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RESEARCH ARTICLE

GRASSHOPPER: RAW MATERIAL IN TRADITIONAL MEDICINE

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ABSTRACT

Reparations and medicaments derived from grasshoppers are mostly used for curing such diseases as respiratory disorders /65%/, wounds /16%/, poisoning /8%/, and trauma /11%/ in the forms of simple extract /35%/, boiled extract /21%/ and bolus /35%/ via swallowing /9%/, drinking /80%/, topical use /14%/ and fumigation /6%/. Specimen as raw materials of medicine from grasshopper can be annually collected from 8 through 10. In total 8 species of grasshoppers of therapeutic values belonging to 4 genera have been identified. Caloric value of raw material 1351,2 joule and contains up to 60..proteins, 16 amino acids, vitamins and various minerals. Toxicity of grasshoppers preparation is 8,1 ml/kg and the preparation has no adverse effects on animal body. The preparation exerts such effects as relieving the severity of edematous pneumonia, prolonging the life span, minimizing the concentration of nitrogen oxide during inflammation as inhibitor, stopping the oxidation and adaptability during oxygen deficiency and thermal exposures.

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INTRODUCTION

The order to collect information about using grasshoppers in traditional medicine was made research work in 14 provinces in Mongolia. Based on the decoction, used in the traditional medicine and, I.A.Muravievs method the preparation named "Acritract", besides, in order to compare the chemical, pharmacological and clinical. Grasshoppers that can be effective for research were chosen after cultivation of the materials and grasshoppers specifics were defined by G.Ya. Bei-Bienko method. Chemical compound, pharmacology inductions, drug action and activity of treatment were defined by specific methods for each of them. Use the preparation results in neutralization of extreme irritation of parasymphatic nerves of smoot muscles and normalizations of structures and functions of bronchial mucosa, alveolar and lobular tissues and cells during inflammation of lung. Fundamental and laboratory work, and statistical analysis of results were performed in the laboratory of traditional therapy and pharmacology, laboratory of pharmacology and toxicology, laboratory of biochemistry and metabolism, laboratory of infectious diseases and immunology, and laboratory of pathology of Institute of veterinary medicine, the laboratory of natural compounds of institute of chemistry and chemical technology, scientific-research laboratory of pharmacology of University of health sciences, Mongolia.

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Materials of the study: Folk traditional methods by using grasshoppers for curative treatments;

- Data from interviews with and experiences of herders;
- Processed raw materials of grasshoppers, which were collected by ourselves from steppe, Gobi and steppe-gobi zones\
- Preparations of raw materials of grasshoppers in the following forms: aqueous extract, powder, ointment and tablet and "Acritract".

Methods of the study: Interviews with herders, veterinarians and other people were made in 14 aimags in order to evaluate data and empiric methods for using grasshoppers as traditional knowledge.

Prepared raw materials were dried in dark place with sufficient ventilation or in the oven at temperature 38-40^{0}C or frozen in the freezer at -25^{0}C, after which, material was grinded fully in pestle and mortar till particle size reached 0.1 mm. In order to study pharmaceutical, pharmacological and clinical properties, and technological features, the preparations from grasshopper were made in the following primary forms: ten percent aqueous extract for clinical trials and comparative study, preparation "Acritract" was made by own technology, based on conventional technology of making preparation forms from animal sources in regard to methodology of I.A. Muraveva, and its biological potency, chemical composition, pharmacological effects and clinical significance were investigated.

For species identification of selected representatives of grasshoppers, which have therapeutic values, method of G.Y. Bei-Bienko and general zoological identification key were used. Total chemical compositions of the raw material were investigated by using conventional methods and caloric value of raw material was obtained by calculation method. Biological potency was measured by dermal probe (4 rabbits) under the effect of Trifanblue. In this case, changes of coefficient of reagents permeability affected by the preparation through dermal capillaries in rabbits were investigated in comparison with control animals. Also biological activity was tested by measuring photochemoluminescence of the preparation.

Toxicity of the preparation was determined by method of Behrens and Pershing, Total effect of the preparation was tested in rabbits (10) and sheep (10), and the animals were observed for 14-21 days. The preparation was parenterally administered at the dose of 5 and 10 ml. Anti-inflammatory effect of the preparation was according to the below methods of : S.Salamon–burn induced inflammation of skin, caused by hot water (80^0C) in white mice (20); D.A.Kharkovich–experimental inflammation of lungs, caused by intra-abdominal injection of 0.18% solution of adrenalin in white mice (20); B.Chirkov –injection of 1% solution of formalin in femoral muscle of white mice (40).

During pneumonia, general condition of animals was examined, and at 24 hour after sacrifice of all animals of both experimental and control groups, development of inflammatory processes in the lungs was observed, lungs were removed and their mass were measured. Then relative coefficient of lungs was calculated. Effectiveness of the preparation to cure burn induced inflammation was evaluated by general condition, necrotic process and viability of the animals. In order to trace inflammatory process in muscle, dimensions and mass of muscles after isolation were measured. Specific anti-inflammatory effect was investigated in the model of inflammatory process in white mice (60), which was caused by experimentally by use of 0.18% soluation of adrenalin. Change of concentration of free radical-nitrogen oxide, produced during inflammation was determined.

Concentration of nitrogen oxide is demonstrated with its intensity of spectrum in the following variants: during inflammation with the effect of adrenalin; during inflammation with the effect of adrenalin and paramagnetic trap; during use of the preparation for curing inflammation. Experiment was performed by use of radiospectrometer RE-1506. Lungs of white mice was removed and preserved in liquid nitrogen (-196^0C), and results of the experiment was assessed with the spectra of free radicals- nitrogen oxide. Anti-oxidation effect of the preparation was investigated by measuring MDA in liver homogenate of white mice (20), which were preliminarily injected with carbon tetrachloride to cause per-oxidation. Histomorphological changes in pulmonary cells and tissues of white mice (16) during use of the preparation for curing inflammation were also studied by conventional methods. Study of the preparation effect on tolerance of animals to action of high temperature (45^0C) and acute oxygen deficiency was undertaken.

Experiment was performed on 40 white mice. Parameters of tolerance of animals to high temperature and acute oxygen deficiency were the lengths of viability of these animals. Wound healing effect of aqueous extract, powder and ointment made from raw material of grasshopper was investigated on artificial wound of 0.03 m^2 area, caused on the spine of rabbit (40). The preparations were used to cure wounds for 21 days.

Effect of the preparation on smooth muscle of isolated intestine of rabbits (27) was traced by the method of Magnus on the ground of using m-cholinoblockers (atropine) and m-cholinomimetics (barium chloride and pilocarpine), and relative changes of frequency and amplitude of intestinal musculature with the effect of the preparations were studied.
Toxin neutralizing effect of "Acritract" was measured by method of weakening toxic substance of kantradin, contained in *Mylabris phalerata*, Based on the obtained laboratory and experimental results, clinical trials were carried out on sheep with diagnosis of chronic pneumonia. With the purpose of comparative study of therapeutic values of the preparations, aqueous extract and "Acritract" were used for the trials. All data were analyzed by using conventional bio-statistical methods (Lakin, G.F., 1990, Purevjav, J, 1981 and 2005).

RESULTS

Tradition of using medicinal raw materials from grasshoppers is one of the profiles folk medicine, having its own essence, goal, theoretical and practical principles and biological and pharmaceutical significance. Since ancient time, medicinal raw materials of grasshoppers were used in the forms of powder, extract, ointment and native state. Grasshoppers and their raw materials for medical use were prepared during late summer and autumn, when they reach full biological development, they were collected and dried in the air and dead, but not-contaminated ones were selected. Our herders use preparation of grasshopper in the form of aqueous extract for treatment of bronchitis, various forms of pneumonia and wounds in various animals.

Independent experiences of 28 persons, recorded by us became fundamental materials for our study. As a result of discussion and analysis of materials on medicines, we developed scientifically based scheme and gave assessments on the essence of empiric methods on the basis of collection of raw materials, regimes of processing, methods of preparation, their forms and objects of the use. From ancient time, grasshoppers in accessible form were used in countries of Asia (Mongolia, India, China and Singapore) and Arab for curing tuberculosis, bronchial asthma, various disorders of respiratory tract, wounds, intoxications and allergic pathologies. Above mentioned data demonstrate that raw material form grasshopper can be collected in the periods of full biological development. Therefore, we manually collected raw material every year, but in the last years we are using special equipment during September and October, and during these periods chemical properties of collected raw material was investigated.
The following species of grasshoppers (Acrididea) have been identified as a result of our study: *Compsorhips Saussure; Oedaleus Fiber; Breodema Fiber; Calliptamus Servile*. Above species are raw materials of therapeutic values.

A hundred gram grinded and dried raw material had 4.41% moisture, contained 81.66% dru matter, 6.47% ash and 7.44% fat, while energetic value of raw material was 1351.2 Joule. Content of proteins is more than 60%. These proteins are total protein compounds, enzymes and peptides. Raw material-grasshopper contains 16 amino acids, including 6 essential and 10 nonessential ones. Essential amino acids are 82.7 mmol and nonessential ones 41.4 mmol. Amino acids with highest contents are glutamic acid (13.2 mmol), valine (8.8 mmol) and methionine (9.0 mmol). Raw material of grasshopper is also source of vitamins. It is evidenced with that it contains vitamin C (79.2 mmol), vitamin B_1 (8.8 mmol) and vitamin B_2 (32.1 mmol).

According to our study on solubility of raw material in various solvents, 30% ethanol solution is seen to be most suitable. Having evaporated completely ethanol in water bath and added adequate amount of distilled water, preparation in the form of 10% aqueous extract was made and it was injected into tail vein of white mouse. Doses from 1 to 3 ml per kg of animal body weight, injected intravenously were seen as acceptable. Injection of 4-6 ml/kg dose resulted in death of 17 mice of experimental group. In experiment using 8-10 ml/kg dos, sudden dyspnoea, ataxia and depression were observed in experimental mice at the first minutes after injection. In this case 24 out of 40 experimental mice were died. Doses of preparation 11 and 12 ml/kg for all mice were lethal. As a result of our experiments, median lethal dose is equal to 8.1 ml/kg or 0.81 g/kg dry matter.

Total biological effects of the preparation was investigated by us on rabbits and sheep. Animals of experimental group received 5-10 ml/kg doses. During the experiments, lasted for 7 days animals of experimental group had no any changes of body temperature, respiration frequency and heart rate. General conditions of the animals remained unchanged. It revealed that the preparation has no total toxic effect on animal body. Preparation at the dose of 0.5 ml/kg was orally given to white mice for 30 days, followed by every day observations and their body weights were measured. Due to effect of the preparation, live weights of animals increased by 17.7% as compared to control group animals.

After artificially caused pneumonia by use of 0.18% adrenalin, mice, which received no treatments, were died showing overt signs of inflammation for 30 minutes. Mice of experimental group survived during 3 hours, when observation continued. Partial hemorrhages, swelling of lobes of lungs and increase of its size were found during examination of isolated lungs. Coefficient of ratio between lungs and body weight for both experimental and control group animals was equal to 0.98 and 1.18 respectively (should be less than 1 at norm). It revealed that the preparation stopped the further development of inflammatory process in lungs. During burn induced inflammation in control group mice, necrotic pathology (infiltration) appeared in the regions of spine, belly and tail. Tails were intensively necrotized and in some cases tails were broken. Above mentioned signs were less in those mice, which received the preparation. During the observation period, 60% of control animals and 30% of experimental animals were died. It demonstrated that the preparation exerts protective effect in case of burn induced inflammation. Preparation of grasshopper during pneumonia had strong effect on respiratory chain of microsome and mitochondria, and inhibits production of free radicals – nitrogen oxide. Content of nitrogen oxide in case caused by adrenalin (typical inflammation) increased by 34.7%. It is greater than the preparation was used. In cases, when adrenalin and paramagnetic trap are effective, content of nitrogen oxide increases by 22.5%. These results evidence that due to effect if the preparation during inflammation production of nitrogen oxide drops and the preparation acts as inhibitor. As a result of the experiment performed in the models representing per-oxidation in liver caused by carbon tetrachloride, production of MDA, a product of per-oxidation was arrested. The preparation acted in this case as an anti-oxidant.

Control group mice were died due to high temperature exposure within 108 minutes in average, whereas experimental group mice within 120.4 minutes (in total 90%) or in other words the preparation increased survival length of experimental group animals by 11.5% ($P<0.010$), which is statistically significant. Administration of the preparation provides the increase of tolerance of animals to temperature influence. In exposure to effect of acute hypoxia the preparation decreases the duration of convulsion and increases survival length of experimental white mice as compared to control animals.

In case of control animals, length of survival is 135 minute in average, while in experimental animals 180 minutes. Results of our observation, where mice in sealed container were treated with the preparation for prevention, are positive. The preparation stops convulsion and increases survival length by 39.1% ($P<0.001$) or significantly. Thus experimental results allow suggest that the preparation of grasshopper has clear anti-inflammatory effect and possess capacity to enhance tolerance of animals to exposures of exogenous adverse factors. The preparation is effective to decrease contraction, amplitude and tonus of muscle of isolated intestine by 6.3%, 7.5% and 11.1% respectively, which reveals the preparation has spasmolythic effect. On the ground of atropine, spasm, amplitude and tonus drop as compared to normal parameters and therefore the preparation acts in synergy with atropine, when all data were at level 0. On the ground of barium chloride and pilcarpin the same results were obtained. From above it was revealed that the preparation has m-spasmolythic effect.

The preparation has no adverse effects on cardio-vascular system. During topical use of the preparation in the form of aqueous extract, ointment and powder the lengths of healing were 20, 12-13 and 10-12 days respectively. In control animals healing of wounds started from day 14, but wound surface was not fully recovered. Of these forms, ointment and powder were effective to cure wounds. In samples of Mylabris phalerata, not weakened by vodka, Acritract and mixture, needle shaped white crystals of kantradin, contained in Mylabris phalerata were detected and their amount was 0.10 g in average. In samples of Mylabris phalerata, weakened by vodka, Acritract and mixture, no traces of kantradin, which causes acute

inflammation of mucous membrane (Ts.Khaidav, 1981), were detected and it was impossible to measure them in the sample. From above it is concluded that due to effect of "Acritract" kantradin, contained in the sample of Mylabris phalerata is neutralized. For study of processes of pathomorphological changes under the effect of preparation, we investigated morphological changes of parenchymatous organs of white mice (4 groups) on the ground of artificially caused inflammation in lungs at hours 24, 48 and 72. Samples in these cases were taken from groups of animals, treated by adrenalin and preparation and control animals. No any lesions were found in liver, kidney, spleen and heart of experimental animals, treated by preparation.

At hour 24 after injection of adrenalin and simultaneous administration of the preparation, smooth muscle contracts at lower tone and mucous membrane is slightly irritated and therefore typical artificial asthma is not caused. In this period, the preparation neutralized acute irritation of parasympathetic nerve. Clearly demonstrated pathological lesions due to effect of adrenalin are decreasing and at hours 48 and 72 pulmonary cells and tissues become normal. The preparation revives the structures of cells and tissues, which were damaged during pathology due to effect of adrenalin.

Hematological parameters changed in control animals, while hematological parameters remained unchanged after administration of the preparation and leukocytes decreased further, that reveals inflammatory process is decreasing and recovery starts in the lungs. Sputum expectorating effect of the preparation was clearly observed. Clinical recovery of patient animals completed at day 21 after administration of "Acrtract" (96.5%), whereas 78% of sick animals recovered clinically due to effect of aqueous extract.

Conclusion

By this study, we have determined following results

- The caloric value of raw materials from grasshopper is 1351.2 Joule and it contains 16 amino acids, Cand B vitamin, up to 60% proteins, and various minerals (zinc content is more than others).
- The raw material from grasshopper is highly solublein 30% ethanol For the treatment, the preparation would be used in various forms, such as ethanol extract8 tablet and oiantment.
- The preparation has prolonging effect to edematous pneumonia of lung and shows inhibitor effect during inflammation by minimizing nitrogen oxide.
- The preparation stabilizes the intestinal contractions and exerts M-spasmolytic effect.
- Toxin neutralization was alse tested and preparation has neutralization effect to toxin of animal origin, such as kantradin.
- Use of the preparation results in neutralization of extreme irritation of parasymphatetic nerves of smooth muscles and normalizations of structures and functions of bronchial mucosa, alveolar and lobular tissues and cells during inflammation of lungs.
- In the future, we want to develop food additive containing protein technology using grasshopper, for that genomic and proteomic analysis are essential.

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