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CYTOTOXICITY SCREENING OF LOCALLY PRODUCED CHITOSAN FILM ON HUMAN DERMAL FIBROBLASTS.

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PURPOSE :
Chitosan has been proposed for biomedical applications because of its biocompatibility and abundance in nature. The chitosan film that was tested on has been locally processed as a chitosan derivative. Therefore, the biosafety of this chitosan product requires an in-vitro evaluation. The objective of this study is to assess the cytotoxicity of locally produced chitosan derivatives film on primary human dermal fibroblasts.

METHOD :
The evaluation of toxicity for the chitosan derivatives film is based on direct contact and indirect extraction approach. Cultured of primary human dermal fibroblast from consented donors was used for the evaluation. The assessment was done at 24, 48 and 72 hours post treatment. At the end of incubation period, chitosan products were removed and cell activity test was performed using qualitative and quantitative means.

RESULT :
Observation using phase contrast microscopy showed no marked morphological changes on O-C and N-CMC treated cells at initial incubation period. Cultures incubated with NO-CMC were graded as slightly changed which more than 20% of the cells are rounded, loosely attached and occasional lysed. Meanwhile, minimal cell damage was observed on O-CMC treated cells. Quantitative assessment revealed similar results. These signify that O-C and N-CMC product was not toxic to fibroblast cells at all time exposure, while NO-CMC showed toxicity effect after 48 and 72 hours exposure.

CONCLUSION :
O-C and N-CMC chitosan film was the materials of choice in this screening process, which shows the least toxic effect. This finding provides evidence of the promising role of locally produced chitosan derivative for usage as wound dressing material. Thus, further research on chitosan derivatives and its activities is required in utilizing this material in clinical setting

PB-40

STUDIES ON GENDER IDENTIFICATION OF THE MOUSE EMBRYO BY POLYMERASE CHAIN REACTION (PCR) AND ITS SUBSEQUENT IN VITRO *DEVELOPMENT*

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PURPOSE :
The study was conducted to identify the gender of eight-cell murine embryos from blastomere biopsies and to observe the effect of the biopsy procedure on subsequent *in vitro* development.

METHODS :
Blastomeres biopsied from eight-cell embryos were lysed and utilized in the PCR. Amplicons of Y- and X-specific sequences observed on agarose gels were then used as the basis for gender identification. Ninety three percent amplification efficiency was obtained when DNA from single blastomere was sexed. The accuracy of the method was found to be 96% when single blastomere results were compared with those of the matched blastocysts. The entire gender identification procedure was performed in less than six hours. The developmental capacity of the embryo after removal of one and two blastomeres was also studied.

RESULTS :
Survivability of embryos following biopsy was found to decline with advancing developmental stages. Removal of a single blastomere resulted in 96.1% morulae, 84.3% expanded blastocysts and 74.5% hatched blastocysts. Removal of two blastomeres resulted in 94.0% morulae, 72.0% expanded blastocyst and 44.0% hatched blastocysts. One- blastomere biopsies did not significantly reduce developmental capacity. However, two-blastomere biopsies significantly reduced the number of hatched blastocysts.

CONCLUSION :
PCR method was found to be efficient in identifying gender and the accompanying biopsy procedure produced high *in vitro* survivability.

PB-41

MICROBIOLOGICAL PATTERN OF LOWER RESPIRATORY TRACT INFECTIONS AMONGST CHILDREN IN A TEACHING HOSPITAL: A ONE-YEAR STUDY

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PURPOSE :
1. To determine the spectrum of bacteria in paediatric patients diagnosed with pneumonia.
2. To determine the antimicrobial susceptibility pattern of the organisms isolated.

METHODS :
i. Design: Laboratory-based study. Retrospective and prospective study over a one-year period from July 2002-June 2003. Respiratory samples (i.e., nasopharyngeal aspirates, tracheal aspirates and sputums) received from the paediatric wards with the diagnosis of pneumonia were reviewed from the microbiology data. *ii. Setting and patients:* Hospital Universiti Kebangsaan Malaysia. Respiratory samples from paediatric patients.

RESULTS :
A total of 4575 respiratory samples were received in the diagnostic microbiology laboratory. Only 148 (3.23%) met the criteria of the study of which 56.8% samples were from children aged less than 2 years old. Less than half of the suitable samples (43%) yielded positive for pathogens. *Pseudomonas aeruginosa (*17.1%), methicillin-resistant *Staphylococcus aureus* and *Acinetobacter* spp. (11.8% respectively) were the three most common organisms isolated. Interestingly, if a patient was infected by either of these organisms, it was found that involvement of the other two organisms was likely to occur and this was observed between 14-19% of the samples. *Haemophilus influenzae* and *Enterobacter spp.* were the two least encountered bacteria, each was isolated from two samples. *Streptococcus pneumoniae* which is the most common organism implicated in community-acquired pneumonia was not isolated in this study. All the *Pseudomonas aeruginosa* strains were susceptible to ceftazidime. The MRSA or methicillin resistant *Staphylococcus aureus* organisms revealed an expected pattern to the antibiotics tested and all were susceptible to vancomycin. *Acinetobacter spp.* were multi-resistant and 70% of the strains were resistant to Imipenem.

CONCLUSION :
Pseudomonas aeruginosa is the most common organism isolated amongst hospitalized paediatric patients diagnosed with pneumonia which presumedly were nosocomial in origin. Majority of the bacteria isolated showed a susceptible pattern to the first-line antimicrobial agents tested except *Acinetobacter* spp were multi-resistant.

PB-42

CYTOTOXICITY EVALUATION OF OLIGO-CHITOSAN AND N, O-*CARBOXYMETHYL CHITOSAN USING PRIMARY NORMAL HUMAN EPIDERMAL KERATINOCYTES AS AN* IN VITRO *TOXICOLOGY MODEL*

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INTRODUCTION :
Chitosan (b-1,4-D-glucosamine) is a deacetylated form of chitin with excellent biological properties in wound management. The natural properties of chitosan have the physical and chemical limitations to be broadly used in biomedical fields. The improvement of the physical and chemical properties of the chitosan with some additional chemicals will alter its biocompatibility. Thus, the biological attribute of the modified chitosan must be evaluated. Our purpose was to evaluate the cytotoxicity of oligo-chitosan (O-C) and *N, O*-carboxymethyl-chitosan (NO-CMC) derivatives (O-C 1%, O-C 5%, NO-CMC 1% and NO-CMC 5%) in the form of film using primary normal human epidermal keratinocytes (NHEK) cultures as an *in vitro* toxicology model.

METHODS :
Pieces of O-C and NO-CMC were assayed via the direct contact method on cultured NHEK. The 3-[4,5-dimethyl-2-thiazolyl]-2,5-diphenyl tetrazolium bromide (MTT) was used as the cell viability assay. Two types of references were used (control and negative control) for the treated NHEK cultures within 72 hours of treatment. The results obtained were graphed. Two comparisons were then made relative to the two references used.

RESULTS :
The O-C 1% resulted as the most compatible chitosan derivative since it was able to steadily sustain more than 70% of viable cells until 72 hours of treatment. This was followed by O-C 5%, NO-CMC 5% and NO-CMC 1%.

CONCLUSION :
O-C was an ideal chitosan derivative compared to NO-CMC.

PB-43

CYTOTOXICITY EVALUATION OF CHITOSAN POROUS SKIN REGENERATING TEMPLATES (PSRTS) USING COMMERCIAL AND PRIMARY NORMAL HUMAN DERMAL FIBROBLASTS: AN IN VITRO *TOXICOLOGY MODEL*

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INTRODUCTION :
Chitosan (b-1,4-D-glucosamine) is a biopolymer that comprises the copolymer mixture of *N*-acetylglucosamine and glucosamine based on the degree of deacetylation (DAA) of chitin. The natural biocompatibility and biodegradability of chitosan have brought its wide applications in the field of pharmaceutical especially in wound management. The chemical and physical availabilities of chitosan which are to be further modified into a variety of forms (*e.g.* film, paste, sheet and porous skin regenerating templates) will influence its biocompatibility and biodegradability to an uncertain degree on human. Our purpose was to pre-evaluate the cytotoxicity of chitosan derivatives in the form of porous skin regenerating templates (PSRTs) using primary normal human dermal fibroblasts (pNHDF) and commercial normal human dermal fibroblasts (cNHDF) cultures.

METHODS :
Six PSRTs were assayed on cultured primary normal human dermal fibroblasts (pNHDF) and commercial normal human dermal fibroblasts (cNHDF). Cultured cells were all standardized at the second passage for cytotoxicity assay using the direct contact method. The 3-[4,5-dimethyl-2-thiazolyl]-2,5-diphenyl tetrazolium bromide (MTT) was used as the colorimetric cell viability assay. Treated pNHDF and cNHDF cultures were referred to the control (cells and medium) and negative control in order to produce relative percentages of viable cells.

RESULTS :
Percentages of viable cells were depicted as graphs. Generally, there were no significant differences of cytotoxicity pre-evaluation between the treated pHNDF and cNHDF cultures. PSRT132 in bilayer form was the most compatible chitosan derivative as it contributed the most number of viable cells after 72 hours post-treatment. This was followed by PSRT88, PSRT87, PSRT82, PSRT89 and lastly PSRT86. All the PSRTs in this study showed a decreased in the number of viable cells during the 72 hours of post-treatment compared with the 48 hours post-treatment. The fact might be due to the lack of nutrients in the culture medium during the third day. Only PSRT89 and PSRT86 showed a continuously decreased number of viable cells in the 72 hours treatment in which PSRT86 appeared as the least compatible chitosan derivative among all.

CONCLUSION :
By using the final 24 hours of post-treatment as the level of cytotoxicity determination, PSRT132 appeared as the most compatible. This was followed by PSRT88, PSRT87, PSRT82, PSRT89 and PSRT86.

PB-44

IDENTIFICATION OF HAEMOGLOBIN AC HETEROZYGOTE IN A MALAY FAMILY : IS IT THE TIME TO DECIDE BETWEEN HAEMOGLOBIN ELECTROPHORESIS AND HPLC?

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INTRODUCTION :

Thalassaemia is a common public health problem among Malays. Haemoglobin C (Hb C) is a haemoglobin beta variant resulting from a single base mutation at the 6th position of the beta globin gene leading to the substitution of glycine for glutamic acid. A rare type of haemoglobinopathy, Hb C is commonly detected in West Africans and in American Africans but has not been reported in Malaysia. It can be falsely diagnosed as the HbE trait in The Malaysian Thalassaemia Screening Program which utilizes cellulose acetate haemoglobin electrophoresis.

CASE REPORT :

This is the first reported case of Hb AC heterozygote in a Malay family, with an unusual splenomegaly in one of the family members.

CONCLUSION :

In most hospitals in Malaysia, cellulose acetate Hb electrophoresis at an alkaline pH is used as a screening procedure for thalassaemia. Citrate agar in acid pH is only used in cases suspected to have sickle cell disease, whereas it may also enable the differentiation between Hb C from O-Arab, C-Harlem and E. HPLC is not routinely used in all hospitals. Hence, it is recommended that it should be introduced into routine practice.

PB-45

EVALUATION OF THE ALPHA OMEGA HIV RAPID TEST KIT FOR DETECTION OF HIV ANTIBODY IN SERA AND WHOLE BLOOD SAMPLES.

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PURPOSE :
The aim of this study was to evaluate and compare the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of Alpha Omega HIV Rapid Test Kit for the detection of HIV antibody between the sera and whole blood samples.

METHODS :
A total of 301blood samples were tested with the Alpha Omega HIV Rapid Test Kit which consisted of sera (n=213; 34 sera positive, 179 sera negative) and whole blood (n=88; 33 positive, 55 negative). Positive samples for both sera and whole blood were taken from consented HIV positive patients who were admitted to Hospital Universiti Sains Malaysia (HUSM) whereas negative samples were taken from consented normal healthy volunteers and hepatitis B positive patients. Out of 179 of the sera negative samples, 168 samples were taken from normal healthy volunteer and 11 from hepatitis B positive patients. Out of 55 whole blood negative samples, 44 were taken from normal healthy volunteers and 11 from hepatitis B positive patients. Sera samples were collected in plain bottles whereas whole blood samples were collected in EDTA bottles. Western Blot was used as a reference test for the positive samples whereas for the negative samples the reference test was MEIA Axsym.

RESULTS :
The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) for the sera samples was 91%, 100%, 100% and 98.4% respectively. For the whole blood samples the sensitivity, specificity, PPV and NPV was 91%, 100%, 100% and 94.8% respectively.

CONCLUSION :
The Alpha Omega Rapid Test Kit was found to give a similar result in sensitivity, specificity and positive predictive value for both types of samples, however the negative predictive value (NPV) for whole blood samples was lower compared to sera samples. Even though the specificity of the test kit for both types of samples was very high, its relatively low sensitivity renders it unsuitable for HIV screening purposes.

PB-46

THE ROLE OF IMMUNOPHENOTYPING IN ESTABLISHING A DIAGNOSIS OF ACUTE LEUKEMIA IN A HYPOPLASTIC OR FAILED MARROW

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INTRODUCTION :

Occasionally cases of acute leukemia preceded by aplastic anemia or presents as hypoplastic marrow. This scenario is rarely faced during the review of bone marrow aspiration which results in diagnostic difficulties. Also we have encountered difficulties in diagnosing when the bone marrow aspiration fails to yield proper marrow fragments and the immunophenotyping remains the only resort to make a diagnosis. Some time immunophenotyping is also not conclusive due to poor specimen and the only choice is to do repeat marrow aspiration.

***CASE REPORT* :**

In this case report series we have studied twenty three (23) diagnostic bone marrows from the period of 2001 till 2006 which were not suitable to make a definitive diagnosis on the basis of morphology. Most of these cases were suspected cases of either acute lymphoblastic leukemia or acute myeloblastic leukemia. Out of these 23 diagnostic marrows five were inconclusive even with immunophenotyping due to poor specimen while the rest of eighteen cases produced conclusive results based on which the definitive diagnosis was made. Only one case of AML was diagnosed while two cases of biphenotypic leukemia were diagnosed by IPT. Rest fifteen cases were diagnosed as ALL B and T lineage (12 & 3) respectively. This also goes with notion that ALL can results into failure of bone marrow aspiration.

CONCLUSION :

With these results we concluded that immunophenotyping is a very important diagnostic tool and aid to morphology for diagnosing acute leukemias.

PB-47

PREVALENCE OF ALPHA THALASSEMIA 1 (SOUTHEAST ASIA TYPE) IN HUSM

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PURPOSE :
The Southeast Asia (SEA) type deletion of alpha thalassemia 1 is the most common type of alpha thalassemia 1 in SEA countries including Malaysia. Affected heterozygous individuals are asymptomatic, thus left undiagnosed. These undiagnosed individuals will be potential carriers to more severe forms of alpha thalassemias, particularly Hydrops fetalis and HbH disease. Therefore, a preliminary study to determine the prevalence of the disease is very important to determine our future strategy in preventing this disease. The objectives of this study was to assess the prevalence of alpha thalassemia 1 (SEA type) among antenatal patients in HUSM.

METHOD :
This study was conducted on 113 peripheral blood samples taken from antenatal patients. Detection of alpha thalassemia 1 (SEA type) was performed by Polymerase Chain Reaction (PCR).

RESULT :
Alpha thalassemia 1 (SEA type) was detected in 12 (10.6%) out of 113 samples.

CONCLUSION :
The prevalence of alpha thalassemia 1 (SEA type) in Malaysia is common. This data alerted us towards the importance of formulation a strategy for the detection and prevention of these disorders in our country.

PB-48

MATERNAL AGE AND CYTOGENETIC PROFILE IN 128 DOWN SYNDROME PATIENTS- EXPERIENCE AT HUMAN GENOME CENTER, USM

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PURPOSE :
Down Syndrome (DS), a variable combination of congenital malformations caused by trisomy 21, is the most commonly recognized genetic cause of mental retardation. The risk of DS, is directly related to maternal age. The purpose of this study was to determine the cytogenetic profiles of Down Syndrome patients in correlation with maternal age, among referrals to the Human Genome Center, USM.

METHOD :
This report included 128 DS patients, clinically diagnosed by characteristic facial features, who were referred to our center, for cytogenetic study during 2001 to 2006. Maternal ages were available for 121 cases and were grouped into A (age below 35 years) and B (age above 35 years). For cytogenetic analysis, peripheral blood lymphocytes of the study subjects were cultured for 72 hours at 37°C, chromosome preparations were made using standard cytogenetic procedures, karyotypes prepared following International System for Human Cytogenetic Nomenclature (ISCN,1995).

RESULT :
Karyotype analysis of 128 patients revealed 95.3% as free trisomy 21 DS, 4.1% as mosaic trisomy 21 DS, and 0.78% as translocation DS. The finding of one case of free trisomy DS with an additional inversion 9q11q13 which had been inherited from the father, was an interesting observation. Among the 128 DS patients, 33.6% were born to group A mothers and 60.9% were born to group B mothers and for 7 DS patients, maternal age was not available. A significantly increased incidence of DS due to increased maternal age is observed in Malay population.

CONCLUSION :
Karyotyping is essential for diagnostic confirmation of DS, for determining the risk of recurrence and to provide a basis for genetic counseling. Although there is no way to prevent DS, when there is an increased risk because of a mother's age (35 years or older), perents may be counseled about prenatal screening and diagnostic tests.

PB-49

GENES EXPRESSION ANALYSIS OF OSTEOBLAST CELL LINE DIFFERENTIATED FROM MESENCHYMAL STEM CELL

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PURPOSE :
To detect the gene expression of specific genetic markers; osteopontin and runx2 genes from osteoblast derived mesenchymal stem cell.

METHOD :
Mesenchymal stem cell was treated with special growth media to differentiate it into osteoblast cell. The cell cultures treated were harvested on 0, 7th, 14th, 21st and 28th day. Preservation of the RNA in the cells was done using *RNAlater* (Ambion, USA). The cells were kept frozen at -80°C until used. The total RNA from the cultured cells was isolated using commercial RNA extraction kit (Invitrogen, USA). The quantity of RNA was determined using spectrophotometer. Using the same quantity of total RNA as the template, the genes were amplified using One Step RT-PCR kit (Qiagen, Germany). Expression of the genes was analysed quantitatively on an agarose gel. The expression of the genes were assesed based on the intensity of bands for different period of cell harvest.

RESULT :
In this study, changes were observed in the band intensities for the amplified region of the osteopontin and runx2 genes. The gene expressions were increasing and declining corresponding to growth media treatment.

CONCLUSION :
The gene expression profile reveals the successful differentiation of the osteoblast from the mesenchymal stem cell.

PB-50

PREVALENCE OF MYELOID ANTIGEN EXPRESSION IN ACUTE LYMPHOBLASTIC LEUKEMIA IN HUSM

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PURPOSE :
Acute lymphoblastic leukemia (ALL) is a malignant (clonal) disease of the bone marrow in which early lymphoid precursors proliferate and replace the normal hematopoietic cells of the marrow. Some recent reports indicate a high frequency of immunophenotypic aberrancy in ALL. The relations of myeloid antigen expression to other features of ALL and to prognosis have been controversial. Our objective was to evaluate the frequency of myeloid antigen expression in ALL

METHODOLOGY :
A retrospective analysis of 52 cases of ALL diagnosed at our institution between 2004 and 2005 was performed. Immunophenotypic analysis was performed on leukemic cells from 52 patients diagnosed as ALL by the FAB (French-American-British) criteria using a comprehensive panel of monoclonal antibodies to lymphoid and myeloid associated antigens by flow cytometry.

RESULTS :
Approximately 64.5% were B-precursor ALL, 31.2% was T-ALL and 4.2% expressed both B and T cell markers. Expression of myeloid antigens was found in 26.9% of the cases, including 7 out of 18 adults (38.9%) and 7 out of 34 children (20.6%). CD13 was positive in 8 patients and CD33 was positive in 5 patients. One patient expressed both CD13 and CD33.

CONCLUSION :
In this study, myeloid antigen expression was present in 26.9% of the cases with CD13 expression being the commonest. The frequency of myeloid expression was higher in adult ALL compared to childhood ALL.

Keywords*: myeloid antigen, acute lymphoblastic leukemia*

PB-51

CRI DU CHAT SYNDROME: ATYPICAL PRESENTATION

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INTRODUCTION :

The Cri du Chat Syndrome (CdCS) is microdeletion syndromes involving the short arm or a segment of chromosome 5 (5p) with a prevalence ranging from approximately 1in15,000 to 1in 50,000 live birth .The hallmark cat-like-cry is a core feature of CdCS and is still regarded as an important early clinical diagnostic feature. Although CdCS is clinically well defined, variation in the clinical features of the syndrome has been reported relating to the size and location of the deleted region within 5p.Here we report a case of Cri du chat syndrome that was presented with atypical clinical features.

CASE REPORT :

A 7 months old, full term Malay boy a first born of non consanguineous parents presented with persistant stidor since birth. Clinical examination revealed multiple syndromic features such as triangular facies, flat occiput, low set ears, hypertelorism and hypotonia. Cytogenetic analysis was carried out at HGC, employing microculture of the peripheral blood lymphocytes of the proband at 37° C for 72 hours. Karyotyping showed a 46,XY,del (5)(p13-15) pattern, which was consistent with a diagnosis of CdCS. Cytogenetic analysis of the parents showed normal karyotype pattern.

Among the patients diagnosed with CdCS, a variation in clinical signs as well as in the size and location (within 5p) of the deleted fragment has been reported. The cat-like cry is mapped to 5p15.3 while remaining features of CdCS are mapped to 5p15.2.In most cases of CdCS, the clinical features,in particular the distinct facial phenotype in combination with the typical cat-like-cry and hypotonia allows to suspect the diagnosis at birth. In the present case, the typical high pitched cry was not prominent. Instead, the patient manifested persistant stidor, which might have blinded the clinician in suspecting the diagnosis of CdCS at birth.

CONCLUSION :

Clinical examination combined with cytogenetic analysis of the deletion maybe of great diagnostic relevance and may aid in a more accurate diagnosis.

PB-52

COMPARISON BETWEEN THE EFFECTS OF TWO DIFFERENT DOSES OF SMILAX MYOSOTIFLORA *(UBI JAGA) RHIZOME ON TESTICULAR 11_-HYDROXYSTEROID DEHYDROGENASE OXIDATIVE ACTIVITY AND PLASMA HORMONAL LEVELS IN CORTICOSTERONE-TREATED NORMAL RATS*

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PURPOSE :
Smilax myosotiflora (Ubi Jaga) rhizome is traditionally used by the indigenous community for its aphrodisiac effects. This study aims to compare between the effects of 8 mg and 16 mg/kg BW of *S. myosotiflora* (UJ) rhizome on testicular 11_-HSD O/A, plasma testosterone and estradiol levels in B-treated normal rats.

METHODS :
Mature Wistar rats were injected intramuscularly with 2.4 mg/kg of B either alone (B group) or in combination with 8 mg or 16 mg/kg BW of *S. myosotiflora* (B+UJ8 or B+UJ16 groups) orally, for 7 consecutive days. Testicular 11_-HSD O/A was expressed as percentage conversion of B to 11-dehydro-B, while plasma hormonal levels were measured using radioimmunoassay kits.

RESULTS :
S. myosotiflora increased testicular 11_-HSD O/A in both B+UJ8 and B+UJ16 groups ($P<0.001$ respectively) compared to B group, towards normal control levels. Similarly, *S. myosotiflora* increased plasma testosterone levels in both B+UJ8 and B+UJ16 groups ($P<0.05$ respectively) compared to B group. Conversely, *S. myosotiflora* decreased plasma estradiol levels in both B+UJ8 ($P<0.001$) and B+UJ16 ($P<0.01$) groups compared to B group. In the B+UJ8 group, testosterone levels were higher ($P<0.01$), while estradiol levels were lower ($P<0.05$) than normal control levels. However, in the B+UJ16 group, both testosterone and estradiol levels did not differ significantly from that of normal controls.

CONCLUSION :
In conclusion, both 8 mg and 16 mg/kg BW of *S. myosotiflora* could counteract the effects of B on testicular 11_-HSD O/A, plasma testosterone and estradiol levels in normal rats. When glucocorticoid levels were increased in normal rats, *S. myosotiflora* at a lower dose of 8 mg/kg BW gave a more marked effect on plasma testosterone and estradiol levels compared to 16 mg/kg BW of *S. myosotiflora*. These results are contrary to that of rats under normal conditions, whereby *S. myosotiflora* at a higher dose of 16 mg/kg BW gave a better effect on plasma testosterone and estradiol levels compared to 8 mg/kg BW of *S. myosotiflora*, as was reported in our prior study.