The abstracts encapsulate selected MS theses of graduate students of the Biochemical Department at the College of Medicine, University of the Philippines.

Use of PCR in the Amplification of the rpoB Gene of some Philippine Mycobacterium Tuberculosis Isolates and SSCP Analysis of Rifampicin Resistance

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Mycobacterial rpoB gene is the structural gene that codes for the synthesis of bacterial RNA polymerase. Presence of mutations in the rpoB gene of *Mycobacterium tuberculosis* alters the sequence of amino acids in bacterial RNA polymerase essential for rifampicin binding. Such mechanism allows for *Mycobacterium tuberculosis* resistance to rifampicin. To detect the presence of mutations in the rpoB gene, 411 bp fragment of the rpoB gene coming from 18 rifampicin resistant (11 hospital and 7 community isolates) and 11 rifampicin sensitive (7 hospital and 4 community isolates) *Mycobacterium tuberculosis (Mtb)* isolates were amplified by PCR (polymerase chain reaction) using TR1 and TR2B primers of Telenti and were subjected to *single strand conformation polymorphism* (SSCP) analysis. H37Rv, a virulent sensitive strain of *Mtb* was used as the *reference* strain. PCR conditions were optimized in two different thermal cyclers, GeneAmp and Master Cycler using 4mM and 2.5mM $MgCL_{2,}$ respectively. PCR reaction was run for 30 cycles using GeneAmp (5 min at 94°C, 30s at 94°C, 30s at 55°C, 30s at 72°C) followed by 10 minutes of final extension at 72°C. In the Master Cycler, the reaction was run for 30 cycles (5 min at 95°C, 20s at 95°C, 20s at 60°C, 30s at 72°C) followed by 10 minutes of final extension at 72°C. SSCP analysis of *Mtb* isolates was carried out under manual and semi-automated procedures.

A clear distinction between the sensitive isolates and resistant isolates were not clearly established under manual SSCP conditions. Two sensitive isolates (1459H and 46aH) demonstrated two different mobility patterns. Despite the presence of minor shifts, similar patterns were found between sensitive isolate 1459H and resistant isolate 24H; and between sensitive isolate 46aH and resistant isolate $455bR_1$ H.

Using the semi-automated PCR-SSCP analysis, eight sensitive isolates demonstrated a consistent pattern when run under 7°C (1463H and 344C) and 10°C (244C, 1463H, 240C, 2122aH, 255C, 1224H). At constant temperature of 7°C using DSFebX denaturing solution, four (1807H, 1348H, 333C, 267C) out of five rifampicin resistant strains demonstrated polymorphisms which were distinct from the pattern of the two rifampicin sensitive isolates. One rifampicin resistant isolate (1365H) had a very similar pattern with that of the sensitive isolates. But at a constant temperature of 10°C using DS3 denaturing solution, all nine (333C, 2321H, 270C, 276c, 473DC, 1259H, 1400H, 306C) rifampicin resistant isolates demonstrated polymorphisms which are distinct from the consistent pattern demonstrated by six rifampicin sensitive isolates. The PCR-SSCP product coming from H37Rv exhibited an electro-mobility pattern similar to that of the rifampicin sensitive isolate (1224H) that exhibited a consistent pattern with the other rifampicin sensitive isolates. The consistent pattern achieved among all six sensitive isolates and the polymorphisms among the nine rifampicin resistant strains suggest that the SSCP temperature condition is optimum at 10°C with the use of DS3 denaturing solution; that mutations are present in the amplified 411 bp fragment of the *Mtb* rpoB gene of the nine rifampicin resistant *Mtb* isolates. These are confirmatory to the phenotypic resistance determined by the drug sensitivity testing for the nine rifampicin resistant *Mtb* isolates.

Similarities in the prominent PCR-SSCP bands were exhibited by three rifampicin resistant isolates **333CS, 2321 R_1 HS, and 270 CS**; three rifampicin sensitive isolates **244 CS, 240 CS and 1463 HS** and two rifampicin sensitive isolates **2122a HS and 255 CS.** Presence of several polymorphic patterns in the resistant isolates other than the two major prominent bands suggests that the mutations harbored by the *Mtb* rpoB gene are different point mutations that alters the key amino acid sequence in the *Mtb* RNA polymerase. PCR-SSCP analysis at 10^0C revealed six polymorphic variants among the rifampicin resistant Philippine *Mtb* isolates and one consistent pattern among the six rifampicin sensitive Philippine *Mtb* isolates which came either from the Lung Center of the Philippines or Department of Health.

Molecular Epidemiology of Hepatitis G Virus (HGV) Infection in the Philippines

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Infections with hepatitis G virus (HGV), a recently characterized member of the hepatitis viruses, had been reported worldwide. The current study details the molecular epidemiology of HGV infection in the Philippines.

A cross-sectional study of 1,088 blood samples from healthy adults (volunteer blood donors n=516), chronic liver disease patients (n=138), hemodialysis patients (n=207) and multiply transfused patients (n=227) was conducted to determine the prevalence of HGV infection, its concurrent infection with other known parenterally transmitted viruses - hepatitis B & C (HBV & HCV), and the HGV genotype present in the Philippines.

HGV RNA was detected in the serum of these patients using reverse-transcription polymerase chain reaction (RT-PCR). RT-PCR was performed with random hexamer primers and a set of PCR primers from the 5′ UTR of the HGV genome. The identity of the PCR products was determined by hybridization using an ELISA-based method with

a specific HGV capture probe. HGV RNA was found in 6/516 (1.2%) healthy adults (volunteer blood donors), 11/138 (8%) chronic liver disease patients, 7/207 (3.4%) hemodialysis patients, and 14/227 (6.2%) multiply transfused patients. Thus, a total of 38/1,088 subjects were HGV RNA-positive.

HGV RNA-positive serum samples were further tested to detect HCV RNA and HBsAg using the Amplicor HCV test ver. 2 and Hexagon HBsAg test, respectively. HCV R.NA was present among the HGV RNA+ samples as follows: 1 of 6 healthy adults, 6 of 11 chronic liver disease patients, 1 of 7 hemodialysis patients, and 6 of 14 multiply transfused patients. HBsAg positivity among HGV RNA+ subjects was 1 of 6 healthy adults, 5 of 11 chronic liver disease patients, 0 of 7 hemodialysis patients and 1 of 14 multiply transfused patients. There was only 1 sample from the chronic liver disease patients that tested positive for HGV RNA, HCV RNA and HbsAg.

In order to determine the genotypic variant of HGV in the Philippines, the PCR products derived from the 5' untranslated region (5'UTR) of HGV RNA+ samples were subjected to nucleotide sequence analysis. Pairwise alignment of sequences and phylogenetic tree construction revealed that among the five known HGV genotypic variants, the Philippine isolates are most closely related to the Asian type (III).

When subjects were infected with HGV alone, the virus does not appear to cause clinically significant hepatitis among the subjects. Further studies are warranted to understand the impact of HGV on concurrent infections with HCV, HBV or both. Future investigations should also focus on studies on viral evolution and continuous monitoring of subjects for development of clinically significant disease.

Biophysical Characterization of the Interaction Between Sense Chimeric RNA-Antisense Partial Intermolecular Duplexes (SCRAPIDU) and Target RNA

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The significance of RNA molecules in various cellular functions has been attributed to their capability to adapt to a wide range of conformations. The numerous potential roles of RNA has led to the exploration of its structure and function in order to combat human diseases. A classification of RNA-based therapeutics are the gene inhibitors which include the conventional antisense RNA. Antisense RNAs employ complementary RNA molecules which target specific messenger RNAs via Watson-Crick base pairing resulting to inhibition of protein synthesis.

Antisense technology provides basis for constructing a Sense Chimeric RNA-Antisense Partial Intermolecular Duplex (SCRAPIDu). A SCRAPIDu is a novel RNA construct containing a chimeric RNA coding for a reporter protein partially hybridized to the antisense of a target RNA. Liberation of the chimeric RNA is via formation of a sense-antisense duplex in the presence of a target RNA sequence. A preliminary investigation of the interaction between the SCRAPIDu and target RNA is necessary to evaluate the feasibility of a SCRAPIDu as a disease treatment strategy.

A SCRAPIDu model which is a potential treatment for neoplastic disorders was constructed and monitored in the presence of the target RNA sequence. These were achieved by monitoring expected product using circular dichroism (CD), agarose electrophoresis and RT-PCR. CD results provided a good estimate of the duration of product formation, which was found to be within an hour of hybridization in sodium-saline-citrate buffer pH 7.0. Visual detection by agarose gel elctrophoresis showed the presence of a duplex RNA after one hour-hybridization. RT -PCR showed the presence of liberated chimeric RNA after an hour of incubation with the target RNA.

Isolation, Purification, and Molecular Characterization of the Putative Asparaginyl-tRNA Synthetase cDNA of Schistosoma japonicum

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The current study was undertaken as part of a bigger project for new drug discovery for parasitic diseases. Specifically, this study aimed to determine the sequence of the putative asparaginyl-tRNA synthetase (AsnRS) cDNA of Schistosoma *japonicum* (Sj), deduce its resulting amino acid sequence, compare both sequences with the respective human and *Brugia malayi (8m)* AsnRS sequences, determine the predicted functions of the deduced Sj polypeptide, and compare its protein domains with the human and *Bm* AsnRS domains.

The possible sequence of the schistosomal AsnRS was first constructed from a series of sequence searches and alignments that initially used the known sequence of the *Bm* AsnRS. Different combinations of gene-specific primers (GSP) designed from the constructed sequence were then used in the attempts to isolate the desired cDNA from a Sj cDNA library. After optimization of thermal cycling conditions, a full-length cDNA containing the entire coding sequence of the putative *S. japonicum* AsnRS was amplified. This was cloned and subsequently sequenced.

From the obtained 1749-base cDNA sequence, the probable amino acid sequence was deduced using the Transeq program, which revealed a 546-residue polypeptide. Both the cDNA and the deduced amino acid sequences of the putative Sj AsnRS were compared with the respective sequences of human and *Bm* AsnRS using the Emboss and ClustalW programs, revealing up to 73% similarity.

Structural analysis through ProDom queries of the amino acid sequences of the *Brugia malayi* and human AsnRS and that of the polypeptide deduced from the isolated Sj cDNA revealed 4 domains identical to all the 3 polypeptides, while a functional analysis done through an InterPro query of the deduced *Sj* amino acid sequence

revealed that it contains domains with tRNA ligase activity for tRNA aminoacylation and domains for nucleic acid binding.

The evidences provided by the results strongly suggest that the Sj AsnRS cDNA had indeed been isolated. With the sequence of the full-length *Sj* AsnRS cDNA now known, new opportunities for the discovery and development of a new drug against *Schistosoma japonicum* have been opened.

Detection of Hepatitis B Virus (HBV) Infection among Apparently Healthy Adults Undergoing Pre-Employment Screening A Comparison between Three Hepatitis B Markers: HBV DNA, HbsAg and Anti-HBC

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The prevention of transfusion-transmitted Hepatitis B virus (HBV) in the country has relied solely on serological screening of blood donors using Hepatitis B surface antigen (HBsAg) using either enzyme immunoassay or dipstick method. However, studies have shown that HBV infection may be missed out by these assays for HBsAg if the infected person is within the immunological window period in which HBsAg is undetectable. This study determines if the addition of other HBV serological markers - the antibody to Hepatitis B core antigen (anti-HBc) and HBV DNA (using pooled serum samples) - could represent as alternatives in detecting the presence of the virus.

A prospective study of 315 serum samples from apparently healthy adults was conducted to determine the prevalence of HBV infection using the three different HBV markers and combinations of these markers.

The presence of HBsAg in the sera of apparently healthy individuals was detected using Hexagon HBsAg test (Human, Germany). The prevalence using this immunochromatographic method was compared to an enzyme immunoassay carried out as part of the pre-employment screening using MONOLISA ® Ag HBs PLUS (Bio-Rad laboratories, France) in detecting the presence of HBsAg. The IgM antibodies to hepatitis B virus core antigen was detected in the sera of healthy adults using enzyme immunoassay using ImmunoLISA ™ HBc IgM kit (Orgenics, Israel). HBV DNA was quantitatively detected using Ampljcor HBV Monitor Test (Roche Diagnostics. Mannheim, Germany) using pooled serum samples.

HBsAg was detected in 3.8% apparently healthy individuals, a rate lower than the prevalence of the general population in the Philippines published in the 1980s. Anti-HBc marker was observed in 0.95% of the subjects. The use of anti-HBc marker in addition to HBsAg has detected two more individuals with HBV, increasing the prevalence of HB infection from 3.8% to 4.4%.

HBV DNA was carried out in minipools of sera, a routine practice among laboratories performing nucleic acid testing. Out of the 32 pools, 22 were HBV DNA-positive. Assuming that one individual in each pool contributed to its positivity, then> 6.98% of the subjects were HBV DNA-positive. All HBsAg-positive sera were also found to be positive for HBV DNA.

The combined HBV markers to detect the presence of HBV have resulted to a higher rate of detection rate for HBV infection (≥7.30%). The use of HBsAg alone as a screening marker is inferior to using a combination of two markers: HBsAg and anti-HBc. Nucleic acid testing when used alone with minipool of blood samples, was the best method for the detection of HBV infection. Future investigations should focus on the feasibility of adapting an improved screening strategy in the Philippines that incorporates the use of a combination of HBsAg and anti-HBc or HBV DNA in routine blood testing.

Effectiveness of Three Antigen Retrieval Techniques for Use in Immunostaining of Formalin-Fixed Schistosoma japonicum Egg Antigens in Mouse Granulomatous Liver

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Antigen retrieval systems have expanded the scope of immunohistochemical studies. Various antigen-unmasking techniques were developed and utilized as pretreatment for formalin-fixed paraffin embedded tissues prior to immunological studies. Antigen retrieval systems are necessary for immunostaining of formalin-fixed specimens because it uncovers the epitopes masked by hydroxy-methylene bridges that cross-links macromolecules in and outside the cells.

In this study, effectiveness of antigen retrieval systems was tested for immunohistochemistry studies. *Schistosoma japonicum*-infected mice granulomatous liver tissues fixed in 10% neutral buffered formalin at either 20-hour or 3O-day fixation periods were utilized. Effect of both fixation times on immunostaining was evaluated.

Four procedures were used in this study, three for antigen retrieval systems and one for no antigen retrieval. The three methods employed as antigen retrieval prior to immunostaining included (1) heat-induced antigen retrieval, (2) proteolytic enzyme digestion, and (3) combination of heat-induced and enzyme digestion. For the heat-induced antigen retrieval, water bath was utilized to incubate specimens at 100°C for 20 minutes. Proteolytic enzyme digestion was done with 0.1 % trypsin at 37°C for 30 minutes. The third antigen retrieval system was a combination of heat-induced and enzyme digestion methods. The same procedure for heat-induced antigen retrieval was applied to the combination treatment but was followed immediately after by enzyme digestion with 0.1 % trypsin for 15 minutes. Citrate buffer was used as the antigen retrieval solution. Polyclonal antibody from serum of infected mice was utilized as primary antibody. Immunohistochemistry was done using DAKO LSAB® 2 kits, and the procedure was followed as provided by the kit.

Three observers composed of two pathologists and the researcher

interpreted the immunostaining results. The readers were blinded during microscopic evaluation of the immunostained slides. The results of the study showed that antigen retrieval is necessary for doing immunohistochemistry on formalin-fixed S. *japonicum* egg antigens. The three AR systems were effective in unmasking epitopes based on the three readings done by the observers. The most sensitive ARS in 20-hour fixed specimens is Enzyme digestion with 80% sensitivity. Statistical analysis using Pearson Chi Square test showed no significant difference in immunohistochemistry among the antigen retrieval systems. The Mann-Whitney test showed no significant difference in immunostaining between tissues fixed for 20 hours and 30 days. However, sensitivity of enzyme digestion decreased from 80% to 73%. Specimens fixed with formalin for more than 48 hours up to 30 days can be utilized for immunohistochemistry studies using *any of the antigen retrieval* systems described. The use of 10% neutral buffered formalin for *S. japonicum* egg antigens was established after successful immunostaining results. Future studies whether for diagnostic or research purposes involving *S. japonicum* egg antigens can be guided by using the information gained from this research paper.

Comparative Study of hsC-Reactive Protein and Lipid Profiles of Members of Four Filipino Families Suspected with Familial Hypercholesterolemia

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C-reactive protein and LDL-cholesterol have a strong linear relation with the incidence of cardiovascular disease (Ridker, 2002). However, Familial Hypercholesterolemia (FH) patient-based data directly comparing these two biologic markers are not available.

In this study, total cholesterol, HDL cholesterol (HDLc), LDL cholesterol (LDLc) and high sensitive C-reactive protein (hsCRP) profiles of members of four Filipino families suspected with FH and age- and gender-matched normal healthy individuals were measured and compared, in order to determine if lipid profile is directly related with hsCRP level in FH patients.

The study initially surveyed some 32,800 patients from the blood chemistry records of the five cooperating hospitals. From these, 352 were found hypercholesterolemic based on their total cholesterol. From the 352, 60 individuals were suspected with FH based on high total cholesterol and LDL cholesterol, history of cardiovascular disease in the family, and normal blood sugar and creatinine. From the 60, 17 subjects belong to four (4) Filipino families. These 17 individuals composed the final population of this study. Seventeen normal, healthy Filipinos, age- and gender-matched of the 17 subjects suspected with FH were recruited to be normal control in the study.

In this study, it was found that among the 17 subjects suspected with FH, the incidence of FH was higher in males than in females. All 17 subjects suspected with FH exhibit cholesterol values consistent with patients diagnosed with FH. The rise of serum total cholesterol and LDLc levels appeared to generally increase with age as observed among ages 50-60 where mean cholesterol levels were highest.

The mean total cholesterol:HDL cholesterol (TC:HDLc) ratio among the FH subjects in this study was 12:1 while among normal healthy subjects, it was 4.5:1. Using the Quintile of cardiovascular diseases (CVD) risk to evaluate TC:HDLc ratio, 16 of the 17 subjects suspected with FH have TC:HDLc ratio falling on quintile 5, being the highest risk for CVD relative risk category, while among normal healthy subjects only one was on quintile 5, while five, four and six subjects fell on quintiles 4, 3 and 2 respectively. This data may suggest that the suspected FH subjects are at greater odds developing CHD.

High sensitivity CRP is an inflammatory marker believed to be of value in the prediction of coronary events. The mean hsCRP (3.92 mg/L) among the subjects suspected with FH was not statistically different from the mean (3.00 mg/L) of normal healthy subjects. High sensitive-CRP data were interpreted based on "quintiles of CVD risk", a series of five clinical cutpoints wherein the relative risks of sustaining CVD increases in a stepwise fashion with Quintile 1 (0.1 - 0.7 mg/L) and Quintile 5 (3.9 -15 mg/L) being the lowest risk and highest risk respectively (Ridker, 2002). Eight of the 17 subjects suspected with FH fell on quintile 5 and seven in quintile 4, while among normal healthy subjects five were on quintile 5 and seven on quintile 4. This means about 76 percent of subjects suspected with FH and about 70 percent of the normal healthy individuals are at high risk for future cardiovascular events. Age and gender showed no significant relationship with hsCRP levels.

This study showed eight of the 17 subjects suspected with FH have 8.7 fold and six subjects have 7.2 fold higher relative risk (RR) of CVD than those in the lowest quintile when hsCRP and TC:HDLc ratio RR were combined. This observation may suggest that the FH subjects in this study are really at greater risk of cardiovascular event. The result also shows dramatic increase in RR of CVD when hsCRP RR is combined with TC:HDLc RR, wherein mean RR of CVD based on TC:HDLc ratio alone was 4, while it dramatically increases to 7.4 when combined. This observation may suggest that when hsCRP value is utilized with lipid values, the CVD risk prediction is better.

The FH subjects' total cholesterol and LDLc levels were weakly correlated with hsCRP levels suggesting each of these parameters may identify different high-risk groups for cardiovascular disease.

The Effect of Lipase-Hydrolyzed and Unhydrolyzed Virgin Coconut Oil (Vco) on the Growth and Beta-Lactamase Activity of Methicillin-Resistant *Staphylococcus Aureus* (MRSA)

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Virgin coconut oil (VCO) has become a popular health product because of its various health benefits including its antimicrobial

properties. However, studies on VCO are limited and most of its celebrated benefits are only based on studies with the medium chain triglycerides and its hydrolyzates, medium chain fatty acids, especially lauric acid.

The present study determined the effect of VCO on the beta-lactamase activity of Methicillin-resistant *Staphylococcus aureus* using an iodometric assay. Inhibition of beta-lactamase was observed with the lipase hydrolyzed-VCO. Lipase hydrolysis produced a potent antimicrobial - ***monolaurin***. Increased amounts of VCO lipase hydrolysis products (diglycerides, monoglycerides and fatty acids) dispersed in culture correlated with the lowering of beta-lactamase activity. This suggests that beta-lactamase inhibition depends on the hydrolysis of VCO into monoglycerides and fatty acids.

The concentrations of unhydrolyzed VCO used in the experiment did not produce a significant inhibition of beta-lactamase activity, suggesting the dependence of MRSA beta-lactamase inhibition on the action of lipases.

Conversely, MRSA treated with olive oil had increased beta-lactamase activity and hydrolysis of the oil further increased the activity of the enzyme, similar to the effect of penicillin - an inducer of beta-lactamase in *Staphylococcus aureus*. The effect of olive oil on MRSA beta-lactamase activity may be due to oleic acid which has nutritive effects on microorganisms.

The growth of MRSA was hindered by both unhydrolyzed and pancreatic lipase hydrolyzed VCO and olive oil, antibiotics and standard lauric lipids (monolaurins, dilaurins and lauric acid) except for trilaurin (TL), which produced higher cell count in culture. This may probably be due to the difficulty of releasing the antimicrobial monolaurin and lauric acid from TL in the culture. Inhibition of MRSA growth by VCO, standard lauric lipids and olive oil may be attributed to increased acidity of fatty acids.

Keywords: Virgin coconut oil (VCO) , beta-lactamase, Methicillin-resistant *Staphylococcus aureus* (MRSA), lauric acid, monolaurin, antimicrobial activity

Determination of the Lymphocyte DNA Resistance to Oxidative Damage Imparted by the Consumption of Soy Bean Supplemented Diet in the Murine Model Using Single Cell Gel ElectrophoresisAssay

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The mammalian cell needs to maintain a delicate balance between the amount of free radicals and its anti-oxidant system for optimum performance. Oxidative stress results when there is an imbalance between these two components, with an increase in the relative amount of free radicals versus anti-oxidants. To counter oxidative stress, the balance should be shifted back by increasing the amount of anti-oxidants.

Several compounds have been identified as potent anti-oxidants.

One of these compounds is known collectively as isoflavonoids. Found primarily in soybeans, isoflavonoids have been shown to possess anti-oxidant properties following *in vitro* and *in vivo* studies. Among the isoflavonoids, genistein is the most important agent that has been extensively investigated for its chemopreventive and anti-cancer activity (Wei *et al., 2003)*.

Oxidative damage can best be demonstrated by observing its effects on DNA resistance in affected cells using various laboratory methods. One such method, called single cell gel electrophoresis (SCGE) assay, had been shown to be the method of choice for evaluating low degrees of DNA damage in individual cells. The SCGE assay had been useful in the study of several important anti-oxidants, such as lycopene from tomatoes, and resveratrol from grapes and soymilk. The SCGE assay was adapted for use in this study.

The overall objective of this study was to determine whether genistein in soybeans can impart protection against DNA damage on lymphocytes using SCGE in the murine model.

.Sixty-four BALB/c ? mice, (32 males and 32 females) about 6 to 8 weeks old, 18 to 22 grams in weight, were randomly divided into four diet/treatment groups. There were three treatment groups consisting of different dosages (50 mg, 250 mg and 500 mg) of genistein per kg diet. One group served as the negative control group. Corresponding intervention was administered *ad libitum* and was carried out for six weeks. Blood were collected and were used for the single cell gel electrophoresis (SCGE) assay for the evaluation of the DNA damage and for the time-resolved fluorescence immunoassay (TR-FIA) for the quantitation of the plasma genistein concentration.

Based on the evaluation of DNA damage, for the treatment groups of both sexes, as the dose increases and the intervention proceeds, the mean relative tail moment and percentage of cell damage increases. Note that there is an inverse effect between the dose of the intervention and the mean outcome.

In addition, plasma genistein concentration of pooled samples as well as the genistein concentration of the respective diets were determined using TR-FIA. There was a significant increase in the plasma genistein concentration as compared to the baseline and this increase was seen to be dose dependent. It was also inferred that there is a correlation between the plasma genistein concentration and level of DNA protection.

It can be concluded from these results that lymphocytes of soybean-supplemented mice were shown to have an increased resistance to oxidative damage when challenged *ex vivo* with 500 µM of hydrogen peroxide in a dose and time dependent manner.

However, death of several female members of the intervention group warrants further investigation. Future studies are recommended on the toxicity and estrogenic effects of soy supplementation with particular attention on the age of the animal subjects.