling and Tyler-Smith 2003). The Y chromosome contains a major proportion of segmental duplications (Skaletsky et al. 2003) and shows cytogenetically observable structural polymorphisms such as length variation (Bobrow et al. 1971; Verma et al. 1978) and inversions (Verma et al. 1982b; Bernstein et al. 1986). The former comprises large Y (Yq + greater than size of chromosome 18) and small Y (Yq- less than size of a G-group chromosome) and the latter comprises pericentric inversion (a rearrangement in which a segment, including the centromere, is rotated) and paracentric inversion (a rearrangement in which a segment of chromosome, excluding the centromere, is rotated). The Y chromosome is more polymorphic in the Asians (3.37%) and Hispanics (1.82%) compared to the Whites or Blacks (Hsu et al. 1987).

Cohen et al. (1966) reported that length of human Y chromosome differs in different racial groups. Like normal populations, Y chromosome length variation has also been documented in association with different clinical conditions such as mental disorders (Funderburk et al. 1978), abortions (Genest 1979), Down syndrome (Verma et al. 1982a), embryo development (Podugolnikova and Blumina 1983), birth complications (Videbech et al. 1984) and bad obstetric history (Minocherhomji et al. 2009).

Pericentric inversion of Y chromosome (inv(Y)) was first documented by Jacobs et al. (1964) and Solomon et al. (1964) and the condition is familial (Verma et al. 1982b). Using over 12,000 prenatal diagnosis cases the incidence of pericentric inversion Y has been found to be 1.15 per 1,000 and a value of 1-2 per 1,000 was estimated in different human populations by Shapiro et al. (1984). While reviewing human population cytogenetics, Bhasin (2005) has reviewed incidence of inv(Y) in various world populations. A high value of the trait (30.5%) was reported in the immigrant Gujarati Muslim community settled in South Africa by Bernstein et al. (1986). The authors traced the origins of the ancestors of the individuals with inv(Y) to a few small villages near the city of Surat and concluded that the polymorphic frequency of the trait observed has probably been produced through random genetic drift in a reproductively isolated community, maintained by strict endogamy based on religious and linguistic affiliations. There was no indication in the study that the inverted Y was associated with any reproductive disadvantages.

Pericentric inversion in different human chromosomes has been observed to be associated with infertility, repeated foetal loss, congenital
anomalies and mental retardation, possibly predisposing for inter-chromosomal effect and nondisjunction (Gardner and Sutherland 1996; Krishna et al. 1992). Polymorphic variants of chromosomes were reported in 28.82% males having primary infertility or repeated miscarriages (Madon et al. 2005). According to Motos Guirao (1989) pericentric inversion of human Y chromosome is only a rare chromosomal heteromorphism and there was no clinical significance of the condition because the fathers and male fetuses had the same pericentric inversion. Similarly, Verma et al. (1982b) concluded that inverted Y chromosome does not affect the sperm production and like normal Y chromosome it is inherited without any clinical significance. The present study was planned to investigate the association, if any, of inversion Y with different clinical conditions in people of Gujarat, West India.

**MATERIALS AND METHODS**

A total of 1,408 male subjects inhabiting Gujarat state in West India were investigated for this cytogenetic work carried out between October 1994 and September 2006. This included 239 children with Down syndrome, 9 children with multiple congenital anomalies, 212 children with ambiguous genitalia and 948 males in couples with recurrent pregnancy loss. Peripheral blood samples were obtained and cultures were established using the original technique of Moorhead et al. (1960) with modifications. The morphology of the Y chromosome was analysed by quinacrine and giemsa banding techniques and inversion Y cases were confirmed using centromere banding.

**RESULTS**

The incidence of inversion Y (inv(Y)) in different clinical conditions in people of Gujarat is listed in Table 1. Figure 1 shows a karyotype showing inv(Y) in a patient in the present study. Table 1 shows that the percentage of this genetic trait ranged from a low of 0.47 in ambiguous genitalia to a high of 55.56 in multiple congenital anomalies with an average of 2.20% for the entire clinical material tested. It may be noted that the frequency range of inv(Y) found in the present study is comparatively somewhat higher than the range reported in normal world populations (1-2 per 1,000).

<table>
<thead>
<tr>
<th>Clinical condition</th>
<th>n</th>
<th>Inversion (Y) cases observed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Down syndrome</td>
<td>239</td>
<td>4 (1.67)</td>
</tr>
<tr>
<td>Multiple congenital anomalies</td>
<td>9</td>
<td>5 (55.56)</td>
</tr>
<tr>
<td>Ambiguous genitalia</td>
<td>212</td>
<td>1 (0.47)</td>
</tr>
<tr>
<td>Recurrent pregnancy loss</td>
<td>948</td>
<td>21 (2.22)</td>
</tr>
<tr>
<td>Total</td>
<td>1408</td>
<td>31 (2.20)</td>
</tr>
</tbody>
</table>

Figures in parentheses are percentages

**DISCUSSION**

The present investigation showed that the incidence of pericentric inversion Y (inv(Y)) in patients of Down syndrome, ambiguous genitalia and recurrent pregnancy loss is low in comparison to rather high percentage of the trait observed in multiple congenital anomalies. This is an interesting observation but it may also be an attribute of small sample size in the latter clinical condition.

In a case study of a Down syndrome reported from California (USA), cytogenetic analysis diagnosed inv(Y) in four male family members studied, including proband (Sparkes et al. 1970). As for Gujarat, there are two case reports of inv(Y) in Down syndrome. In one of the study by Krishna Murthy et al. (1989), a 13 year old boy with Down syndrome was found to have inv(Y). The proband’s father and two elder brothers also had inv(Y) with normal phenotype but the mother had two spontaneous abortions. In another case study, a boy with Down syndrome and his normal father showed inv(Y) (García Sagredo et al. 1975) and the authors concluded that the presence of this trait in Down syndrome was by chance and no association can be made. The present study revealed that the incidence of inv(Y) in Down syndrome cases of Gujarat is 1.67%.

Association between multiple congenital anomalies and inversion of different human chromosomes has been reported in literature. In a subject from California (USA) inversion of chromosome 4 was observed with multiple congenital anomalies (Wilson et al. 1970). As for India, Sasikala (1990) showed the presence of pericentric inversion of chromosome 9 in children with this clinical condition. On the other hand, association between inversion Y (inv(Y)) and multiple congenital anomalies is rarely docu-
mented. In a cytogenetic study carried out on 50 patients having multiple congenital anomalies and mental retardation, only one patient showed inversion Y (Magnelli 1976). Like high frequency of inv(Y) reported in normal immigrant Gujarati population settled in South Africa (30.5%), the present multiple congenital anomalies patients studied from Gujarat showed a rather high value of the trait (55.56%). Further studies are desirable to confirm this observation.

A cytogenetic study reported from USA by Liou et al. (1997) showed paracentric inversion in Yq region in a White subject with ambiguous genitalia. The molecular analysis revealed that the short arm of proband’s inversion Y was identical to his father. The incidence of the trait in the present Gujarati subjects with this clinical condition was found to be 0.47%. A study in China showed that 22% males in couples with recurrent spontaneous abortion had pericentric inversion Y (Zhou et al. 2006). By contrast, the present figure of 2.22% in cases of this clinical condition investigated from Gujarat was about one-tenth.

To conclude, although inversion Y (inv(Y)) is generally considered to be of no significance in various clinical conditions, the present study from Gujarat in West India has provided some evidence for its role in multiple congenital anomalies. Studies from other states of India on the patients along with controls are required to confirm this finding.

REFERENCES


Genoprotective Effect of Indian Gentian in Type 2 Diabetes Mellitus (T2DM): Comet Assay, Sister Chromatid Exchange and Protein Oxidation Studies

Jayesh J. Sheth1,2, Ushma J. Shah1,2, Frenny J. Sheth1, Navneet Shah2, Rama A. Vaidya1 and Ashok D. B. Vaidya1

1. Institute of Human Genetics, “FRIGE HOUSE”. Jodhpur Gam Road, Satellite, Ahmedabad 380 015, Gujarat, India
2. Sheth V. S. General Hospital and NHL Medical College, Ellisbridge, Ahmedabad 380 006, Gujarat, India
3. ICMR Advanced Centre for Reverse Pharmacology, MRC, Kasturba Health Society, Mumbai 400 056, Maharashtra, India

KEYWORDS
T2DM. Indian Gentian. Comet Assay. SCEs. Protein Oxidation

ABSTRACT The present study was undertaken to study the effect of Indian Gentian (Enicostemma littorale Blume), a herb, as a genoprotective agent in type 2 diabetes mellitus (T2DM) patients. For this, a total of 52 T2DM patients were investigated, of which 38 received 500 mg - 1 g Indian Gentian thrice daily escalated over three months (Group 1). The remaining 14 patients were not given the herb (Group 2). Fifteen age and sex matched non diabetic healthy volunteers served as controls (Group 3). All three groups were studied for DNA damage by comet assay and Sister Chromatid Exchanges (SCEs); Group 1 was also investigated for protein oxidation. Paired and unpaired t tests were performed at 95% confidence interval. Results of comet assay and SCE studies revealed that in Group 1, post Indian Gentian treatment, normal cell population increased, whereas moderately damaged, highly damaged and apoptotic cell population and SCE decreased as compared to Group 1 (pre-treatment patients) and Group 2 (without treatment patients). In comet assay, statistically significant difference between Group 1 (post-treatment patients) and Group 3 (controls) suggested that the herb was able to decrease the DNA damage but not as low as non-diabetic healthy controls. On the other hand, SCE analysis showed that the herb can reduce such exchanges to as low as the controls. In protein oxidation assay, no significant difference was found between the pre- and post-treatment T2DM patients of Group 1. The present study therefore indicated that overall Indian Gentian may have a significant effect on reducing DNA damage and attenuating SCEs in T2DM patients.

INTRODUCTION
Diabetes mellitus, a chronic metabolic disorder, is known to have several micro- and macrovascular complications that contribute to an increase in the morbidity and mortality (Giugliano et al. 1996). Increased generation of Reactive Oxygen Species (ROS) due to hyperglycemia causes oxidative stress. This results in endothelial damage that leads to vascular complications (Giugliano et al. 1996; Son 2007). The ROS induced Advanced Glycation End products (AGEs) damage several macromolecules, including lipids, proteins, and nucleic acids (Son 2007). In addition, the release of pro-inflammatory cytokines by ROS leads to chronic inflammation. The latter mechanism is emerging as an important causative consequence of oxidative stress leading to DNA damage that predisposes to age related diseases, including diabetes, atherosclerosis, osteoporosis and cancer (Khansari et al. 2009; Hamada et al. 2009). The damage to DNA in the peripheral blood lymphocytes can be revealed by the comet assay (single cell gel electrophoresis) (Sheth et al. 2006). Beside double-strand and single-strand breaks, this technique measures DNA damage in somatic cells after a variety of genotoxic insults, including in vivo and in vitro radiation.

The Sister Chromatid Exchange (SCE) refers to the exchange of certain homologous stretches of DNA sequence between two pairing chromatids and higher frequency of such exchanges is associated with certain pathological conditions. A study conducted by Sheth et al. (2006) revealed an increased frequency of SCEs in diabetes as compared to the healthy controls. Carbonyl groups result from protein oxidation and their level in tissues and plasma is indicative of AGEs due to oxidative damage.

tification of these proteins in the peripheral blood is widely used to measure the extent of AGEs (Trombetta et al. 2006).

*Enicostemma littorale Blume* (Family: Gentianaceae) is an herbal medicinal plant, commonly known as Indian Gentian and is widely used in West India for the treatment of diabetes. The herb has been reported to have blood glucose lowering potential in alloxan induced diabetic rats and humans (Maroo et al. 2002; Upadhyay and Goyal 2004). Treatment with Indian Gentian has been shown to decrease the elevated cholesterol, triglyceride and creatinine levels in non insulin dependent diabetes mellitus (NIDDM) rats (Murali et al. 2003). In the present study, genoprotective effect of Indian Gentian in T2DM patients has been studied by comet assay and SCEs tests, in addition to assessment of protein oxidation in the treated patients.

**MATERIAL AND METHODS**

**Selection of Study Subjects**

An independent Ethics committee approved the study of Indian Gentian trial in diabetes mellitus prior to the patient recruitment. After written informed consent, recruitment of the subjects was carried out by organizing camps in Gujarat state and patients were selected as per the inclusion and exclusion criteria listed in Table 1.

| Table 1: Inclusion and exclusion criteria for Groups 1 and 2 T2DM patients |
|-----------------------------|-----------------------------|
| **Inclusion Criteria**      | **Exclusion Criteria**       |
| Age                         | Type 1 diabetic patient     |
| Fasting Blood Sugar (FBS)   | Pregnant woman              |
| Post Prandial Blood Sugar (PPBS) | Lactating mother          |
| Glycosylated Hemoglobin (HbA1c) | Patient with recent stroke or unstable angina or coronary artery disease in previous 6 months |
| Body Mass Index (BMI)       | Presence of ketone bodies in patient’s urine |
|                             | Patient receiving any type of thiazolidinedione group drug(s) |
|                             | Patient suffering from major systemic illness(es) |

The criteria of selection of patients with T2DM were that the upper limit of fasting plasma glucose was 234 mg % and post prandial it was 360 mg % to avoid the hyperosmolar problem or associated complications. It was also felt that higher exclusion levels would not be advisable for early study of a standardized natural product such as Indian Gentian. The highest dose of oral hypoglycemic agents (OHAs) taken by the patient was 20 mg sulphonyl urea and 1.5 to 2 g of metformin.

A total of 52 clinically diagnosed T2DM patients were selected for the study. Thirty eight of these patients were considered for Indian Gentian treatment (Group 1) and the remaining 14 patients (Group 2) were not given any such treatment. Fifteen, age, sex and Body Mass Index (BMI) matched non diabetic volunteers were selected as healthy controls (Group 3).

Herbal drug Indian Gentian was selected as an insulin sensitizer for T2DM patients, under a CSIR funded research project. The herb is commercially available and is manufactured by Shree Dootpapeshwar Ltd (Panvel), Mumbai. All the three groups were studied for genotoxicity by comet assay and SCEs, and Group 1 also for protein oxidation by protein carbonyl estimation. In Group 1, post-treatment follow up was done after 12 weeks. A battery of biochemical parameters including plasma glucose, lipid profile, serum insulin and glycosylated haemoglobin (HbA1c) were measured in both pre- and post-treatment patients in Group 1.

**Comet Assay**

The comet assay was performed under alkaline conditions following the protocols of Klau-de et al. (1996) and Tice et al. (2000) with minor modifications. A freshly prepared cell suspension from theuffy coat of the centrifuged blood sample was mixed in 0.5% low melting agarose and casted on microscope slide pre-coated with 1% normal melting agarose. The cells were then lysed for 1 hour at 4°C in a buffer composed of 1.25 M NaCl, 100 mM Tris, 50 mM EDTA, 1% Triton X-100 and 10% Dimethyl sulfoxide (DMSO) pH 10. After lysis, DNA was allowed to unwind for 20 minutes in electrophoresis buffer consisting of 10 N NaOH, 200 mM EDTA and 10% DMSO pH >13. Electrophoresis was carried out at 0.7 to 1.0 V/cm for 20 minutes in a refrigerator in dark. The slides were neutralized with 0.4 M Tris pH 7.0 and stained with 10 µg/ml ethidium bromide and covered with cover slips and scanned under a fluorescence micro-
scope (Olympus BX-51) attached with a CCD camera. Using appropriate filters 10 images were captured per slide and comet tail DNA was measured using the Adobe Photoshop. According to tail length, the cells were differentiated as normal or as mildly, moderately and highly damaged, and apoptic cells.

### Sister Chromatid Exchanges (SCEs)

Peripheral blood was subjected to the standard cytogenetic culture technique. Differential chromatid staining method of Schneider et al. (1978) was employed and 10 µl / ml Bromodeoxy-uridine (BrdU) was added after 24 hours of the initiation of the culture and harvested after 96 hours. The slides were prepared and mounted with Bisbenzimide solution (150 µg / ml) (Hoechst), followed by exposure to sunlight for 7-8 hours. These were dipped in 2 X saline sodium citrate solution for 30 minutes at 50ºC in a water bath, followed by staining with Giemsa. SCEs were observed under a microscope and a minimum of 25 metaphases were scored in second cell cycle. Results were recorded as SCEs per metaphase and SCEs per chromosome.

### Protein Carbonyl Estimation

Protein carbonyl estimation was carried out as per the method of Reznick and Packer (1994). The assay involves derivitization of the carbonyl group with dinitrophenylhydrazine (DNPH), followed by an anti-DNP antibody detection. DNPH reacts with protein carbonyls and the amount of protein-hydrazone produced was measured spectrophotometrically at 375 nm using Shimandzu UV-1700.

### Statistical Analysis

Data on Group 1 pre- and post-treatment T2DM patients using Indian Gentian for the comet assay, SCEs, protein carbonyl, plasma glucose, lipid profile, serum insulin and glycosylated haemoglobin (HbA1c) were analysed using paired *t* test at 95% confidence interval. Such data on Group 1 post-treatment T2DM patients were compared with Groups 2 and 3 using unpaired *t* test at 95% confidence interval. All *t* tests were performed using GraphPad QuickCalcs Web sites http://www.graphpad.com/quickcalcs/ttest1.cfm?Format=C and http://www.graphpad.com/quickcalcs/ttest1.cfm?Format=SD (accessed June 2011).

### RESULTS

Paired *t* test results (Table 2) of Group 1 pre- and post-treatment T2DM patients with Indian Gentian using comet assay, SCE and protein carbonyl tests showed variable results. Statistically the herb was found to significantly increase normal cells (*p* = 0.0020), and decrease moderately damaged (*p* = 0.0279), highly damaged (*p* = 0.0165) and apoptic (*p* = 0.0014) (Table 2, Fig. 1) cells. It also decreased mildly damaged cells but the result was not appreciable (*p* = 0.9309). Significant decrease was observed in SCEs (*p* = 0.0002) (Table 2, Fig. 2), while no significant difference was observed in protein carbonyl levels (*p* = 0.9038) (Table 2). In addition, paired *t* test results showed that there is no significant effect of Indian Gentian in Group 1 post-treatment T2DM patients for fasting plasma glucose and lipid levels (*p* > 0.05). However, fasting and post prandial insulin (*p* = 0.0308 and 0.0187, respectively) and HbA1c levels (*p* = 0.0070) did decrease significantly after 12 weeks of the treatment (Table 2).

Table 3 shows comparison of the comet assay and SCE results of Group 1 post-treatment with Groups 2 and 3. The unpaired *t* test between Group 1 post-treatment and Group 2 showed highly significant difference in normal (*p* < 0.0001), moderately damaged (*p* = 0.0141), highly damaged (*p* = 0.0001) and apoptic (*p* < 0.0001) cells, and SCEs (*p* < 0.0001), while mildly damaged cells showed no such difference (*p* = 0.6424). Similarly, unpaired *t* test between Group 1 post-treatment and Group 3 showed highly significant difference in normal (*p* < 0.0001), mildly damaged (*p* = 0.0001), moderately damaged (*p* = 0.0003), highly damaged (*p* < 0.0001) and apoptic (*p* < 0.0001) cells, while SCEs showed no significant difference (*p* = 0.6070).

### DISCUSSION

DNA damage and impaired DNA repair have been shown in T2DM by Blasiak et al. (2004). In an *in vitro* study, OHA gliclazide showed DNA repair (Sliwinska et al. 2008). The significant antglycemic effect of Indian Gentian has been well documented in T2DM patients. The present
Table 2: Paired t test results for Group 1 pre- and post-treatment T2DM patients

<table>
<thead>
<tr>
<th>Test</th>
<th>Parameter</th>
<th>Pre-treatment</th>
<th>Post-treatment</th>
<th>t</th>
<th>d.f.</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Mean ± S.D.</td>
<td>n</td>
<td>Mean ± S.D.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Comet assay</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal cells (%)</td>
<td>38</td>
<td>57.18 ± 13.95</td>
<td>29</td>
<td>68.90 ± 8.36</td>
<td>3.3991</td>
<td>28</td>
</tr>
<tr>
<td>Mildly damaged cells (%)</td>
<td>38</td>
<td>16.33 ± 7.83</td>
<td>29</td>
<td>13.57 ± 5.17</td>
<td>0.0875</td>
<td>28</td>
</tr>
<tr>
<td>Moderately damaged cells (%)</td>
<td>38</td>
<td>13.01 ± 6.16</td>
<td>29</td>
<td>9.01 ± 4.14</td>
<td>2.3198</td>
<td>28</td>
</tr>
<tr>
<td>Highly damaged cells (%)</td>
<td>38</td>
<td>9.61 ± 4.70</td>
<td>29</td>
<td>6.28 ± 2.50</td>
<td>2.5519</td>
<td>28</td>
</tr>
<tr>
<td>Apoptic cells (%)</td>
<td>38</td>
<td>3.85 ± 2.26</td>
<td>29</td>
<td>2.09 ± 1.51</td>
<td>3.5501</td>
<td>28</td>
</tr>
<tr>
<td>SCE per metaphase</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein oxidation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein carbonyl (nmol/mg)</td>
<td>38</td>
<td>1.20 ± 0.56</td>
<td>27</td>
<td>1.25 ± 1.41</td>
<td>0.1221</td>
<td>26</td>
</tr>
<tr>
<td>Plasma glucose</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting Blood Sugar (FBS) (mg/dl)</td>
<td>29</td>
<td>134.03 ± 27.60</td>
<td>27</td>
<td>131.78 ± 35.06</td>
<td>0.3490</td>
<td>26</td>
</tr>
<tr>
<td>Lipid profile</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>29</td>
<td>201.90 ± 23.46</td>
<td>27</td>
<td>198.93 ± 28.12</td>
<td>0.8715</td>
<td>26</td>
</tr>
<tr>
<td>Triglycerides (TG) (mg/dl)</td>
<td>29</td>
<td>145.10 ± 40.93</td>
<td>27</td>
<td>145.44 ± 54.83</td>
<td>0.1611</td>
<td>26</td>
</tr>
<tr>
<td>High Density Lipoprotein (HDL) (mg/dl)</td>
<td>29</td>
<td>43.97 ± 5.14</td>
<td>27</td>
<td>43.56 ± 4.93</td>
<td>0.8534</td>
<td>26</td>
</tr>
<tr>
<td>Low Density Lipoprotein (LDL) (mg/dl)</td>
<td>29</td>
<td>135.93 ± 24.55</td>
<td>27</td>
<td>127.89 ± 20.30</td>
<td>1.9064</td>
<td>26</td>
</tr>
<tr>
<td>Serum insulin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting Insulin (IU/ml)</td>
<td>29</td>
<td>17.63 ± 11.77</td>
<td>27</td>
<td>11.87 ± 8.46</td>
<td>2.2836</td>
<td>26</td>
</tr>
<tr>
<td>Post Prandial Insulin (IU/ml)</td>
<td>29</td>
<td>61.90 ± 35.29</td>
<td>26</td>
<td>44.02 ± 31.95</td>
<td>2.5159</td>
<td>25</td>
</tr>
<tr>
<td>Glycosylated haemoglobin (HbA1c) (%)</td>
<td>29</td>
<td>7.68 ± 0.72</td>
<td>27</td>
<td>7.19 ± 0.70</td>
<td>2.9311</td>
<td>26</td>
</tr>
</tbody>
</table>

*Statistically significant (p ≤ 0.05)

Fig. 1. Comet assay results of a Group 1 subject: (a) pre-treatment showing large number of damaged cells (b) post-treatment showing reduced number of damaged cells

Fig. 2. SCEs results of a Group 1 subject: (a) pre-treatment showing excessive chromatid exchanges (b) post-treatment showing less chromatid exchanges
study showed decline in mean glycation of haemoglobin, which is likely to be due to glucose induced insulin release through K(+-) ATP channel dependant pathway as observed in diabetic rats (Maroo et al. 2002). However, the cellular effect of Indian Gentian was not known which has been demonstrated by comet assay, SCE and protein oxidation tests.

In the present study, in comet assay, a significant increase in normal cell population and decrease in damaged cell population was observed in T2DM patients after treatment with Indian Gentian as compared to that observed in pre-treatment, and Group 2 patients receiving only OHAs. Among DNA repair pathways, nucleotide excision repair (NER) is able to recognize and process a wide variety of DNA lesions. Effect of Indian Gentian could be comprehended as an increased DNA repair due to significant role of the herb in NER activity. There was a significant difference between Group 1 (post-treatment) and Group 3 (controls), which suggested that the herb was able to decrease the DNA damage but not as low as in non diabetic healthy subjects.

Significant decrease in SCEs in T2DM patients receiving Indian Gentian treatment can be explained by two different mechanisms of SCEs, one operating at replicating points probably utilizing the machinery of DNA replication, and the other acting only in the post replication DNA stage. Cells can achieve error-free repair of repair DNA double-strand breaks (DSBs) by homologous recombination through gene conversion with or without crossover (Kato 1977). Eukaryotes have developed several mechanisms to repair DSBs, including non-homologous DNA end-joining (NHEJ) and homologous recombination (HR) (Paques and Haber 1999). It can be postulated that Indian Gentian may have efficiency to play significant role in DNA repair activity by inducing homologous recombination, with or without cross over, that reduces SCEs per se. No significant difference between Group 1 (post-treatment T2DM patients) and Group 3 (controls) was observed in this study suggesting that the herb reduced SCEs per metaphase as low as the latter group.

In conclusion, the present results indicated that Indian Gentian may have potential beneficial effect in correcting DNA damage produced by oxidative stress in T2DM patients. It would be desirable to confirm these findings in a larger sample elucidating the possible molecular/cellular mechanism(s) involved in genoprotective effect of the herb.

**ACKNOWLEDGEMENTS**

We gratefully acknowledge the research grant from the Council of Scientific and Industrial Research (CSIR), New Delhi for the project entitled “Herbal Based Preparation for Degenerative Disorders: Type II (NIDDM) Diabetes Mellitus with Emphasis on Insulin Sensitization” (vide no. 5/258/12a/2002-NMITLI). Thanks are due to Dr M. H. Makwana for providing facility.

---

**Table 3: Unpaired t test results for Group 1 (post-treatment T2DM patients) with Group 2 (T2DM patients without Indian Gentian treatment) and Group 3 (healthy controls) using comet assay and SCE tests**

<table>
<thead>
<tr>
<th>Test Parameter</th>
<th>Group 1</th>
<th>Group 2</th>
<th>t</th>
<th>d.f.</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Mean ± S.D.</td>
<td>n</td>
<td>Mean ± S.D.</td>
<td></td>
</tr>
<tr>
<td>Comet assay Normal cells (%)</td>
<td>29</td>
<td>68.90 ± 8.35</td>
<td>14</td>
<td>48.61 ± 21.89</td>
<td>4.417</td>
</tr>
<tr>
<td>Mildly damaged cells (%)</td>
<td>29</td>
<td>13.57 ± 5.16</td>
<td>14</td>
<td>14.38 ± 5.58</td>
<td>0.4678</td>
</tr>
<tr>
<td>Moderately damaged cells (%)</td>
<td>29</td>
<td>9.01 ± 4.14</td>
<td>14</td>
<td>13.31 ± 6.83</td>
<td>2.5653</td>
</tr>
<tr>
<td>Highly damaged cells (%)</td>
<td>29</td>
<td>6.28 ± 2.50</td>
<td>14</td>
<td>13.33 ± 8.34</td>
<td>4.2224</td>
</tr>
<tr>
<td>Apoptic cells (%)</td>
<td>29</td>
<td>2.09 ± 1.51</td>
<td>14</td>
<td>10.85 ± 10.23</td>
<td>4.5673</td>
</tr>
<tr>
<td>SCEs SCEs per metaphase</td>
<td>29</td>
<td>7.32 ± 1.10</td>
<td>4</td>
<td>13.00 ± 5.05</td>
<td>5.6436</td>
</tr>
</tbody>
</table>

*Statistically significant (p ≤ 0.05)
ties at V.S. Hospital, to Dr. Zarna Rawal, Dr. Jignasha Thakkar and Mrs. Meghna Patel for technical assistance and to the subjects for participating in the study.

REFERENCES


Khansari N, Shakiba V, Mahmoudi M 2009. Chronic inflammation and oxidative stress as a major cause of age related diseases and cancer. Recent Pat Inflamm Allergy Drug Discov, 3: 73-80.


