EFFECT OF SOYBEAN DIET ON THE BLOOD BIOCHEMICAL PARAMETERS OF FEMALE RATS (RATTUS SPP.) AND THEIR OFFSPRING

Iryna Matiukha¹
Halyna Tkachenko²
Natalia Kurhaluk²

¹ National Academy of Agrarian Science
Institute of Animal Biology
V. Stusa Str. 38, 79034 Lviv, Ukraine
e-mail: inenbiol@mail.lviv.ua
² Pomeranian University in Słupsk
Department of Zoology and Animal Physiology
Institute of Biology and Environmental Protection
Arciszewski Str. 22b, 76-200 Słupsk, Poland

ABSTRACT

The aim of our study was to study the impact of soybean feeding on the antioxidant defense and biochemical parameters in the blood of female rats (Rattus spp.) and their offspring, as well as reproductive ability. Dynamic analysis of these parameters on two generations of rats in combination with estimates of changes in growth also have been assessed. Experiment performed with two generations of rats: females parental (F0) and first generation (F1). Our results demonstrate influence of prolonged effects of soy diet on female rats and their offspring. Soy diet provided decrease of antioxidant defenses and lipid peroxidation, activation of phagocytic activity in the blood of offspring. The decrease of livability of newborn offspring from soy-treated rats also was demonstrated. However since biological effects are dependent on many factors including dose, duration of use, protein binding affinity, individual metabolism and intrinsic estrogenic state, further clinical studies are necessary to determine the potential health effects of these compounds.

Key words: amphibians, spring migrations, amphibian road mortality, mortality index, Gdańsk

INTRODUCTION

Soybean and its components are widely used in almost all sectors of agriculture, food industry, veterinary and human medicine (Ørgaard and Jensen 2008, Sakai and...
Soybean is the product with balanced content of essential amino acids, polyunsaturated fatty acids, vitamins, mineral elements and lecithin, biological active substances, antinutritive components, and attracts scholars and researchers of different industries. The important place in soy content belongs to biological active substances, which have very wide specter of influence on organism. The health benefits associated with soy consumption have been linked to the content of isoflavones, the main class of the phytoestrogens (Ørgaard and Jensen 2008). As a result of their structural similarities to endogenous estrogens, isoflavones elicit weak estrogenic effects by competing with 17-beta-estradiol (E2) for binding to the intranuclear estrogen receptors (ERs) and exert estrogenic or antiestrogenic effects in various tissues. The estrogenic activities of soy isoflavones are thought to play an important role in their health-enhancing properties (Ørgaard and Jensen 2008). Consumption of traditional soy food containing isoflavones is associated with reduced prevalence of chronic health disorders (Masilamani et al. 2012). Recent evidence indicates that soy isoflavones play a beneficial role in obesity, cancer, osteoporosis, and cardiovascular disease (Sakai and Kogiso 2008). Several epidemiological studies correlated high dose consumptions of soy isoflavones with multiple beneficial effects on breast and prostate cancers, menopausal symptoms, osteoporosis, atherosclerosis and stroke, and neurodegeneration (Branca and Lorenzetti 2005). Their perceived health beneficial properties extend beyond hormone-dependent breast and prostate cancers and osteoporosis to include cognitive function, cardiovascular disease, immunity and inflammation, and reproduction and fertility (Dixon 2004).

Soybeans are rich in isoflavones such as genistein, daidzein, and glycine. These isoflavones are well-known antioxidants, chemopreventive and anti-inflammatory agents. Isoflavones are considered to be phytoestrogens because of their ability to bind to estrogen receptors (Masilamani et al. 2012). They are naturally occurring chemical constituents that may interact with estrogen receptors to produce weak estrogenic or antiestrogenic effects. They are composed of a wide group of nonsteroidal compounds similar in structure and function to human estrogens (Leclercq and Heuson 1979). A conspicuous feature of the chemical structure of phytoestrogens is the presence of a phenolic ring that, with few exceptions, is a prerequisite for binding to the estrogen receptor (Fig. 1).

For this reason, phytoestrogens can act as weak estrogen agonists, partial agonists, or as antagonists to endogenous estrogens (such as estradiol) and xenoestro-
gens (including phytoestrogens) with estrogen receptors in both animals and humans. Therefore, working as estrogen mimics, phytoestrogens may either have the same effects as estrogen or block estrogen’s effects. There are three major classes of plant chemical compounds that have estrogen-like actions in the body. They are lignans (enterolactone, enterodiol), isoflavones (genistein, daidzein, biochanin A), and coumestans. The two major chemical classes of phytoestrogens found in people’s diets are lignans (enterodiol and enterolactone) and isoflavones (daidzein, genistein, and glycine) (Leclercq and Heuson 1979) (Fig. 1).

The two primary isoflavones in soybeans are daidzein and genistein and their respective glycosides genistein and daidzein. Soy foods typically contain more genistein than daidzein, although this ratio varies among different soy products. Isoflavones are contained in soybean or soy foods in two chemical forms, i.e., aglycone (unconjugated form) and glycosides (bound to a sugar molecule). The main dietary source of genistein is the biologically active glucoside genistein (Rowland et al. 2003). Bioavailability of isoflavones requires an initial hydrolysis of the sugar moiety by intestinal beta-glucosidases to allow the following uptake by enterocytes and the flow through the peripheral circulation. Following absorption, isoflavones are then reconjugated mainly to glucuronic acid and to a lesser degree to sulphuric acid. Gut metabolism seems key to the determination of the potency of action (Branca and Lorenzetti 2005). Fermentation and digestion of soybeans or soy products results in the release of the sugar molecule from the isoflavone glycoside, genistein, leaving the isoflavone aglycone, genistein (Rowland et al. 2003). Before genistein can act it first needs to be released from genistein. This normally happens in the stomach (acid hydrolysis) and intestine (bacterial enzymes action).

There is considerable individual variation in the absorption and metabolism of ingested genistin and genistein. There are some data suggesting that genistein may be more bioavailable than genistin. However, other data suggest that the extent of absorption of genistein is similar for the aglycone and the glucoside forms. There is little data available on the tissue distribution of genistein. The pharmacokinetics of genistein in humans is complex and not well understood. Genistein affects the process by which signals at the cell surface are transferred to the interior of the cell and inhibits the activity of several enzymes intimately involved in controlling cell growth and regulation (Sarkar et al. 2006).

The complete metabolic fate of exposure to genistein is not known. Genistein is the most studied of the soy isoflavones with regard to antioxidant activity. It is thought that genistein may be a more potent antioxidant than daidzein. There are few studies comparing the antioxidant activity of the two isoflavones (Physicians’ Desk Reference (PDR) Health, Soy Isoflavones, Verheus et al. 2007).

Isoflavones currently heralded as offering potential alternative therapies for a range of hormone-dependent conditions, including cancer, menopausal symptoms, cardiovascular disease and osteoporosis. Epidemiologic evidence and experimental data from animal studies that have been reviewed (Messina et al. 1994, Setchell 1995, 1998, Cassidy 1996, Knight and Eden 1996, Anderson and Garner 1997, Murkies et al. 1998) are highly suggestive of beneficial effects of isoflavones on human health. The soybean, in particular, provides the most abundant source of isoflavones, and therefore most soy foods will provide a significant dietary source of these bioactive nonnutrients (Coward et al. 1993, Reinli and Block 1996).
The effect of isoflavone genistein, plant-derived compounds with estrogenic and antioxidant properties, on immunity is immune cell-dependent (Sakai and Kogiso 2008). Genistein suppresses antigen-specific immune response in vivo and lymphocyte proliferation response in vitro. However, genistein enhances the cytotoxic response mediated by NK and cytotoxic T cells and the cytokine production from T cells. Due to its unique effect on immune function, genistein has been used for the treatment of the diseases in animal models and it has been found that genistein inhibits allergic inflammatory responses (Sakai and Kogiso 2008). The immune system may be compromised after menopause because of the effects of aging and diminishing concentrations of estrogen, an immune-modulating hormone (Ryan-Borchers et al. 2006). Soy milk and supplemental isoflavones modulate B cell populations and appear to be protective against DNA damage in postmenopausal women and may offer immunologic benefits to women during this stage of life (Ryan-Borchers et al. 2006). Pretreatment with a phytoestrogen genistein has been shown to attenuate the development of pulmonary hypertension and prevent associated right heart failure (Matori et al. 2012). Ming et al. (2013) demonstrated functions and action mechanisms of flavonoids genistein and icariin in regulating bone remodeling and preventing bone loss. Genistein has dual functions on bone cells, able to inhibit bone resorption activity of osteoclasts and stimulate osteogenic differentiation and maturation of bone marrow stromal progenitor cells and osteoblasts (Ming et al. 2013).

However, studies of soy isoflavones in experimental animals suggest possible adverse effects as well (e.g. enhancement of reproductive organ cancer, modulation of endocrine function, anti-thyroid effects) (Doerge and Chang 2002). On the other hand, there are a number of components (protease inhibitors and lectins) present in soybeans that exert a negative impact on the nutritional quality of the protein (Liener 1994). Protease inhibitors exert their antinutritional effect by causing pancreatic hypertrophy/hyperplasia, which ultimately results in an inhibition of growth. The lectin, by virtue of its ability to bind to glycoprotein receptors on the epithelial cells lining the intestinal mucosa, inhibits growth by interfering with the absorption of nutrients. Of lesser significance are the antinutritional effects produced by relatively heat stable factors, such as goitrogens, tannins, phytoestrogens, flatus-producing oligosaccharides, phytate, and saponins. Other diverse but ill-defined factors appear to increase the requirements for vitamins A, B₁₂, D, and E (Liener 1994). Soy effects on the thyroid involve the critical relationship between iodine status and thyroid function (Doerge and Sheehan 2002). Divi et al. (1997) were observed that an acidic methanolic extract of soybeans contains compounds that inhibit thyroid peroxidase (TPO) catalyzed reactions essential to thyroid hormone synthesis. the components responsible for inhibition of TPO-catalyzed reactions coeluted with daidzein and genistein. In the presence of iodide ion, genistein and daidzein blocked TPO-catalyzed tyrosine iodination by acting as alternate substrates, yielding mono-, di-, and triiodoisoflavones. Genistein also inhibited thyroxine synthesis using iodinated casein or human goiter thyroglobulin as substrates for the coupling reaction (Divi et al. 1997).

The aim of our work was to study the impact of soybean feeding on the antioxidant defense and biochemical parameters in the blood of female rats (Rattus spp.) and their offspring, as well as reproductive ability. Dynamic analysis of these parameters on two generations of rats in combination with estimates of changes in growth also have been assessed.
MATERIAL AND METHODS

Animals and experimental design. Experiment performed with two generations of rats: females parental (F0) and first generation (F1). The study was carried out in the Institute of Animal Biology National Academy of Agrarian Sciences of Ukraine (Lviv, Ukraine) in two stages according scheme (Fig. 2). All manipulations with animals were carried out according to European convention for the protection of vertebrate animals used for experiments and other scientific purposes (1985).

Fig. 2. Experiment’s scheme

Soybean before feeding had heat treatment at a temperature of 140°C for 2 h for disposal anti nutritive substances and the reduce urease activity. Diets of all groups of animals comply with the requirements and accepted standards. After 40 days of feeding soybean, females of all groups were paired, and then held control of their clinical status, progress and duration of pregnancy.

After 23-25 days, 6 females from each group were subjected to ether anesthesia and decapitation on the last period of pregnancy. From each female were selected blood samples for laboratory tests, to determine the number of fetuses and their morphometric parameters (weight, size, physiological and anatomical characteristics).

The remaining females were submitted so that they gave offspring for control postnatal development (number, viability, growth and development offspring) and researches in the second generation. After weaning, offspring (n = 8) of each two groups were fed the same diet during experiment. At 4 months aged, 4 young females from each group were decapitated and sampled of blood for physiological and biochemical assays.

Reproductive ability of females was assessed by postnatal developing of the offspring F0, viability and survival of the offspring. During the first month of life according to the number of alive and stillborn offspring, the dynamics of morphometric parameters, the general physiological and postnatal development were also assessed.
Blood samples. Blood samples were collected in K-EDTA tubes after decapitation. After centrifugation, plasma samples were frozen at -20°C and stored until analysis. For isolation of erythrocytes, blood samples were transferred to tubes with potassium ethylenediaminetetraacetic (K-EDTA), and held on ice until centrifugation at 3,000 g for 10 min. The plasma was removed and stored at 4°C; the erythrocytes were washed three times with five volumes of saline solution and centrifuged at 3,000 for 10 min.

Biochemical analysis. Thiobarbituric acid (TBA), oxidized and reduced glutathione (GSSG and GSH), NADPH, and 5,5-dithiobis-2-nitrobenzoic acid, ethylenediaminetetraacetic acid (EDTA), thrichloroacetic acid (TCA), hydrogen peroxide, ammonium molybdate, sodium aside, t-butylhydroperoxide, ammonium iron (II) sulfate, ammonium thiocyanate, phosphowolframic acid, nitro-blue tetrazolium (NBT) were of analytical grade. All enzymatic assays were carried out at 22±1°C using a Specol 220 spectrophotometer. The enzymatic reactions were started by adding the blood or plasma. The specific assay conditions are presented subsequently. Each sample was analyzed in duplicate. The protein concentration in each sample was determined according to Bradford (1976) using bovine serum albumin as a standard.

Lipid hydroperoxides (LHP) assay. The method was described by Mironchyk (1982). To 0.2 ml of plasma sample were added 2.8 ml ethanol and 0.05 ml 50% TCA and vortexed vigorously. The mixture was centrifuged at 1,000 g for 10 min. The resulting supernatant was resuspended in ethanol, 1% ammonium iron (II) sulfate (Mohr’s Salt), and 20% ammonium thiocyanate. The reaction was initiated by adding 20% ammonium thiocyanate. Absorbance at 480 nm was measured during 10 min. The lipid hydroperoxides level was expressed as E per ml.

2-Thiobarbituric acid reactive substances (TBARS) assay. The level of lipid peroxidation was determined by quantifying the concentration of 2-thiobarbituric acid reacting substances (TBARS) with the Korobeynikova (1989) method for determining the malondialdehyde (MDA) concentration. Briefly, 0.5 ml of plasma was added to 5 ml of 20% phosphowolframic acid, vortexed, and incubated at -4°C for a minimum of 15 min. The mixture was centrifuged at 3,000 g for 10 minutes. The sedimentation was mixed with 1 ml of 2-thiobarbituric acid reagent. The mixture was heated in a boiling water bath for 60 minutes at which the color development was virtually completed. After cooling, the mixture was centrifuged at 3,000 g for 10 minutes. The composition of MDA was monitored at 535 and 580 nm. The nmol of MDA (malondialdehyde) per ml of blood was calculated by using $1.56 \cdot 10^5 \text{ mm}^{-1} \text{ cm}^{-1}$ as extinction coefficient.

Superoxide dismutase activity assay. Superoxide dismutase (SOD, E.C. 1.15.1.1) activity was assayed spectrophotometrically as the inhibition of photochemical reduction of nitro-blue tetrazolium (NBT) at 540 nm by Dubinina et al. (1983) method. The reaction mixture with no enzyme developed maximum color due to maximum reduction of NBT. A non-irradiated reaction mixture did not develop color and served as the control. The reduction of NBT was inversely proportional to the SOD activity. One unit for SOD activity was expressed as the enzyme protein amount causing 50 percent inhibition in NBT reduction rate. Activity is expressed in units of SOD per mg of protein.
Catalase activity assay. Catalase (CAT, E.C. 1.11.1.6) activity was determined by measuring the decrease of H$_2$O$_2$ in the reaction mixture using a spectrophotometer at the wavelength of 410 nm by the method of Koroliuk et al. (1988). The reaction was initialized by adding 0.1 ml of plasma into the incubation medium (2 ml of 0.03% solution of H$_2$O$_2$). The duration of this reaction was 10 min at room temperature. The reaction was terminated by rapid adding of 1.0 ml of 4% ammonium molybdate solution in 12.5 mm H$_2$SO$_4$ and 1 ml 125 mm H$_2$SO$_4$. Blank assay instead of plasma included 0.1 ml of distilled water. All samples were centrifuged at 3,000 g for 5 min. The absorbance of the obtained solution was measured at 410 nm and was compared with that of the blank. One unit of catalase activity is defined as the amount of enzyme required for decomposition of 1 mmol H$_2$O$_2$ per min per mg of protein.

Glutathione peroxidase activity assay. Glutathione peroxidase (GPx, E.C. 1.11.1.9) activity in the blood was measured spectrophotometrically as described by Moin (1986). The assay mixture contained 0.8 ml of 0.1 M Tris-HCl with 6 mm EDTA and 12 mm sodium aside (pH 8.9), 0.1 ml of 4.8 mm GSH, 0.2 ml of plasma sample (1:20), 1 ml of 20 mm t-butylhydroperoxide, and 0.1 ml of 0.01 m 5,5-dithiobis-2-nitrobenzoic acid. The rate of GSH reduction was followed spectrophotometrically at 412 nm. GPx activity is expressed as nmol GSH per min per mg of protein.

Glutathione level assay. The method of Woodward and Fray was used for the determination of glutathione in the blood (Travina 1995). This titrimetric method involves the oxidation of sulfhydryl groups with potassium iodate using a starch indicator. Glutathione total, glutathione reduced and oxidized was measured and its levels were expressed as mg per l.

Phagocytic activity of neutrophils assay. Phagocytosis is a main mechanism of natural resistance as a main element of induction and formation of specific immune responses. Assessment of phagocytic activity of the blood was performed by the Gosteeva method (Chumachenko et al. 1999). As test-culture of microorganisms, inactive daily culture of laboratory strain of E. coli (1033 F41, S form) was used. Phagocytic reaction of neutrophils evaluated by phagocytic activity (PA), number (PN), and phagocytosis index (PI). Briefly, 0.2 ml of blood was added in a test tube to standardized $2 \times 10^6$ per ml suspension of daily culture E. coli. Content of tubes was well shaked and placed in a water bath at a temperature of 37°C for 30 minutes. Samples were dried on the air and painted by the method of the Romanovski-Giemsia. In each of the samples counted 100 neutrophils. For complete description of phagocytosis, phagocytic activity by the number of active leucocytes from 100 of counted were determined and expressed as a percentage.

Alanine (ALT) and aspartate aminotransferase (AST) activity assay. ALT and AST activity will be analyzed spectrophotometrically by standard enzymatic method (Reitman and Frankel 1957). The ketoacids produced by the enzyme action reacts with 2,4-dinitrophenylhydrazine producing hydrazone complex measured colorimetrically at 530 nm. ALT and AST activities were expressed as mmol pyruvate per h per l of blood (µkat per l).

Determination of reproductive ability. Viability and survival of the offspring reproductive function in female rats by the postnatal development of the offspring of F0 was evaluated. Postnatal development during the first month of life according to
the number of alive and stillborn offspring, the dynamics of morphometric parameters, the general physiological development was also assessed.

**Statistical analysis.** Results are expressed as mean ± S.E.M. All variables were tested for normal distribution using the Kolmogorov-Smirnov test (p > 0.05). In order to find significant differences (significance level, p < 0.05) between values of control and experimental groups, paired samples by the Mann-Whitney U test was applied to the data (Zar 1999). All statistical analyses were performed using Statistica 10.0 software (StatSoft, Poland).

**RESULTS AND DISCUSSION**

The activities of catalase, superoxide dismutase and glutathione peroxidase was decreased by 20.5%, 17.1% and 26.9%, respectively in the blood of experimental animals compared to control group (Figs 3A, B, and C), whilst the activities of these enzymes in the blood of F1 generation from experimental group were increased, compared with control group.
Todaka et al. (2005) study demonstrates placental transfer of phytoestrogens from mother to fetus. The goal of their study was to investigate fetal exposure to phytoestrogens, estrogenic compounds derived from plants, by measuring serum concentrations of phytoestrogens in maternal and cord blood. This study included 51 mothers scheduled for cesarean section, to obtain the serum of mother and fetus at almost the same time. Serum concentrations of phytoestrogens, including genistein, daidzein, coumestrol, equol, and sulfate-conjugated genistein, were measured in maternal and cord blood samples. It is suggested that the metabolic and/or excretion rates of phytoestrogens are different between mother and fetus and once phytoestrogens are transferred to the fetus, they tend to stay in the fetal side longer than in the maternal side (Todaka et al. 2005). Nagata et al. (2006) also examined the hypothesis that maternal soy intake may be inversely associated with pregnancy hormone levels. The concentrations of hormones (estradiol, estriol, and testosterone) and isoflavones (genistein, daidzein, and equol) were measured in the maternal urine and serum, and umbilical cord blood of 194 women during pregnancy and at delivery. Soy intake during pregnancy was assessed by 5-day diet records at approximately the 25th week of pregnancy. High correlations were observed for isoflavone levels between maternal samples and umbilical cord blood, indicating that isoflavone can be transferred from the maternal to the fetal compartment. There were a few significant associations between maternal hormone levels and isoflavone measures during pregnancy, but their patterns of associations varied by gestational week and differed depending on whether isoflavone exposure was measured by diet records, urine or serum (Nagata et al. 2006).
In the US, typical diets are low in soy products, and the fetus is thus hypothesized to be exposed to low levels of genistein. In Asian cultures consuming soy products, the fetus is exposed to genistein as a result of maternal soy product intake, yet little or no toxicity is reported. Pregnant women are advised to avoid the use of genistein/genistein-containing supplements pending long-term safety studies (Physicians’ Desk Reference (PDR) Health, Genistein). Our results demonstrated that soy diet had different effect on blood antioxidant defenses of females and their offspring (Fig. 3). Accordingly, reducing the intensity of accumulation products peroxide oxidation in rat’s blood group II F1 generation was noted (Figs 4A and B).

Fig. 4. Impact of soybean feeding on the lipid hydroperoxides (A) and 2-thiobarbituric acid reacting substances (TBARS, B) in the blood of female rats (n = 12) and their offspring (n = 12). For legend * and ** – see fig. 3
Such changes in the blood of females indicate that the components of soybeans, which have antioxidant properties, apparently prevent the intensification of oxidative processes exactly in the prolonged effect that was found in offspring of F0 generation.

Except phytoestrogens possessing antioxidant properties, some activity of phytic acid in soy even after heat treatment is remain. Phytic acid is a potent inhibitor of native and fortification iron absorption (Hurrell 2004). It is a chelating agent, which indicates its ability to attach metal atoms. This metal binding is essential for the action of antioxidants. Phytic acid readily binds iron atoms. When iron is exposed to oxygen, may form free radicals, which are potentially harmful to DNA. Phytic acid bind iron atoms, preventing their interaction with atoms of oxygen and thus protects cells from damages (Halliwell 1994).

Researches demonstrated that genistein has antioxidant properties and antiproliferative effects may be responsible for anticarcinogenic effect. Its high content in soybeans and relatively high bioavailability favor genistein as a promising candidate for the prevention against human cancers (Wei et al. 1995). Genistein displays a wide array of biological activities, but it is best known for its ability to inhibit cancer progression, especially for hormone-related ones such as breast cancer. Genistein has been shown to bind both the estrogen receptor alpha (ERα) and the estrogen receptor beta (ERβ), although it has a higher affinity for the ERβ. The ERα/ERβ ratio is a prognostic marker for breast tumors, and ERβ expression could indicate the presence of tumors more benign in state, whereas ERα indicates malignant tumors. Nadal-Serrano et al. (2013) investigate the effects of genistein on oxidative stress and mitochondrial functionality through its interaction with the estrogen receptor in breast cancer cell lines with different ERα/ERβ ratios. The lower ERα/ERβ ratio T47D cell line showed lower oxidative stress and greater mitochondrial functionality, along with an up-regulation of uncoupling protein 2 and sirtuins. Their results show different genistein effects depending on ERα/ERβ ratio for oxidative stress regulation, mitochondrial functionality, and modulation of UCPs, antioxidant enzymes and sirtuins in breast cancer cell lines (Nadal-Serrano et al. 2013). Genistein supplementation also decreases oxidative stress markers and increases antioxidant activity in brain tissues of rats (Evseen et al. 2013).

Activity of alanine and aspartate aminotransferases in the blood is very important diagnostic feature of many diseases. The increase of these enzymes activity in the blood led recognize pathological conditions involving violation protein metabolism and tissue metabolism in the liver. Particularly informative determining ALT activity for early diagnosis of metabolic disorders in the liver, since the activity of serum ALT starts to increase already in the prodromal stage of the disease such violations, when other signs are not detected (Henderson 1986).

The activity of aminotransferases remained at physiological levels indicating about the normal course of metabolic processes in liver after adding soy to the diet (Figs 5A and B). In the blood of female rats of the parental generation, nothing destructive changes no pathological changes aminotransferases activity were found. Thus, in the second group of rats from F1 generation, decrease of ALT and AST activity was noted (by 4% and 9%, respectively) (Fig. 5).
Fan et al. (2013) investigated genistein’s influence on the relationship between the activation of uridine diphosphate glucuronosyltransferase (UGTs) and the protection against acetaminophen-induced liver toxicity. Animal experimental results revealed that genistein (50, 100 or 200mg/BW kg) significantly ameliorated the biomarkers alanine aminotransferase, alanine aminotransferase, lactate dehydrogenase and malondialdehyde, as indicators of acute liver damage caused by APAP (200mg/BW kg). Their results suggest that genistein can prevent and protect against APAP-induced liver toxicity due to the inhibition of APAP biotransformation and the resistance to oxidative stress via the modulation of the activities of metabolism and the antioxidant enzyme (Fan et al. 2013).

Huang et al. (2013) also examined the effect of genistein isolated from Hydrocotyle sibthorpioides on chronic alcohol-induced hepatic injury (underwent intragastric administration of alcohol (5.0-9.5 g/kg) once a day for 24 weeks) and fibrosis. A subset of rats were also intragastrically treated with genistein (0.5, 1 or 2 mg/kg) once a day. Genistein exerts a preventative effect to ameliorate developing liver injury and even liver fibrosis induced by chronic alcohol administration in rats. Genistein significantly decreased the plasma alcohol concentration, inhibited the activities of alanine and aspartate aminotransferases and decreased levels of inflammatory mediators, including interleukin 6, tumor necrosis factor-α and myeloperoxidase, via downregulation of nuclear factor-κB, effectively inhibited collagen deposition and reduced pathological tissue damage as determined by hepatic fibrosis biomarkers, such as total hyaluronic acid, laminin, and type III collagen, markedly reduced lipid peroxidation, recruited the anti-oxidative defense system, inhibited CYP2E1 activity, promoted extracellular matrix degradation by modulating the levels of tissue inhibitor of matrix metalloproteinase-1 and matrix metalloproteinase-2, induced HSC apoptosis by downregulating B-cell lymphoma 2 mRNA, and inhibited the expression of α-smooth muscle actin and transforming growth factor β(1) proteins (Huang et al. 2013).

Reactive oxygen and nitrogen species (ROS-RNS) and other redox active molecules fulfill key functions in immunity. Beside the initiation of cytoidal reactions within the pathogen defense strategy, redox reactions trigger and shape the immune response and are further involved in termination and initialization of cellular restorative processes.
Regulatory mechanisms provided by redox-activated signaling events guarantee the correct spatial and temporal proceeding of immunological processes, and continued imbalances in redox homeostasis lead to crucial failures of control mechanisms, thus promoting the development of pathological conditions (Gostner et al. 2013). Cordle et al. (2002) examined immune cell populations of infants fed soy protein isolate formulas with and without added nucleotides for 1 year. Newborn, term infants studied in a masked 12-month feeding trial were assigned randomly to soy formula groups with and without added nucleotides (n = 94, n = 92). A nonrandomized human milk/formula-fed cohort (n = 81), was concurrently enrolled. Blood samples were collected at 6, 7, and 12 months. Thirty-two immune cell populations were characterized using three-color flow cytometry. Cellular markers were chosen to assess general pediatric immune status, emphasizing maturation and activation of B, T, and NK lymphocytes. All cell populations, number and percentages, were within age-related normal ranges. The only significant difference found between soy formula and human milk/formula-fed infants was the percentage of CD57 + NK T cells at 12 months (human milk/formula > soy formula, P = 0.034). There were significant differences at some time points between human milk/formula-fed and nucleotide-supplemented soy formula-fed infants in populations of lymphocytes, eosinophils, total T, helper T, naive helper, memory/effector helper, CD57 – T, and CD11b + CD8 + NK cells. None of the cell populations differed between infants fed soy formula versus soy plus nucleotides. Their results suggest that infants fed this commercial soy formula demonstrated immune cell status similar to human milk/formula-fed infants, consistent with normal immune system development. The addition of nucleotides to soy formula did not significantly change specific individual immune cell populations but tended to increase numbers and percentages of T cells and decreased numbers and percentages of NK cells (Cordle et al. 2002).

Intergroup differences in indexes of immunobiological status of the organism of female rats of both generations was found. In particular, in the blood of F0 animals tendency to increase index of phagocytosis and phagocytic number (by 2% and 19.8%, respectively) was observed. However, in the blood of rat from F1 generation growth phagocytic immunity was similar to other indicators, which indicate about prolonged effect of soy components (Figs 6A and B).
Researchers proposed mechanisms of phytoestrogen’s action. Cooke et al. (2006) reported that genistein induces thymic atrophy in mice, and decreases both humoral and cell-mediated immunity. These thymic effects of genistein occur via estrogen receptor (ER)-mediated and non-ER-mediated pathways. Genistein injections produced the most pronounced effects, but dietary administration to mice that produced serum genistein concentrations similar to those reported in human infants consuming soy formula also had demonstrable effects. Microarray analysis of the effects of estradiol and genistein on neonatal thymus indicated that estradiol affected genes involved in transcription, apoptosis, cell cycle, and thymic development and function;
genistein had similar effects on many estradiol target genes, but also had unique actions not replicated by estradiol. Despite extensive work showing inhibitory effects of genistein on immunity, other rodent studies reported that genistein or other phytoestrogens stimulate various aspects of immune function (Cooke et al. 2006). Our results are in agreement with previous studies and indicate that prolonged soy diet stimulate phagocytic immune system (Fig. 6).

To assess the postnatal development of animals counted the number of live and stillborn fetuses, to control vitality, physiological condition and live weight of rat’s infant during the first two months of life. Typically, the number of offspring in the experimental and control groups were in the range of physiological norm; dead animals among newborns was not found. Offspring from females of the control group was characterized by high viability and thus for the first 2 months of life, there were no deaths rats (Figs 7A and B), whilst the mortality rate of rats from experimental group during the first 5 days was 14.6% (Fig. 7A). White rats are characterized by relative variability of indices of reproductive capacity, but their offspring survival is high and amounts to 96-99%. Offspring mortality in the experimental group were in the first 5 days of life; it may indicate about a breach of embryonic development, and, as a result, the birth of physiologically weakened and less viable offspring.

Fig. 7. Impact of soybean feeding on the reproductive ability of rats – the viability of offspring (A) and dynamics of animal mass (B)

* The level of significance is set at p < 0.001 compared between reproductive ability of rats from control and experimental groups (paired samples by the Mann-Whitney U test)

No significant intergroup differences in weight of newborn and young rats were noted; all parameters were correspond to physiological norms for young rats at this age. However, the mean of weight of the rats aged 20 days from experimental group was lower by 1.7% (p > 0.05) in comparison to the control (Fig. 7B). All groups of rats aged 20 days were within the physiological norm. The mean of weight of the rats aged 2 months from experimental group was higher by 21.8% (p < 0.001) compared to the control (Fig. 7B). This increase in body weight of animals treated soy diet indicates a positive level of assimilation and high nutritive components of soy.

The study of the reproductive capacity of female rats fed diet consisting of soybean suggest a slight decrease in fertility in animals from experimental group. Be-
fore-implantation mortality was higher in females of the experimental group compared with controls, indicating about the influence of phytoestrogens on embryonic development, confirming studies by other authors (Jefferson et al. 2006, Marty et al. 2009, Hooper et al. 2009).

Postnatal mortality in the experimental group was higher than in the control group that was marked 100% fertility. This confirms the possibility of influence of phytoestrogens and other biologically active substances within soybeans, resulting in a violation of embryonic development and as a consequence of birth physiologically weak and no viable offspring.

The obtained results are in agreement with literature data of numerous authors. Prolonged fed of animals with phytoestrogens before pairing causes an increase in fetal loss. Jefferson et al. (2006) demonstrated that developmental exposure to genistein alters murine reproductive differentiation, resulting in abnormal ovarian development (multioocyte follicles) and uterine neoplasia later in life. Further, reproductive function was altered. Prolonged estrous cyclicity was observed following neonatal genistein treatment (0.5-50 mg/kg) on days 1-5 with dose- and age-related increase in severity. Fertility, determined at 2, 4, and 6 months, showed decreased numbers of genistein-treated females (0.5 or 5 mg/kg) delivering live pups and reduced numbers of pups. At 6 months, 60% of 0.5 mg/kg and 40% of 5 mg/kg groups delivered live pups compared to 100% of controls. At 2 months, half the mice treated with 25 mg/kg of genistein and none treated with 50 mg/kg delivered live pups, although half of the latter group showed signs of pregnancy with few small implantation sites. Ovarian function was disrupted in the low genistein-dosed mice with increased numbers of corpora lutea (CLs) compared to controls and increased ovulated oocytes following exogenous gonadotropins treatment. In contrast, mice treated with high genistein doses had decreased numbers of CLs; ovulation could be restored with exogenous gonadotropins. Thus, neonatal treatment with genistein at environmentally relevant doses caused adverse consequences on ovarian development and reproductive function (Jefferson et al. 2006). Jefferson et al. (2007) revealed that developmental exposure to genistein causes deleterious effects on the reproductive system. Oral exposure to genistin (25 mg/kg) increases uterine weight at 5 days of age similar to subcutaneous injection of genistein (20 mg/kg) suggesting that subcutaneous injection of genistein is a suitable model for oral exposure to genistin. Mice treated neonatally by subcutaneous injection of genistein (0.5-50 mg/kg) exhibit altered ovarian differentiation leading to multioocyte follicles (MOFs). Ovarian function and estrous cyclicity were disrupted in genistein treated mice with increasing severity over time. Reduced fertility was observed in mice treated with genistein (0.5, 5, or 25 mg/kg) and infertility was observed at 50 mg/kg. Females generated from genistein 25 mg/kg females bred to control males have increased MOFs suggesting these effects can be transmitted to subsequent generations. Thus, neonatal treatment with genistein at environmentally relevant doses caused adverse consequences on reproduction in adulthood (Jefferson et al. 2007). Cimafranca et al. (2010) also assessed acute and chronic effects of oral genistein administration in neonatal mice. Mouse pups were dosed orally with genistein in a soy formula-corn oil emulsion from Postnatal Day (PND) 1 to PND 5, then effects on reproductive and non-reproductive organs were assessed after dosing and during subsequent de-
velopment. Neonatal treatment resulted in changes both at the completion of dosing (PND 5) and in adult animals. At PND 5, neonatal genistein treatment caused increased relative uterine weight and down-regulation of progesterone receptor in uterine epithelia. Estrogenic effects of genistein were also seen in the neonatal ovary and thymus, which had an increase in the incidence of multioocyte follicles (MOFs) and a decrease in thymic weight relative to body weight, respectively. The increased incidence of MOFs persisted into adulthood for neonatally treated genistein females, and estrous cycle abnormalities were seen at 6 month of age despite normal fertility in these mice (Cimafranca et al. 2010).

Summarizing, our results demonstrate influence of prolonged effects of soy diet on female rats and their offspring. Soy diet provided decrease of antioxidant defenses and lipid peroxidation, activation of phagocytic activity in the blood of offspring. The decrease of livability of newborn offspring from soy-treated rats also was demonstrated. However since biological effects are dependent on many factors including dose, duration of use, protein binding affinity, individual metabolism and intrinsic estrogenic state, further clinical studies are necessary to determine the potential health effects of these compounds.

REFERENCES


European convention for the protection of vertebrate animals used for experiments and other scientific purposes. 1985. Coun. of Europe, Strasbourg.


SUMMARY

Soybeans are rich in isoflavones such as genistein, daidzein, and glycitein. These isoflavones are well-known antioxidants, chemopreventive and anti-inflammatory agents. Isoflavones are considered to be phytoestrogens because of their ability to bind to estrogen receptors (Masilamani et al. 2012). However, studies of soy isoflavones in experimental animals suggest possible adverse effects as well (e.g. enhancement of reproductive organ cancer, modulation of endocrine function, anti-thyroid effects) (Doerge and Chang 2002). On the other hand, there are a number of components (protease inhibitors and lectins) present in soybeans that exert a negative impact on the nutritional quality of the protein (Liener 1994). The aim of our study was to study the impact of soybean feeding on the antioxidant defense and biochemical parameters in the blood of female rats (Rattus spp.) and their offspring, as well as reproductive ability. Dynamic analysis of these parameters on two generations of rats in combination with estimates of changes in growth also have been assessed. Experiment performed with two generations of rats: females parental (F0) and first generation (F1). Soybean before feeding had heat treatment at a temperature of 140°C for 2 h for disposal anti nutritive substances and the reduce urease activity. Diets of all groups of animals comply with the requirements and accepted standards. After 40 days of feeding soybean, females of all groups were paired, and then held control of their clinical status, progress and duration of pregnancy. After 23-25 days, 6 females from each group were subjected to ether anesthesia and decapitation on the last period of pregnancy. From each female were selected blood samples for laboratory tests, to determine the number of fetuses and their morphometric parameters (weight, size, physiological and anatomical characteristics). The remaining females were submitted so that they gave offspring for control postnatal development (number, viability, growth and development offspring) and researches in the second generation. After weaning, offspring of each two groups were fed the same diet during experiment. At 4 months aged, 4 young females from each group were decapitated and sampled of blood for physiological and biochemical assays. Reproductive ability of females was assessed by postnatal developing of the offspring F0, viability and survival of the offspring. During the first month of life according to the number of alive and stillborn offspring, the dynamics of morphometric parameters, the general physiological and postnatal development were also assessed. Reducing the intensity of accumulation products peroxide oxidation in rat’s blood group II F1 generation was noted. Such changes in the blood of females indicate that the components of soybeans, which have antioxidant properties, apparently prevent the intensification of oxidative processes exactly in the prolonged effect that was found in offspring of F0 generation. In the blood of female rats of the parental generation, nothing destructive changes no pathological changes aminotransferases activity were found. Thus, in the second group of rats from F1 generation, decrease of ALT and
AST activity was noted (by 4% and 9%, respectively). Intergroup differences in indexes of immunological status of the organism of female rats of both generations was found. In particular, in the blood of F0 animals tendency to increase index of phagocytosis and phagocytic number (by 2% and 19.8%, respectively) was observed. However, in the blood of rat from F1 generation growth phagocytic immunity was similar to other indicators, which indicate about prolonged effect of soy components. The number of offspring in the experimental and control groups were in the range of physiological norm; dead animals among newborns was not found. Offspring from females of the control group was characterized by high viability and thus for the first 2 months of life, there were no deaths rats, whilst the mortality rate of rats from experimental group during the first 5 days was 14.6%. White rats are characterized by relative variability of indices of reproductive capacity, but their offspring survival is high and amounts to 96-99%. Offspring mortality in the experimental group were in the first 5 days of life; it may indicate about a breach of embryonic development, and, as a result, the birth of physiologically weakened and less viable offspring. Soy diet provided decrease of antioxidant defenses and lipid peroxidation, activation of phagocytic activity in the blood of offspring.