Tuesday, July 29, 2014

Poster Session: 9:30 AM - 5:00 PM

Animal Clinical Chemistry

A-428

Estimated complete blood count (CBC) reference ranges for aged adult male rhesus monkeys (Macaca mulatta) as measured on the Beckman Coulter HmX analyzer

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Introduction: The clinical laboratory was asked by veterinary services to provide CBC's for routine checkups on a small number of aged adult male rhesus monkeys. As we had no reference values for this population, results were analyzed as if from a control group representing a well population. We report these results obtained using the Beckman HmX analyzer, and compare reference range estimates to available literature.

Methods: Whole blood K-EDTA samples were collected from 12 male rhesus monkeys (age: 23.1 ± 1.3 years; age range: 21 to 25 years). CBC analysis was performed within 3 hours of collection using the Beckman Coulter HmX analyzer. Results were analyzed according to expectation of a normal distribution by correlation of data with the normal distribution predicted by the calculated mean (x) and standard deviation (s). Outliers were defined as samples deviating from x by more than 3s when excluded from the group. Parametric reference ranges were defined by convention as the central 95% of results (x-2s to x+2s).

Results: shown in Table. Despite low sample number, distributions for all CBC component tests were consistent with normal distributions (expected r^2 for n= 12: r^2>0.9). Calculated uncertainty in the width of the estimated reference ranges was ±20% for n=12. Mean values for WBC, MCV, MCHC and platelets were substantially different from literature data (|z|>2). These differences may be due to the substantially advanced age of the animals (life expectancy of 4 years in the wild).

Conclusions: Reference ranges for CBC component tests using the Beckman Coulter HmX analyzer were estimated based on a small dataset for an aged adult population of male rhesus monkeys. Datasets for each test were well characterized as normal distributions. Estimated reference ranges for four CBC component tests (WBC, MCV, MCHC and platelets) for this population differed substantially from available literature.

A. Test	B. UNITS	C. RR	D. n	E. r^2	F. LIT RR	G. z
WBC	10^9/L	2.8-7.4	11	0.970	12.5-18.9	* -6.63
RBC	10^12/L	4.7-6.4	12	0.940	4.9-5.8	0.98
Hb	g/dL	11.9-14.8	12	0.925	11.8-13.8	1.15
HCT	%	36.0-46.9	12	0.958	39.1-44.9	-0.38
MCV	fL	67.8-80.8	12	0.981	75.0-82.2	* -2.39
MCH	pg	21.6-26.2	12	0.911	22.9-25.1	-0.18
MCHC	g/dL	31.0-33.4	12	0.965	29.6-31.4	* 3.86
RDW	%	11.5-14.4	12	0.912	12.3-13.7	-0.15
PLT	10^9/L	118-317	11	0.954	287-431	* -3.95

A. CBC Component Test
C. Parametric reference range (RR)from data
F. Literature reference range (LIT RR): Chen et al., Xenotransplantation 2009;16:496-501
G. z value of data mean relative to mean and standard deviation of LIT RR; *|z|>2.0

A-430

Modulation of the inflammatory response induced by carragenan in a murine model by Effect of Jungia sellowii Less.

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Background: Jungia sellowii Less. is a native plant from Brazil used in traditional medicine to treat inflammatory diseases.

Objective: The aim of this study was to evaluate the anti-inflammatory effect of the crude extract(CE) from Jungia sellowii Less. its derived aqueous fraction(Aq), and isolated compounds, succinic acid(SA) and lactic acid(LA) on leukocytes, exudation, myeloperoxidase(MPO) and adenosine-deaminase(ADA) activities and nitric oxide(NOx), interleukin-1β(IL-1β), tumor necrosis factor-α(TNF-α) and interleukin-17A(IL-17A) levels, using a murine model of pleurisy induced by carrageenan(Cg,1%).

Methodology: Fresh Jungia sellowii Less leaves were extracted with ethanol/water to obtain the CE, which was partitioned with solvents of increasing polarity, yielding a residual Aq fraction. The compounds, SA and LA, were isolated from this fraction and their structures were determined by nuclear magnetic resonance(1H NMR). Swiss mice were used throughout the experiments (Brit.J.Pharmacol.183.811-19.1996). The study was approved by Committee for Ethics in Animal Research of Federal University of Santa catarina (protocol: PP00757). Different groups of animals (n=5) were treated with CE(10-50mg/kg), Aq fraction(1-25mg/kg), SA(0.5-2.5mg/kg) or LA(0.5-2.5mg/kg) administered by intraperitoneal route, 0.5h prior to the intrapleural injection of Cg to analyze the effect of the herb on leukocytes and exudation. A group of animals received a gingival injection of Evans blue dye(25mg/kg) 10min before herb treatment to evaluate the exudation. The Evans blue dye was measured by colorimetric assay on enzimaimmunoassay (ELISA) plate reader. The leukocytes were determined on veterinary automatic counter. Other groups of animals were pretreated (0.5h) with CE(25mg/kg), Aq(5mg/kg), SA(1mg/kg) or LA(1mg/kg) to evaluate the effect of the herb on MPO and ADA activities, NOx , IL-1β, TNF-α, and IL-17A levels. The MPO and ADA, and NOx, were analysed in accordance with methods described by Giusti and Galanti, 1984; Rao et al., 1993, and Green et al., 1982, respectively. The IL-1β, TNF-α, and IL-17A levels, were determined using commercially available ELISA kits. All the inflammatory parameters were analyzed after 4h of pleurisy induction. Statistical differences between groups were determined by ANOVA complemented by Newman-Keuls test. Values of $p<0.05$ were considered significant.

Results: The herb inhibited leukocytes (CE:42.8±2.9% to 66.8±5.8, Aq:47.4±5.0% to 60.7±5.2, SA:24.9±6.8% to 54.4±2.9% and LA:31.2±4.3% to 66.3±5.3%), neutrophils: (CE:40.3±3.4 to 65.8±6.0%, Aq:45.9±5.3% to 59.8±5.2, SA:25.6±6.3% to 53.2±3.3%, and LA:31.8±4.2% to 66.2±5.2%), and exudation (CE:31.2±3.8 to 51.4±3.3%, Aq:41.4±2.7 to 73.4±3.2%, SA:15.0±2.6% to 42.9±4.8%, and LA:23.4±2.9% to 52.6±3.4%)($p<0.05$). Additionally, this plant inhibited MPO (CE:60.1±1.6%; Aq:67.5±1.1%; SA:58.8±3.9%; LA:65.9±2.8%), ADA (CE:45.2±2.3%; Aq:63.9±5.8%; SA:37.5±6.0%; LA:64.4±6.7%), NOx (CE:40.7±1.1%; Aq:70.4±0.8%; SA:73.8±2.6%; LA:76.5±1.4%), IL-1β (CE:78.3±2.0%; Aq:74.2±1.5%; SA:24.6±1.2%; LA:14.9±1.3%), TNF-α (CE:61.9±3.4%; Aq:55.1±2.7%; SA:82.4±2.3%; LA:63.3±2.7%), and IL-17A (CE:64.0±6.4%; Aq:54.3±2.6%; SA:41.93±4.0%; LA: 21.2±5.4%)($p<0.05$).

Conclusion: J.sellowi less.showed an important modulation of the inflammatory response induced by carrageenan into the mouse pleural cavity by inhibiting the leukocytes content and the degree of exudation. These inhibitory effects were associated with the decrease of MPO and ADA activities and NOx, IL-1β, TNF-α and IL-17A levels.

A-431

Effects of the IL-10 gene deficiency on Mouse liver function

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Background: Interleukin (IL)-10 is an important immunoregulatory cytokine produced by many cell populations. Numerous investigations suggest that IL-10 plays a major role in chronic liver diseases. Our aim is to investigate the effect and mechanism of IL-10 deficiency on mouse liver.

Methods: Using the automatic biochemical analyzer to analyze serological biomarkers of liver function between IL-10 gene knockout mice and IL-10 wild type control. The pathological morphological changes were observed with the light microscope. The levels of iNOS and IL-1βgenes in liver tissues were determined by real-time

fluorescence quantitative PCR and enzyme-linked immunosorbent assay(ELISA).

Results: Compared with the wild type, the serum levels of ALB,TP,TBIL and DBIL of IL-10 deficient mice were significantly decreased(P<0.05), no obvious differences were found in AST,ALT and liver pathological morphology(P>O.05). The expression of iNOS and IL-1 βgenes, the serum levels of iNOS and IL-1β were significantly higher in IL-10 deficient mice than in wild type mice (P<0.05).

Conclusion: Endogenous IL-10 deficient mice can significantly decreased serum ALB and BIL. The effect may be related to the upregulated expression of iNOS and IL-1β.

A-432

Analytical Evaluation of an Assay Kit Incorporating New Ready to Use Liquid Stable Reagents for the Determination of Glucose, Through Conversion by Hexokinase, in Different Biological Fluids

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Background: Carbohydrates provide the human body with glucose, a simple sugar used as source of energy by the cells. The body must maintain proper glucose levels to ensure that a person remains healthy. Glucose determination is useful in the diagnosis and monitoring of carbohydrate metabolism disorders (i.e. diabetes mellitus, hypoglycaemia, pancreatic islet cell carcinoma) as well as in research and drug discovery processes. This study reports the evaluation of an assay for the determination of glucose by hexokinase-mediated reaction in serum, plasma, urine, cerebrospinal fluid (CSF) samples. This assay is applicable to automated systems and incorporates new ready to use liquid stable reagents, which facilitates the application in test settings by simplifying the experimental procedure and reducing handling errors. Methods: The assay involves a series of steps, initiated by the conversion of glucose to glucose-6-phosphate by hexokinase. The glucose-6-phosphate is then oxidized by glucose-6-phosphate dehydrogenase, causing the reduction of oxidized nicotinamide adenine dinucleotide (NAD) to reduced nicotinamide adenine dinucleotide (NADH). The absorbance of NADH is measured as endpoint reaction at 340/410 nm. The assay is applicable to a variety of analysers. The reagents are liquid stable and ready to use. On-board and calibration stabilities were tested by storing two lots of reagents uncapped on the analyser for a period of 60 days. Within-run and total precision were assessed by testing serum samples at defined medical decision levels, 2 replicates of each sample were assayed twice a day for 10 days. Correlation studies were conducted using a commercially available assay system. Results: The reagents presented an on-board stability of 60 days and calibration frequency of 60 days. The assay was linear from 4 mg/dL up to 700 mg/dL for serum, plasma, urine and CSF. The within-run and total precision for different concentration levels, expressed as %CV, was ≤ 2.0. In the correlation studies 99 serum patient samples, 88 plasma samples (lithium heparin), 87 plasma samples (potassium EDTA), 51 urine samples and 113 CSF samples were tested and the following linear regression equations were achieved: y = 1.001x + 0.3; r = 1.0 (serum, range 5-676 mg/dL), y = 1.001x + 0.2; r = 1.0 [plasma (lithium heparin), range 5-686 mg/dL), y = 1.002x + 0.0; r = 1.0 [plasma (potassium EDTA), range 5-676 mg/dL), y = 0.989x - 0.3; r = 1.0 (urine, range 4-664 mg/dL), y = 1.005x - 0.1; r = 1.0 (CSF, range 20-654 mg/dL). Conclusion: The results of this evaluation indicate that this assay is applicable to the determination of glucose in different biological fluids. This assay kit exhibits good correlation with existing commercial assay systems for all the analysed matrices. Furthermore, it incorporates liquid stable reagents, which simplifies the experimental procedure and reduces handling errors.

A-433

Effects of Chronic Ozone Exposure on the Oxidant-Antioxidant System of Brain Tissue

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Background: Ozone treatment entails exposing a body cavity or circulation system to a mixture of oxygen and ozone, and has been used in conjunction with other therapies to treat various pathologies. Although repeated ozone treatment has been purported to stimulate an antioxidant response, research characterizing long-term oxidant-antioxidant homeostasis has not yet been reported. As a therapeutic treatment for peritoneal adhesion, rats were exposed to ozone by one of two regimens. During this study, we evaluated oxidant and antioxidant parameters from brain tissues retrieved from these rats.

Methods: For this study, 24 Sprague-Dawley male rats were separated into three groups. The adhesion model for this study was established by making an incision in the cecum of the rats, followed by suturing. Two groups were administered ozone treatment for 15 days; however, each group was subjected to a different regimen: one group was treated with ozone immediately following surgery, whereas the other group was treated 24 h post-surgery. After 15 days, and while anesthetized, surgery was performed to open the abdomen in order to evaluate the adhesion site and to excise the brain tissue. Malondialdehyde (MDA), superoxide dismutase (SOD), carbonilized protein (PCO), and glutathione peroxidase (GSH-Px) levels were measured in the excised brain tissues.

Results: Brain tissues from rats immediately treated with ozone following surgery exhibited higher levels of SOD activity compared with the other two groups. By contrast, the MDA levels observed in this group were significantly lower. There was no difference between groups in terms of PCO levels. We determined that both groups exposed to ozone (i.e., 0 h and 24 h post-surgery) exhibited significantly higher GSH-Px activities in comparison with the control group.

Conclusion: Our findings indicate that long-term ozone treatment supports the antioxidant system in brain tissue. Furthermore, it should be noted that the time it takes to receive treatment is critical, as quicker ozone treatment more effectively stimulated an antioxidant response.

A-435

Evidence of the anti-inflammatory properties of Ageratum conyzoides L. in a murine model of pleurisy induced by carrageenan

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Background: A.conyzoides L. is used in Brazilian folk medicine as analgesic and anti-inflammatory agent. The aim of this study was to evaluate the anti-inflammatory effect of the Crude Extract(CE), and it's derived fractions: Ethanol(EtOH) and Hexane(HEX), and isolated compounds: Methoxy Nobiletin(MeONOB), 1,2-Benzopyrone(BP) and Eupalestin(EP) from A.conyzoides on: leukocytes, exudation and myeloperoxidase(MPO) and adenosine-deaminase(ADA) activities, and nitrate/nitrate(NOx) and cytokines (TNF-alpha and IFN-gamma) levels in a murine carrageenan-induced pleurisy.

Methodology: The aerial parts of A.conyzoides were air-dried at 50ºC, crushed and stored at 8ºC. The CE was prepared by maceration with ethanol, concentrated in rotary evaporator. The EtOH and HEX fractions were obtained by, extraction of CE, with different solvents of increasing polarity: ethanol and n-hexane. EtOH was partitioned with solvents of increasing polarity: n-hexane, dichloromethane and ethyl acetate. The dichloromethane fraction obtained from EtOH was chromatographed on silica gel "flash" column using dichloromethane and methanol gradient as eluent, being collected 100 fractions. Fractions 27-34 resulted in BP and fractions 35-69 yielded a mixture of methoxylated flavonoids were re-chromatographed on silica gel "flash" column using dichloromethane and methane gradient as eluent to obtain EP and MEONOB. Swiss mice were used though the experiments (Br.J.Pharmacol.183.811-19.1996). This study was approved by the local Ethical Committee (protocol: PP00757/CEUA/2012). Different groups of animals (n=5/group) were treated with CE(10-200mg/kg), EtOH(5-25mg/kg), HEX(25-50mg/kg), MeONOB(2.5-10mg/kg), BP(2.5-10mg/kg) or EP(1.0-10mg/kg) administered by intraperitoneal route, 0.5h before carrageenan-induced pleurisy(Cg,1%) administered by intrapleural route(i.pl.). The inflammation was analyzed after 4h. The leukocytes were analyzed using an automatic counter. A group of animals was previously challenged with Evans blue dye (25mg/kg, i.v.) to evaluate the exudation. The doses of CE(50mg/kg), EtOH(10mg/kg), HEX(50mg/kg), MeONOB(5mg/kg), BP(5mg/kg) or EP(5mg/kg) administered 0.5h before were selected to evaluate the effect of the herb on MPO and ADA activities, and NOx level which were analyzed by colorimetric assays. Analysis of cytokines were conducted using mouse inflammation cytometric bead array kit (BD Biosciences) only in EtOH and its isolated compounds. Statistical differences were determined by ANOVA and Student-Newman-Keuls post-hoc analysis. Values of p<0.05 were considered significant.

Results: CE(50-200mg/kg), EtOH(10-25Mg/kg), HEX(50mg/kg), MeONOB(5-10mg/kg), BP(5-10mg/kg) or EP(5-10mg/kg) inhibited leukocytes(CE:35.0±6.8 to 79,3±1.8%; EtOH:57.0±6.6 to 75.6±0.3%; HEX:56.7±7.5%; MeONOB:58.5±2.2 to 60.0±6.2%; BP:59.6±3.2 to 60.9±5.8%; and EP:64.7±1.6 to 69.5±1.4%), neutrophils(CE:37.4±7.1 to 80.1±1.7%; EtOH:68.3±2.2 to 77.0±0.2%; HEX:54.3±8.1%; MeONOB:63.1±2.0 to 63.8±5.5%; BP:64.3±5.1 to 71.9±2.2%; and EP:69.7±1.8 to 74.1±0.8%), exudation(CE:26.5±2.8 to 68.2±2.9%; EtOH:58.5±3.9 to 63.3±2.9%; HEX:33.3±2.4%; MeONOB:51.3±7.2 to 54.3±2.7%; BP:40.8±3.6 to 44.1±3.6%; and EP:39.9±8.1 to 42.1±6.9%), MPO(CE:34.7±5.6%;

EtOH:31.6±5.3%; HEX:29.6±4.2%; MeONOB:28.6±2.3%; BP:22.4±2.9%; and EP:18.3±1.3%), and ADA activities(CE:70.5±6.3%; EtOH:71.0±4.9%; HEX:72.1±4.8%; MeONOB:67.3±2.1%; BP:27.4±8.4%; EP:54.6±3.7%) and NOx level (CE:79.1±7.2%; EtOH:63.2±12.9%; HEX:71.2±9.4%; MeONOB:55.5±13.9%; BP:53.8±14.1%; EP:80.8±0.3%). Also EtOH and its isolated compounds inhibited TNF-alpha(CE:24.7±1.6%; EtOH:25.9±4.5%; MeONOB:26.6±2.2%; BP:31.5±1.3%; and EP:21.4±5.4%) and IFN-gamma(CE:15.7±1.6%; EtOH:11.0±1.4%; MeONOB:13.6±0.5%; BP:6.4±2.0%; and EP:11.0±0.6%)($p<0.05$).

Conclusion: A.conyzoides presented important anti-inflammatory properties not only by inhibiting leukocytes migration but activated neutrophils. This effect was also associated with the decrease of exudation and NOx and pro-inflammatory enzymes (MPO and ADA). This effect appears to be mainly related to the EtOH fraction and its isolated compounds: MeONOB, BP, and EP which inhibited all the inflammatory parameters, including TNF-alpha and IFN-gamma.

A-436

Effects of methanolic leaf extract of African mistletoes (Loranthus micranthus) on male sexual function in streptozotocin-induced diabetic Wister rats

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Background: The leaves of African mistletoes (*Loranthus micranthus*) have been shown in traditional African setting to improve sexual function in diabetic males but data on scientific proofs of this therapeutic action of these leaves is scanty hence this study. In this study, the effect of methanolic extract prepared from the leaves of *L. micranthus* on serum testosterone levels, sperm count and motility in diabetic male wistar rats was studied.

Method: The animals were randomly divided into four (4) groups made up of six (6) rats each and diabetes was induced in the rats by the administration of alloxan (100mg/kg) for 7 days. Group A served as the control (untreated diabetes), groups B and C were treated with 150mg/kg and 300mg/kg respectively of the extract while group D received the 100mg/kg of the standard antidiabetic drug (chlorpropamide). The duration of substance administration was fourteen days. On the fifteenth day, all the animals were lightly anaesthetized with ether and their blood collected for testosterone analysis. The rats were further dissected and the caudal epididymis of each incised and seminal fluid collected for sperm count and motility tests.

Results: Table 1 showed diabetes to decrease male sexual functions (group A) when compared with standard reference value. Also, significant increase ($p<0.05$) in the level of serum testosterone, sperm count and sperm motility which was dose depended was showed with the extract administration (groups B and C) compared with control (group A). Chlorpropamide treated rats (group D) also showed a significantly increased male sexual function compared with the control, however, mistletoe was more potent.

Conclusion: From the findings of this study, we suggest that leaf extract of African mostletes be studied in detail so as to know its therapeutic dose for it possible use as a therapeutic agent in the treatment of male infertility secondary to testosterone/sperm abnormalities.

Table 1: Effects of leaf extract of African mistletoes (*Loranthus micranthus*) on serum testosterone level, sperm count and sperm motility of diabetic male wister rats

Group	Testosterones level	Sperm count (x10)	Sperm Motility (%)
A (n=6)	1.21± 0.22	8.25±2.00	67.50±1.73
B (n=6)	6.63 ± 2.15*	9.90 ± 2.46*	92.50 ±22.64*
C (n=6)	7.76 ± 2.29*	10.90 ± 2.62*	105.00± 25.45*
D (n=6)	2.53 ± 0.67*	10.85 ± 2.89*	95.00± 22.66*

Values are mean ± S.D; * = significantly difference from the control (Group A) at $P < 0.05$.

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Effects of Uvaria chamae Extracts on Blood Glucose,Inflammatory Markers,Hematological and Renal Status in Streptozotocin-induced Diabetic Rats

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Background: Uvaria chamae is a medicinal plant that is used in some regions of the world in the treatment of diabetes, and as an antifungal, antimalaria, and bacteriostatic herb. The chemical constituents of Uvaria chamae, include C-benzylated monoterpenes, aromatic oils, flavanones, C-benzylated flavanones, and C-benzylated dihydrochalcones. Traditionally, a decoction of the root is used in the treatment of many diseases, including diabetes. However, the use of this plant extracts in the treatment of diabetes have not been scientifically validated. In this study, we determined some blood analytes in streptozotocin-induced diabetic rats administered aqueous or ethanolic extract of the root of Uvaria chamae. Methods: Thirty six (eighteen adult normal and eighteen streptozotocin-induced diabetic rats) Sprague rats were administered aqueous or ethanolic extract (300 mg/kg body weight) of Uvaria chamae for 35 days [6 rats per group, average body weight (265.23 ± 7.20 g)]. The six groups were composed as follows: Healthy rats receiving de-ionized water (Normal Control); Normal rats receiving aqueous extract (Normal plus Aqueous Extract); Normal rats receiving ethanolic extract (Normal plus Ethanolic Extract); Diabetic rats receiving de-ionized water (Diabetic Control); Diabetic rats receiving aqueous extract (Diabetic plus Aqueous Extract); and Diabetic rats receiving ethanolic extract (Diabetic plus Ethanolic Extract). Diabetes was induced using a single injection of streptozotocin (Sigma-Aldrich, 60 mg/Kg body weight in 0.05 M-citrate buffer, pH 4.5) intraperitoneally. Animals were euthanized by decapitation on day 35 after commencement of the feeding trial. Blood was collected for assays. Results: There was a significant ($p<0.05$) decrease in blood glucose level in the treated diabetic groups compared to the diabetic control. We also noted significant ($p<0.05$) increase in BUN in the diabetic control compared to the normal control. The administration of aqueous or ethanolic extract to the diabetic rats did not restore the level of BUN to that of normal control group. The diabetic groups administered aqueous or ethanolic extract showed increasing trend in the level of MCV toward the normal control group compared to the diabetic control. The levels of MCHC and WBC were significantly ($p<0.05$) lower in the diabetic groups administered aqueous or ethanolic extract compared to diabetic control. The levels of RBC, Hgb, PCV, platelets, monocytes and granulocytes were not significantly ($p>0.05$) altered among the groups. We noted reducing trend in the levels of IL-6 and IL-β in the diabetic groups administered aqueous or ethanolic extract compared to the diabetic control. However, serum creatinine level was slightly elevated in the diabetic group administered ethanolic extract. Conclusion: Overall, the consumption of aqueous or ethanolic extract of Uvaria chamae lowers blood glucose level which may be beneficial in the management of diabetes. The increasing trend in MCV level due to the administration of ethanolic or aqueous extract may protect against the development of anemia that is associated with diabetes. The inflammatory cytokine (IL-6) normally up-regulated in diabetes was depressed by the aqueous or ethanolic extract administration. However, the increased serum creatinine level is indicative of the potential adverse effect of the ethanolic extract on renal function.

A-438

Validation of Automated Immunoglobulin A, G, and M in Non-human Primate Serum to Support Pre-Clinical Toxicology Studies

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Background: Immunoglobulins are glycoprotein molecules that are produced during an immunogenic response, and are divided into five classes, based on the differences in the amino acid sequence found on the heavy chains. Immunoglobulin A (IgA), Immunoglobulin G (IgG) and Immunoglobulin M (IgM), are the three most common immunoglobulins found in serum. Measurement of immunoglobulin concentrations are used to aid in the diagnosis of immune response or abnormal protein metabolism. Here, we validated automated human IgA, IgG and IgM assays for use in pre-clinical primate studies.

Methods: Siemens IgA (02194102), IgG (02193432), and IgM (02193483) assays are PEG-enhanced immunotubidimetric assays performed on the Siemens Advia 1800. Serum samples are pre-diluted by the instrument, then mixed with specific antiserum to form a precipitate that can be measured turbidimetrically at 340/694 nm. The measured absorbance is then compared to an established calibration curve (Advia Chemistry Liquid Specific Protein Calibrator, 07711199) and the concentration is determined and reported as mg/dL. Bio-Rad Liquid Assayed Multiqual® (Level 1: 694, Level 2:695, Level 3: 696) quality control (QC), AUDIT® MicroCV™ Protein Linearity (K702M-5), and colony primate (*Macaca fascicularis*) serum were analyzed to test the performance and dynamic range of the assays.

Results: Intra-assay precision testing was performed using 4 primate serum samples, and 2 levels of QC. Samples were analyzed a minimum of 5 replicates in a single assay run. All samples demonstrated %CV ≤ 3.6. Accuracy and inter-assay precision testing was performed using 3 levels of QC run in triplicate for 5 runs. Mean and imprecision were calculated and fell within the manufacturer's established 2SD range, and demonstrated %CV ≤ 3.0. Three primate serum samples were analyzed in duplicate over 4 separate assay runs, demonstrating %CV values ≤ 3.6. Commercially available linearity standards were analyzed and demonstrated reportable assay

analytical ranges of 31.4 - 669.4, 140 - 3009, and 17.4 - 393.8 mg/dL for IgA, IgG and IgM, respectively, confirming the manufacturer's stated analytical range. Dilutional linearity was performed with 3 primate serum samples with high concentrations. Samples diluted starting at a 1:2 dilution in 0.9% saline and analyzed in duplicate. Dilutional linearity was established as 1:16, 1:8, and 1:4 for IgA, IgG and IgM, respectively. Spike recovery was performed with pooled primate serum spiked with 10%, 7.5% and 5% of the highest level of linearity material. All spiked sample results were within 20% of the expected value. Sample frozen stability and freeze/thaw stability (-80°C) was performed with eight primate serum samples with varied IgA, IgG and IgM results. The samples were assayed neat then analyzed after 1, 3, and 6 months and after 1, 2, and 3 freeze/thaw cycles. All samples had appropriate percent recovery (80 - 120%).

Conclusion: The Siemens IgA, IgG, and IgM assays met all outlined criteria for validation and are appropriate for use in non-human primate serum samples to support pre-clinical toxicology studies.